Acetyl-L-carnitine to enhance nerve regeneration in carpal tunnel syndrome; a randomized controlled trial

By

Matthew Curran

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science
In
Experimental Surgery

Department of Surgery
University of Alberta

Abstract

Background Carpal tunnel syndrome (CTS) is the most common form of peripheral nerve injury, involving approximately 3% of the population. While surgery is effective in mild and moderate cases, neurologic and functional recovery are often not complete in severe cases. Therefore, there is a need for adjuvant methods to improve nerve regeneration in those cases. Acetyl-l-carnitine (ALCAR) a small molecule involved in lipid transport, has been shown to be effective in various forms of neuropathies. However, it has not been used in compressive peripheral nerve injury.

Purpose To test the hypothesis that ALCAR can promote peripheral nerve regeneration and improve function in patients with severe CTS.

Methods In this pilot study we utilized a double-blind randomized placebo controlled design. Inclusion criteria included adult patients with severe CTS, confirmed by nerve conduction studies and motor unit number estimation (MUNE). Eligible patients were randomized to receive 3000mg/day of ALCAR orally or placebo following carpal tunnel release surgery for 2 months. The primary outcome was MUNE with supplementary secondary outcome measures that focus on sensation and hand function. To follow post treatment recovery and monitor safety, patients were seen post-operatively at 3 months, 6 months and 1 year. The outcome measures were analyzed using two-way ANOVA with treatment assignment and time points being the independent factors.

Results Twenty patients were enrolled in the study. Patients were predominantly female (60%) with an average age of 59.9±2.2 (Mean±SD). Demographic data and baseline measures were similar between the two groups. There was no difference in the primary outcome MUNE between the groups at 12 months follow-up. Similarly, there was no difference in any of the secondary outcomes between the treatment groups at 12 months. Both groups improved with

time over the course of the study. The treatment was well tolerated. There was no difference between vital signs or blood work parameters in each treatment arm. There were only 4 adverse events, with no difference in either group.

Conclusion ALCAR did not improve nerve regeneration nor functional recovery in patients with CTS. The use of ALCAR to enhance peripheral nerve regeneration in compression neuropathy is therefore not supported.

Preface

The methods section of this thesis has been previously published in the journal *Trials*. It can be found in Chapter 2: Protocol for a randomized control trial for peripheral nerve regeneration.

Ming Chan is an experienced clinician scientist in physical medicine and rehabilitation who is responsible for evaluating the treatment outcomes and to oversee the project. Matthew Curran, a resident in the division of plastic surgery was responsible for recruitment of patients, evaluating treatment outcomes and safety, data analysis and manuscript preparation. Jaret Olson and Michael Morhart are plastic surgeons who were responsible for patient recruitment and performing the carpal tunnel release surgery. Dory Sample is a nurse with an extensive background in clinical trial management. All investigators were involved in the study design and have a role in result analysis and writing of the manuscript.

Ethics

The study has ethical approval from the *Health Research Ethics Board* (Pro00045538) at the University of Alberta. The trial was conducted in keeping with the principles of *Good Clinical Practice*. The trial was registered with clinicaltrials.gov (NCT02141035; 20 April 2015).

To my father, James

Acknowledgements

I would like to express my deepest gratitude to my Supervisor Dr. Ming Chan, Supervisory Committee Drs. Jaret Olson and Doug Zochodne, and Dr. Michael Morhart. All have played an integral role in the completion of this project. Their guidance had a strong impact clinically and academically on me. I will be a stronger researcher and clinician in the future from the skills I have learned from each of these individuals. I would also like to thank the administrative staff, particularly Bonnie Monteith and Lynn Boulanger for their assistance with patient scheduling and putting up with my ever frequent questions and calls. I would like to thank my lab mates, particularly Dr. Akiko Hachisuka for their assistance with patient coordination and data input. Finally, my thanks to the friends and family who have provided me with a supportive environment for the past 2 years.

Table of Contents

List of Figures

List of Tables

Abbreviations

2PD	Two Point Discrimination
ACN	Acute Compression Neuropathy
AIN	Anterior Interosseous Nerve
ALCAR	Acetyl-L-carnitine
APB	Abductor Pollicis Brevis
ATN	Antiretroviral Toxic Neuropathy
BCTQ	Boston Carpal Tunnel Questionnaire
CCN	Chronic Compression Neuropathy
CIPN	Chemotherapy Induced Peripheral Neuropathy
CMAP	Compound Motor Action Potential
CNS	Central Nervous System
CTS	Carpal Tunnel Syndrome
CV	Conduction Velocity
DASH	Disabilities of Arm, Shoulder, and Hand Questionnaire
DRG	Dorsal Root Ganglion
DM	Diabetes Mellitus
EMG	Electromyography
ERK	Extracellular Regulated Kinase
FCR	Flexor Carpi Radialis
FDP	Flexor Digitorum Profundus
FDS	Flexor Digitorum Superficialis
FPB	Flexor Pollicis Brevis
FPL	Flexor Pollicis Longus

GAP-43	Growth Associated Protein - 43
GSS	Global Severity Scale
HIV	Human Immunodeficiency Virus
MAPK	Mitogen Associated Protein Kinase
MEK	MAPK/ERK kinase
MUNE	Motor Unit Number Estimation
NCS	Nerve Conduction Studies
NGF	Nerve Growth Factor
PNS	Peripheral Nervous System
QST	Quantitative Sensory Testing
RAG	Regeneration Associated Gene
RCT	Randomized Controlled Trial
SC	Schwann Cells
SNAP	Sensory Nerve Action Potential
SWMT	Semmes-Weinstein Monofilament Test
TCL	Transverse Carpal Ligament

Chapter 1

Epidemiology, anatomy, pathophysiology, and management of carpal tunnel syndrome; use of carpal tunnel syndrome as a model of peripheral nerve injury to evaluate novel therapeutics

Introduction

Peripheral nerve injuries are common and involve all populations from newborns to the elderly. The term peripheral nerve injury represents a spectrum of injury from compression neuropathies to crush and transection axotomies. The etiology of these injuries ranges from idiopathic to high velocity blunt trauma or penetrating trauma. Of all of these injuries by far the most common is compression neuropathy with carpal tunnel syndrome (CTS) being the prototypical example.

In 1854 Sir James Paget first described CTS in 2 patients with paresthesias(Paget, 1854). Putnam produced the first case series of patients with 37 predominantly female patients that presented with numbness that "in some cases simply letting the hand hang over the bed or shaking bit around for some moments would drive away the numbness". This would stand as the first description of the flick sign that is commonly used in the diagnosis of CTS (Putnam, 1880). Although the condition was recognized over a century ago, treatment of CTS did not progress as quickly. Though surgical decompression was postulated in 1913 (Marie & Foix, 1913) the presence of cervical ribs as thoracic outlet compression of the brachial plexus was felt to be the causative etiology (Sargent, 1921). Not until 1933 did Learmonth describe what has become the open carpal tunnel release (CTR), an operation that has been refined but is still performed today (Learmonth, 1933). Phalen would eventually thoroughly describe the diagnosis and its treatments in the 1950s and 1960s which brought the disease widespread recognition (Phalen, 1966; Phalen *et al.*, 1950)

Despite the widespread recognition and prevalence there is a relative paucity of literature especially on pathophysiology. Interestingly, despite only a fraction of the incidence, nerve injuries leading to axonal damage have been much more extensively studied. Consequently, for

a syndrome initially described over 150 years ago, there is still debate about many major aspects of the disease from etiology to treatment.

The lack of pathophysiologic understanding can be problematic when attempting to develop new treatments. Our understanding of the earlier stages of CTS is quite poor (Pham & Gupta, 2009). Thankfully, the treatments used in mild and moderate CTS are quite efficacious though certainly can be improved (Steinberg, 2002). However, for severe CTS this does not hold true. The outcome of the disease is dependent on the chronicity of the compression, comorbidities, and age of the patient; all of these prognostic factors make a complete resolution of symptoms unlikely (Fu & Gordon, 1997; Mondelli *et al.*, 2004). When CTS progresses to this severity there is typically severe axonal loss (M. Mondelli *et al.*, 2002; Padua *et al.*, 1997). Manipulating the molecular pathways associated with nerve regeneration to increase axon outgrowth has long been a desired outcome of peripheral nerve surgery. It provides hope for increasing our outcomes farther than current paradigms.

An example therapeutic would be acetyl-L-carnitine (ALCAR). A naturally occurring peptide which plays a role in fat metabolism, ALCAR has been shown to have neuroprotective effects in the central nervous system and enhance peripheral nerve regeneration in the peripheral nervous system (Chan *et al.*, 2014). While ALCAR has been used in systemic neuropathies, only a single animal study has been conducted for the use of ALCAR in compression neuropathy, with some benefit (Kotil *et al.*, 2007). As ALCAR is a natural product, with a favorable side effect profile, it is an attractive target to explore for human use.

In this chapter I will examine CTS, nerve regeneration and therapeutics that can enhance nerve regeneration with particular attention to ALCAR. This will form the basis of a randomized

control comparing the use of ALCAR after CTR in patients with severe CTS to increase nerve regeneration.

Epidemiology of Carpal Tunnel Syndrome

Carpal tunnel syndrome is the most common peripheral nerve injury and prototypical model of compression neuropathy. It was found to have a prevalence of 3% in a general population study of 3,000 Swedish individuals (Atroshi *et al.*, 1999). In a second study on a Dutch population, found an overall prevalence of 5.8% in females though 3.4% a previous diagnosis of CTS (de Krom *et al.*, 1992). The same study found the male prevalence to be 0.6%. The incidence of CTS diagnosis ranges from 72 to 346/100,000 person years (Jenkins *et al.*, 2012; M. Mondelli *et al.*, 2002; Nordstrom *et al.*, 1998; Stevens *et al.*, 1988). Due to an aging population and increasing rates of obesity, the incidence of CTS appears to be increasing (Bland & Rudolfer, 2003; Bongers *et al.*, 2007)

Demographic data for CTS was first described by Phalen which showed a predominately female population with peak incidence between age 55 to 60 (Phalen *et al.*, 1950). The predisposition of females to CTS has been supported by numerous studies ranging from a frequency of 2:1 to 23:1 compared to male patients (Ahn *et al.*, 2000; Bland & Rudolfer, 2003; M. Mondelli *et al.*, 2002). The peak age of incidence from 50 to 65 appears similar in most studies. However, the distribution of these populations has shown some variability. Bland and Liss both showed a bimodal distribution for females with an initial peak at approximately age 50 and a second at ages greater than 79 (Bland & Rudolfer, 2003; Liss *et al.*, 1992). In contrast, among Italians, Mondelli found a bimodal distribution of male cases and a unimodal age distribution in females (M. Mondelli *et al.*, 2002). However, a population study out of Rochester failed to show a bimodal distribution for either gender (Stevens *et al.*, 1988).

CTS has been linked to many non-occupational and occupational risk factors. Non-occupational risk factors for CTS include systemic diseases such as diabetes, obesity, rheumatoid arthritis, renal failure, hypothyroidism, and pregnancy all of which can alter the course of management of CTS (Becker *et al.*, 2002; Karadag *et al.*, 2012; Karpitskaya *et al.*, 2002) Occupational risk factors have been much more difficult to prove. This is due to the highly heterogeneous nature of work included in large population studies. However, it appears that occupations that are exposed to vibration and repetitive motions may have a higher risk of CTS (Kozak *et al.*, 2015).

Frequently the treatment of CTS requires operative treatment. Over half a million procedures are performed in the US per year (Palmer & Hanrahan, 1995). In the UK, over 18,000 procedures were performed in a year (Boniface *et al.*, 1994) with the number increasing (Jerosch-Herold *et al.*, 2014). Of the Swedish general population used to identify the prevalence of CTS, 25 % progress to require surgery (Atroshi *et al.*, 1999). The incidence of patients requiring surgical decompression ranges from 109 - 136/10,000 patient years (English & Gwynne-Jones, 2015; Hui *et al.*, 2005).

Significant socioeconomic burdens and health care costs can arise from CTS and its treatments (Bitar *et al.*, 2002). CTS accounts for more cases and days of work missed than any other illness in the American workforce (Leigh & Miller, 1998). It has been shown that income loss per CTS patient is $45,000 to $89,000 USD over 6 years (Foley *et al.*, 2007). In the US it is estimated that health care costs associated with CTS exceed $2B USD (Palmer & Hanrahan, 1995). The average time to work was 26 days post CTR (Feinstein, 1993) with 22% of patients remaining off work at 12 months (Katz *et al.*, 1997).

While the link between certain occupation and CTS remains controversial there is nevertheless a significant burden of patients with work-induced CTS. Indeed, the number of cases, cost of claims and periods of days claimed are on the rise (Fisher. B *et al.*, 2004). The mounting costs of CTS require a more thorough understanding of pathophysiology and treatment options.

Pathophysiology of Compression Neuropathy

Anatomy of the Nervous System

The human nervous system is divided into the central (spinal cord and brain) and peripheral nervous systems. The function of the peripheral nervous system (PNS) is to connect the CNS to its end target muscle and sensory organs. It consists of the cranial nerves, the spinal nerves with their roots and rami, the peripheral nerves, and the peripheral components of the autonomic nervous system. Sensory nerves are the afferent connection transmitting signals via receptors in the skin, muscle, tendon and joints toward the brain. Motor fibers act as the efferent pathway carrying information from the brain to the end plates of skeletal muscle to effect motion.

The principle cell of the nervous system is the neuron. Structurally, neurons consist of a cell body which contains multiple dendrites whose branch-like projections reach out to detect stimuli from the environment. The cell body extends out to the axon which acts to transport signals and neurotransmitters to the synapse, the terminal end of a neuron. The cell bodies of motor neurons exist in the ventral horn of the spinal cord, whereas those of the sensory neuron reside in the dorsal root ganglia (DRG) just outside the spinal cord.

Neurons are supported by various cell lines called neuroglia which exist both in the CNS and PNS. Oligodendrocytes and astrocytes make-up the neuroglia of the CNS. The equivalent of

oligodendrocytes in the PNS are Schwann cells (SC). In the PNS, Schwann cells have a 1:1 ratio with myelinated axons while unmyelinated fibers form groups known as Remak bundles. In the CNS, oligodendrocytes myelinate multiple axons.

Connective tissue then organizes groups of axons into four distinct layers. The endoneurium is the innermost layer that is associated with multiple nerve fibers. The basal lamina separates nerve fibers from the endoneurial space, a continuous space from the CNS to the terminus of a nerve fiber. Contained with the endoneurial space is the endoneurial fluid which plays a role in the pathogenesis of CTS. The nerve fibers and endoneurium are then grouped into fascicles by the perineurium. The perineurium is responsible for maintaining the physiologic balance of the conducting elements in the axon. The epineurium forms the outer sheath of a nerve trunk that encloses the grouped fascicles with blood vessels and connective tissue. The adventitial mesoneurium is the final layer where external blood vessels are contained. It also plays a significant role in nerve gliding (Birch *et al.*, 1998) (see Figure 1-1).

The Somatic Motor System

The motor system controlling our movements originates in the neurons of the pre-central gyrus. The axons of these neurons descend via the internal capsule of the midbrain, and the pyramids of the medulla. At the decussation of the pyramids most nerve fibers cross to the contralateral side and descend in the white matter as the lateral corticospinal tract. The motor neuron cell bodies of the peripheral nerve arise in Lamina IX of the grey matter of the ventral horn. Here the fibers exit the spinal cord via spinal roots before forming peripheral nerves where the terminal branches synapse with motor end plates in the muscles. The base functional unit of contraction is the motor unit which consists of an axon and all the muscle fibers it innervates. Groups of motor units will act together to produce muscle contraction

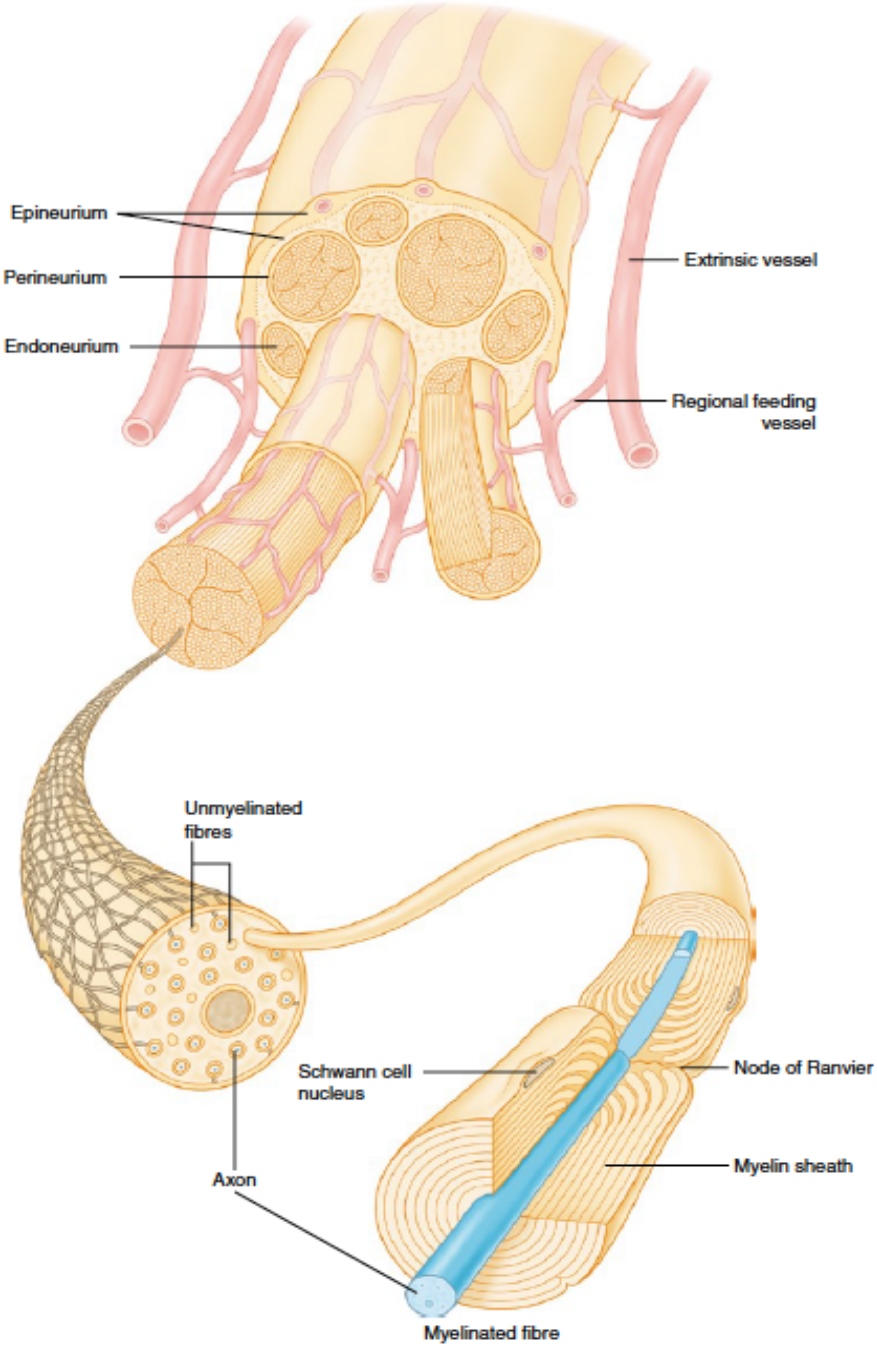


Figure 1-1 Components of a peripheral nerve.

Fascicles are organized by perineurium which are encased by the epineurium. Vaso nervorum runs along the perineurium and epineurium providing blood supply to the nerve through branches to the endoneurieum. Adapted from (Birch *et al.*, 1998).

There are two types of motoneurons found in lamina IX: α motoneurons and γ motoneurons. α motoneurons are responsible for initiating muscle contraction through innervation of the extrafusal muscle fibers of skeletal muscle while γ motoneurons innervate intrafusal muscle fibers. γ motoneurons are not directly involved in muscle contraction but rather allow for the activation of α motoneurons by regulating the tension in intrafusal muscle fibers. Activation of γ motoneurons produce a feedback loop to fine tune the speed and force of muscle contraction.

The Somatosensory System

The human sensory system consists of a multitude of organs enabling a large variety of senses. The somatosensory system allows for conscious appreciation of various senses. The sensitivity of the somatosensory system is anatomically specific. As evident from the organization of the homunculus, the human hand has one of the greatest sensory inputs in the body (Figure 1-2). The somatic senses include touch, vibration, pressure, proprioception, pain and temperature. Some of these sensory functions utilize different sensory receptors located at different depths in the skin, while other senses such as pain are convened via free nerve endings.

Touch is conveyed depending on the type of skin. In hairy skin the hairy follicle acts as the primary mechanoreceptor. In glabrous skin of the hand four main receptors convey touch: Merkel cells in the epidermis, Meissner corpuscle and Ruffini endings in the dermis and the Pacinian corpuscle in the subdermal tissue (see Figure 1-3). Meissner corpuscles detect light touch, skin motion, and vibration. Merkel cells are able to detect form and texture allowing tactile discriminations. Ruffini endings are poorly understood but appear to have a role in sensing skin stretch. Pacinian corpuscles play a role in sensation of vibrations.

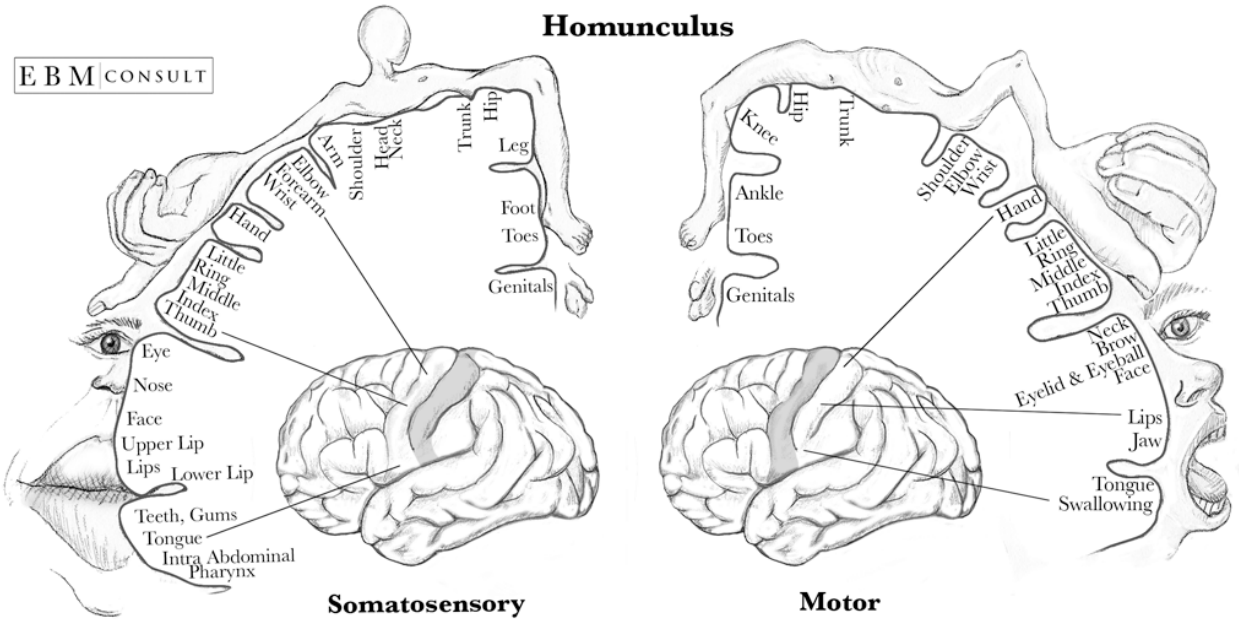


Figure 1-2 The somatosensory and motor homunculus in humans.

The hand has a large representation of cortex both in terms of motor and somatosensory function (Busti & Kellogg, 2015).

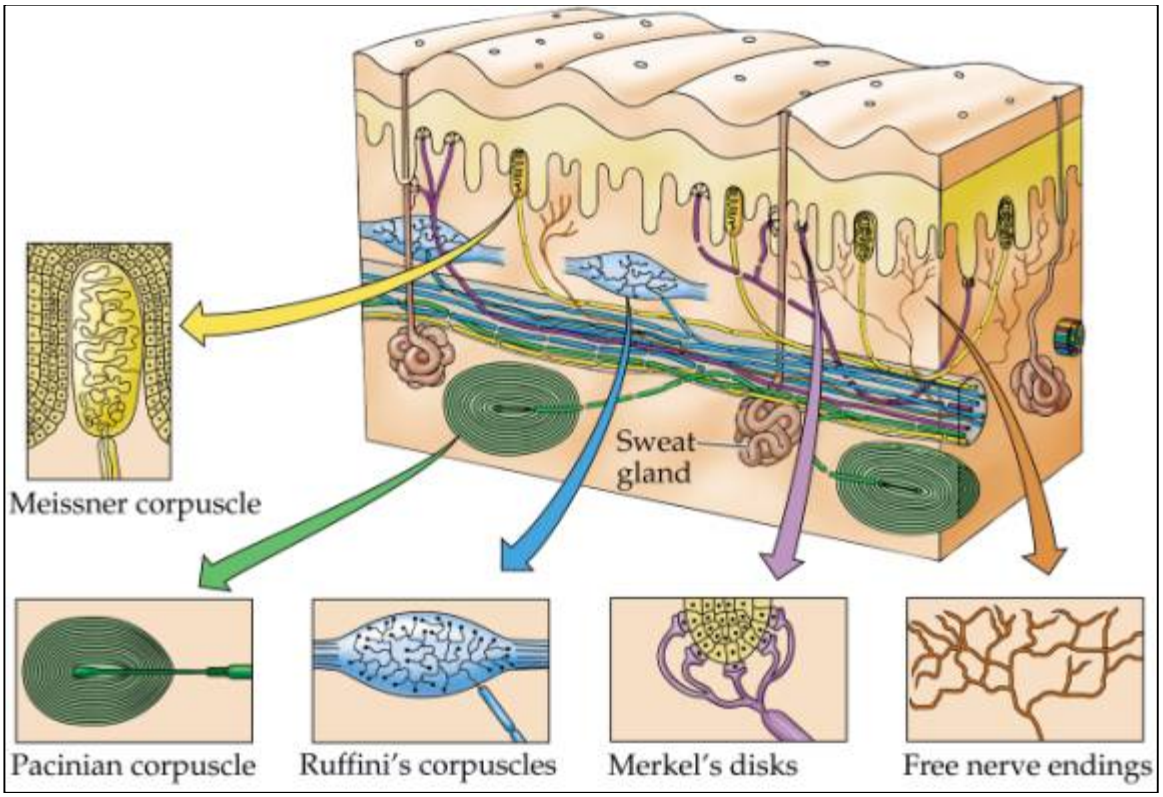


Figure 1-3 Mechanoreceptors in the skin

Merkel cells, Meissner corpuscle, Ruffini's corpuscle and Pacinian corpuscle all transduce touch sensory information and transmit via Aβ fibres. Free nerve endings transmit temperature and pain signals via Aδ and C fibres (Joseph, 2000). Not depicted are the epidermal nerve fiber endings.

The function of all of the receptors is dependent on their ability to adapt and the size of the receptor field they possess. The deeper a structure is located in the skin the larger the receptive field it possesses. While the Meissner corpuscle and the Merkel cells have small receptive field sizes, the Ruffini ending and Pacinian corpuscle have large receptive field sizes. The receptors are also classified based upon how quickly they are able to adapt to stimuli. Meissner and Pacinian corpuscles are rapidly adapting receptors because they respond to any change in stimulus (ie pressure) while the Merkel cell and Ruffini ending are slowly adapting. Sensations are transmitted by these receptors to nerves, the spinal cord and the post-central gyrus of the cortex where the information is processed.

There are four subsets of axons that carry the afferent sensory information back to the CNS. Axons are classified based on size and degree of myelination. This has functional importance as the larger the axon and higher degree of myleination the faster the conduction velocity the nerve possesses (See Figure 1-4). The axons that carry information regarding touch, pressure and vibration are the Aβ fibres with axon diameters 6-12 microns and relatively fast conduction velocity of 35-75 m/s.

For the sensation of temperature and pain there are no specific receptors that transduce nociceptive information to their respective afferents. Nociceptive information is largely detected by the free nerve endings of two types of axons: unmyelinated C fibers which have free nerve endings in the epidermis and myelinated Aδ fibers with endings in the dermis. There are a variety of nociceptors including mechanical nociceptors (Aδ) fibers, polymodal nociceptors, mechanoheat nociceptors, and cold nociceptors which can be associated with either fibre. Typically, temperature stimuli are felt to be identified by Aδ fibers while pain is transmitted

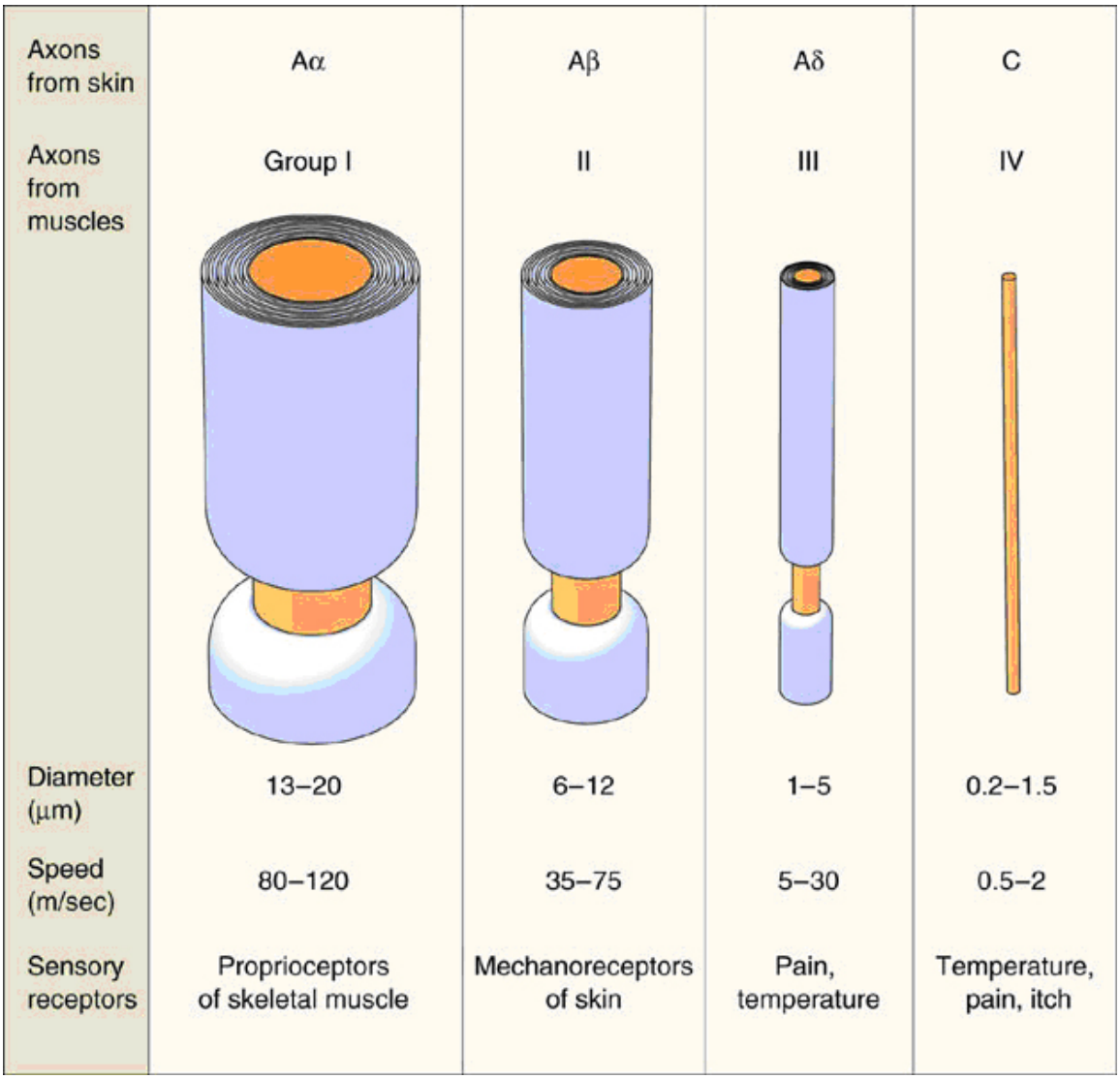


Figure 1-4 Subtypes of afferent axons in the somatosensory system

Conduction velocity is dependent on the size of the axon and the amount of myelin it contains. The speed of propagation of axon potentials dictates the function of the nerve. Large fiber nerves (Aα and Aβ) provide proprioception and light touch. Small nerve fibers (Aδ and C) are responsible for temperature and pain sensation (Joseph, 2000)

from C fibers (Zochodne, 2008). Whereas the small myelinated Aδ fibres have an axon diameter of 1-5 microns and conduction velocity of 5-30m/s, the nonmyelinated C fibres have axon diameters of 0.2-1.5 microns and even slower conduction velocity of 0.5-2m/s.

Most afferents for proprioception are generated in muscles and joints, with some contribution from skin stretch. There are three groups of receptors: Group Ia, Ib, and II. Group Ia and II afferents are associated with muscle spindle fibers and carry somatosensory information for muscle length, while Group Ib are found in the golgi tendon organs carry information regarding muscle force. These fibers are amongst the largest caliber afferents with 13-20 micron axon diameter and a rapid conduction velocity of 80-120 m/s. Knowledge of the somatic motor and somatosensory system is required to understand the different methods electrodiagnostics and sensory testing that are employed to measure diagnose and follow recovery after nerve injury.

Compression Neuropathy

Compression neuropathy can broadly be classified based on its chronology as acute or a chronic disease process. Despite both arising from compression of nerve as the inciting event, acute compression neuropathy (ACN) and chronic compression neuropathy (CCN) have significant differences in terms of pathophysiology. Acute compression neuropathy typically occurs similar to crush injuries and arises from a solitary traumatic event. Chronic compression neuropathy results from multiple etiologies with repetitive insults over a longer time course.

Traumatic nerve injuries are classified depending on the structures involved in the nerve. Initially classified by Seddon, injuries were grouped into three classes; neurapraxia, axonotmesis, and neurotmesis (Seddon, 1943). Neurapraxia represents an injury leading to focal demyelination but leaves the connective tissue and axons intact. In axonotmesis, the connective tissue and epineurium of the nerve remains in continuity but the axons have been damaged.

Neurotmesis represents the final stage of injury in the classification. In neurotmesis, the nerve has been transected with no continuity, with resultant damage to the axons and connective tissue.

Sunderland classified nerve injuries into five groups based on the anatomical structures that were injured. Sunderland class I and II represent neurapraxia and axonotmesis as described by Seddon. Sunderland class III are an axonotmetic injury with disruption of the endoneurium but the perineurium and epineurium remains intact. In Sunderland class IV injuries all structures are damaged except the epineurium which remains intact. Sunderland class V represents a neurotmesis where the nerve is no longer in continuity (Sunderland, 1951)(See Figure 1-5). Mackinnon added an additional class to the Sunderland classification describing a mixed injury as the VI class (Mackinnon & Dellon, 1988). The classification represents progressive severity of injury based on anatomical structures. Compression neuropathy are typically grade I or II unless severe. Not included in the figure is the grade VI injury that represents a mixed injury (Mencora *et al.*, 2013).

Pathophysiology of Acute Compression Neuropathy

Most of the studies elucidating the mechanisms after an acute compression neuropathy come from work of transection and crush injuries. The mechanisms of axonal degeneration post injury are complex and remain only partially understood. The complete mechanisms are reviewed elsewhere and will only be examined here in brief.

When axonal damage is present an organized pattern of degeneration occurs. The cell body undergoes chromatolysis, a process of increased nucleolar cytoplasm, dissolution of Nissl bodies and nuclear eccentricity (Zochodne, 2008). This process sets the stage for the

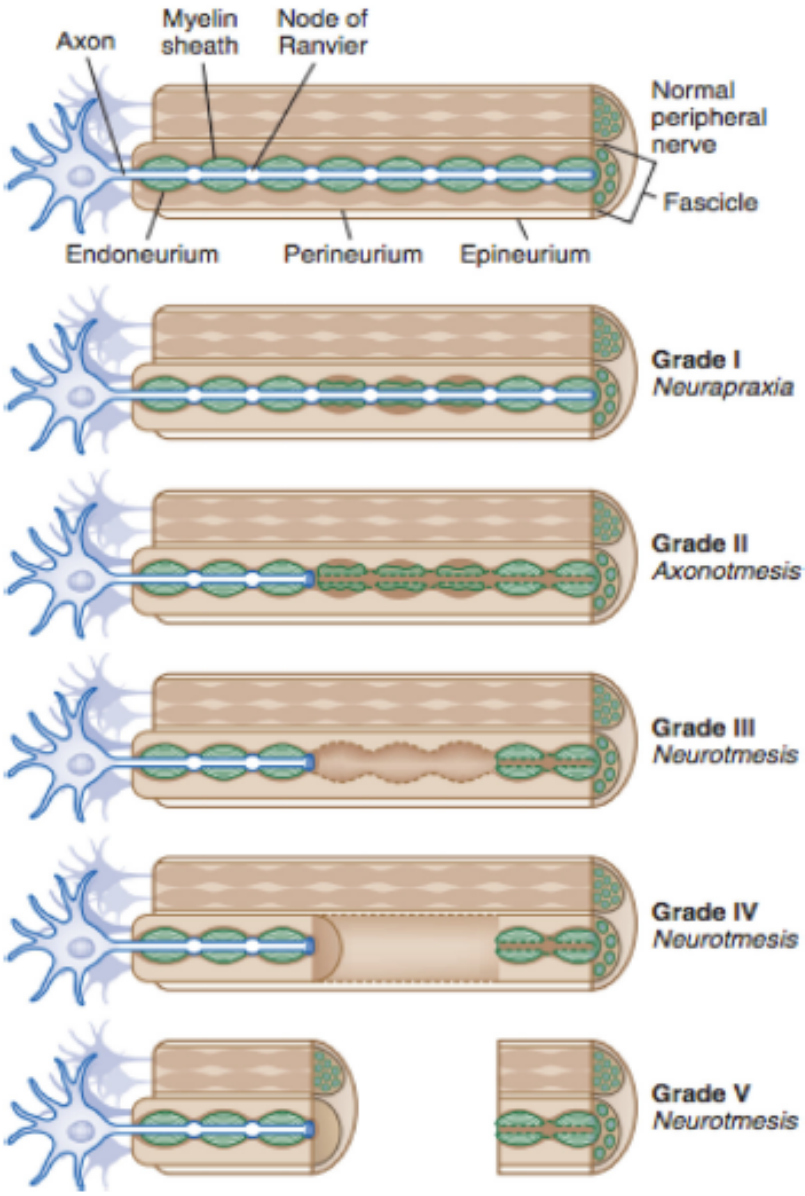


Figure 1-5 Seddon and Sunderland classification of nerve injury.

The Seddon and Sunderland classification system is used to describe nerve injuries and can determine treatment. Less severe injuries (Grade I and II) can often improve with conservative management but more severe injuries require operative intervention (Mencora *et al.*, 2013).

neuron to either regenerate or undergo apoptosis. If the cell body maintains its integrity neurotrophic factors induce the expression of regeneration associated genes (RAGs) including growth associated protein - 43 (GAP-43), actin, tubulin among others (Boyd & Gordon, 2003).

The proximal nerve segment will degenerate to the first node of Ranvier. The proximal segment will then form the growth cone. In a pro-regenerative environment, the growth cone will form filopodia that lengthen through polymerization of actin and microtubules in the search for an end target.

Augustus Waller initially described the phenomenon of distal stump breakdown in 1850. While working on the axotomy of the glossopharyngeal and hypoglossal nerves in frogs he found that the distal aspect of the nerve would disappear after injury. The mechanism Waller described would later be named in his honour (Waller, 1850). Wallerian degeneration is an organized process consisting of the breakdown of axons and Schwann cells to establish an environment suitable for regeneration. While the complete mechanism is still not fully understood, there have been mechanistic insights largely helped by a novel transgenic model. Work with Wlds mutants, an animal model with a single locus model that demonstrates slowed Wallerian degeneration with normal function has garnered insight into Wallerian degeneration. Through this model, Wallerian degeneration has been shown to be an active process (Coleman & Freeman, 2010). Initial events involve a Ca^{2+} - mediated dissolution of the neurofilaments leading to axon breakdown. Myelin breakdown is triggered rapidly after injury by interaction of SC phospholipases with lipophsophocholine (Gaudet *et al.*, 2011). The neural debris from this breakdown is cleared by phagocytosis. Initially this is the responsibility of SC which expresses Mac-2 mediating non-immune opsonin-dependent phagocytosis (Reichert *et al.*, 1994). Additionally, SCs undergo a transformation from a pro-myelination phenotype to a pro-

regenerative phenotype (Avellino *et al.*, 2004). This allows SC to secrete cytokines that are critical for nerve regeneration. Eventually, macrophages are recruited into the injury site. Around day 1-4, as the blood nerve barrier breaks down, the macrophages assist in the phagocytosis of debris. Interleukins and other cytokines secreted by macrophages aid in the dedifferentiation to a proliferating SC (Arthur-Farraj *et al.*, 2012). The process of clearing the debris allows the SC to proliferate to form endoneurial sheaths called the Bands of Bungner which guides regenerating axons towards their end targets (Zochodne, 2008).

Pathophysiology of Chronic Compression Neuropathy

The pathophysiology of chronic compression neuropathies was initially derived from work with crush injuries. In fact, it was believed that the pathophysiology behind CCN was a mild form of Wallerian degeneration. However, with the development of animal models and some human data available it appears that CCN is its own pathophysiological entity, at least early in the disease process (Mackinnon *et al.*, 1985; Mackinnon *et al.*, 1986; O'Brien *et al.*, 1987). Human nerve biopsies are rare due to the morbidity that ensues. However, from the data that is available we know that histologically the nerve has decreased axoplasmic and vascular flow after extraneural compression (Dahlin & McLean, 1986). This leads to the degeneration of the blood nerve barrier which allows the development of endoneurial edema (Dahlin & McLean, 1986). Once endoneurial edema develops, after approximately 1 week, the peripheral nerve fascicles undergo demyelination while remyleination begins at 2 weeks post injury. Mackinnon would correlate these histological findings in terms of symptoms and sensory testing results (Mackinnon, 2002) (See Figure 1-6).

Contrary to the view that CCN is a mild form of Wallerian-like degeneration was work examining SC biology (Gupta & Steward, 2003; Mackinnon, 2002)and the neuromuscular

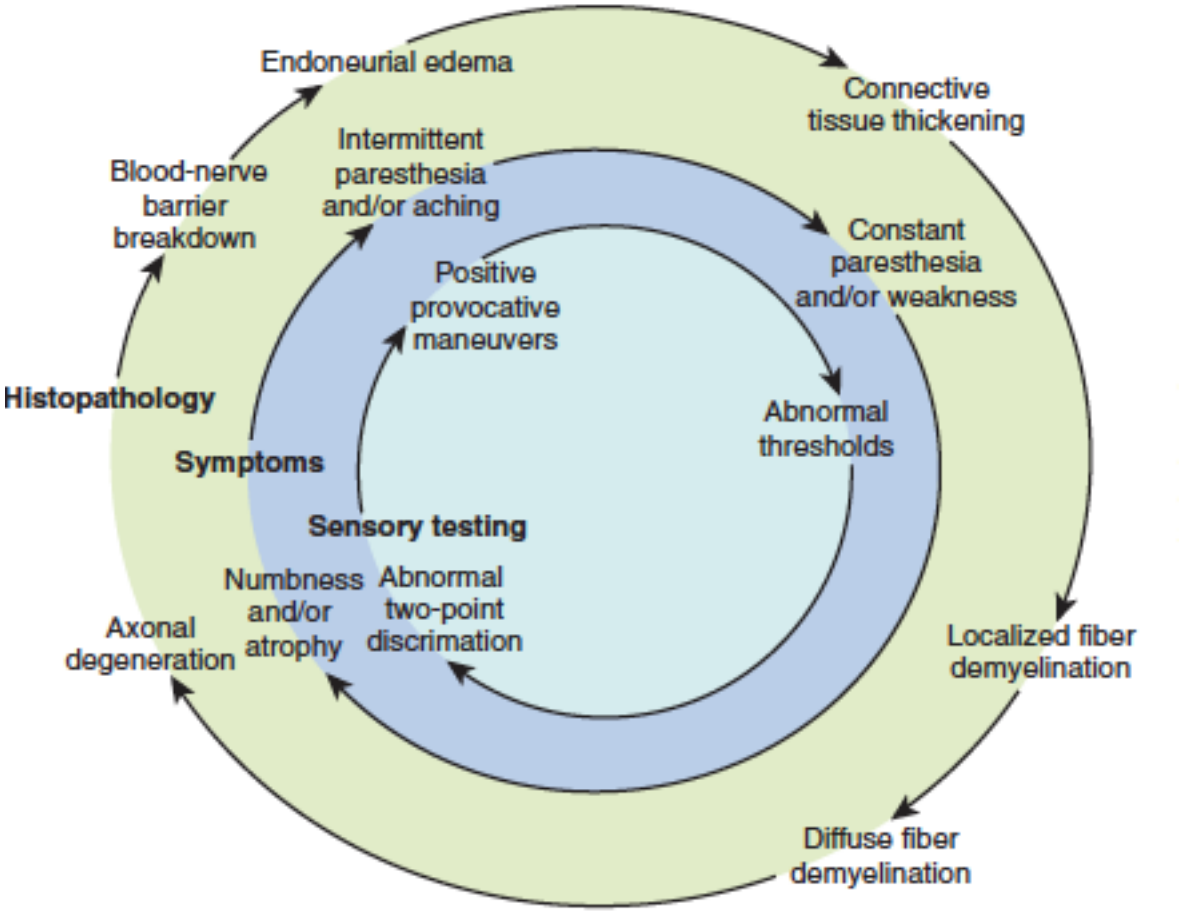


Figure 1-6 Clinicopathological description of compression neuropathy.

The histopathology of compression neuropathy. Pathologic changes are correlated with signs and symptoms allowing for determination of severity of disease from history and physical exam (Mackinnon, 2002).

junction in CCN (Mozaffar *et al.*, 2009). It was found that axons do not have the debris associated with Wallerian degeneration nor the muscle atrophy associated with chronic denervation. However, when CCN becomes advanced and axonal involvement ensues, the targeted axons will then undergo Wallerian degeneration (Mackinnon *et al.*, 1986). After injury SC undergo a dedifferentiation into a proliferative state. It has been shown that SCs proliferate significantly with a peak at 4 weeks post compression. At the same time SCs also undergo apoptosis but no signs of Wallerian-like degeneration are present (Gupta & Steward, 2003). It was also discovered that the myelin surrounding axons was quite thin. Comparing g-ratios of axons (ratio of axon to fiber cross-sectional area) it was found that newly regenerated axons have lower ratios. This supports a demyelinating and remyleinating phenomena since remyelinated intermodal segments take time to mature and reach normal lamellar structure. The thinner myelin results in a decreased conduction velocity seen in keeping with mild to moderate carpal tunnel syndrome while CMAPs are unaffected (Mozaffar *et al.*, 2009).

The conversion of SC to a proliferative state does not appear to be regulated by macrophages. Initial theories postulated that macrophages may have a role in the dedifferentiation due to the crush injury model where macrophage recruitment and interleukins play a role in changing the SC phenotype. However, in CCN models SC are able to dedifferentiate independent of macrophages (Gray *et al.*, 2007). Evidence from in vitro studies showed that mechanical stresses are able to cause the proliferation of SC indicating that myelinated neurons are sensitive to mechanical stresses. Also, mechanical stresses are able to downregulate the expression of MAGs and myelin basic protein which allows axon sprouting (Gupta *et al.*, 2005).

Molecular Mechanisms of Neural Regeneration

The pathways leading to nerve regeneration are complex interactions between multiple neurotrophins, receptor tyrosine kinases (RTK) and downstream signaling pathways (Reviewed in Figure 1-7). These pathways play an important role in neuronal survival and neurite outgrowth. They also represent targets for pharmaceutical agents to enhance nerve regeneration.

PI3K/Akt signaling

The activation of the PI3K (phosphatidylinositol-3-kinase)/Akt cascade plays an important role for neuron survival by blocking apoptosis but also supports the growth and differentiation of neural cells (Boyd & Gordon, 2003). The binding of NGF to the Trk receptor stimulates the activation of PI3K/Akt via upstream activation of Ras and Gab-1. Activation of Ras and Gab-1 allow for PI3K to phosphorylate and thus activate Akt. Activated Akt has roles in many signaling cascades. Akt reduces the amount of apoptosis via inhibition of the transcription factors forkhead and BAD (Vanhaesebroeck & Alessi, 2000). Akt also upregulates growth cone formation through inactivation of glycogen synthase kinase 3β (GSK-3β) removing its inhibition (Read & Gorman, 2009).

Phosphate and tensin homolog deleted on chromosome 10 (PTEN) is a phosphatase that inactivates the PI3K/Akt pathway by lysing the signalling molecule phosphatidylinositol-3 - phosphate preventing activation of Akt. PTEN has been shown to be expressed on both cytoplasmic and nuclear surfaces in nerves and is thought to be an important molecule blocking nerve regeneration. Christie showed that blocking the action of PTEN can result in increased nerve outgrowth in sensory axons (Christie *et al.*, 2010).

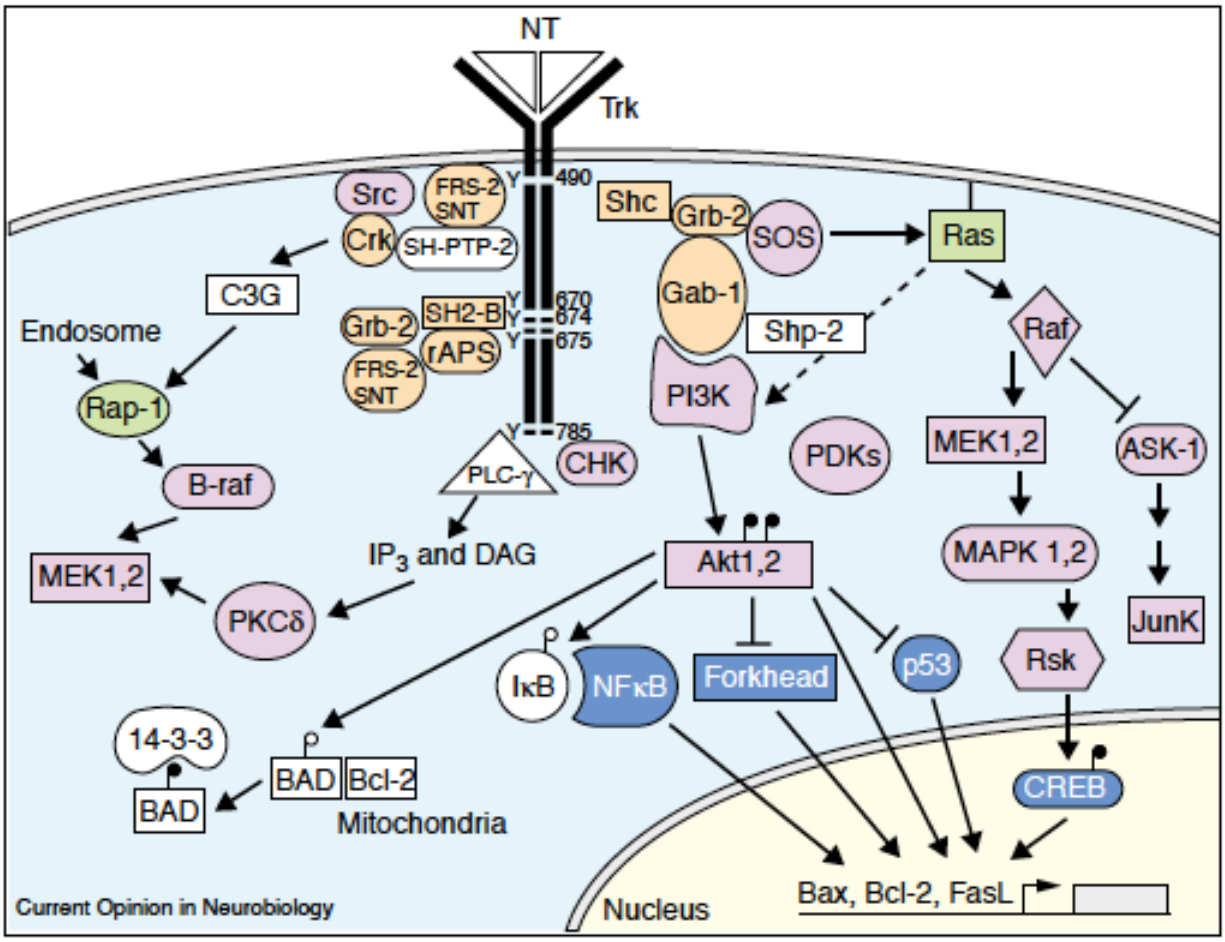


Figure 1-7 Mechanisms of neurotropin signaling

Multiple pathways are activated by neurotrophins binding to Trk leading to downstream activation of regeneration associated genes and eventual nerve regeneration. Pictured are the PI3K and the ERK1/2 pathways. Other pathways not depicted included cAMP signaling and Rho pathway (Patapoutain & Reichardt, 2001).

Ras/ERK pathway

The activation of Ras after binding of NGF to TrkA activates other pathways in addition to the PI3K/Akt pathway. Activation of Ras activates the MAPK/ERK pathway where Ras activates Raf leading to the activation of MAP/ERK kinase (MEK) and finally activation of extracellular regulated kinase (ERK). ERK has been shown to be important for neurite outgrowth (Kaplan & Miller, 2000)and reduces apoptosis through the activation of anti-apoptotic Bcl-2 via cAMP response element binding (CREB) (Mazzoni *et al.*, 1999). This provides a common pathway with PI3K/Akt activation as phosphorylated Akt activates Bcl-2 via the removal of inhibition by BAD.

Cyclic AMP signaling

Cyclic AMP (cAMP) has been found to be a crucial molecule for regeneration in the PNS and the CNS (Neumann *et al.*, 2002). In peripheral nerves, cAMP upregulation causes neuronal survival and nerve outgrowth. Cyclic AMP activates protein kinase A (PKA) which activates CREB leading to the increased expression of arginase I. The increased expression facilitates neurite outgrowth by increasing polyamine synthesis (Cai *et al.*, 2002). PKA also phosphorylates the RhoA GTPase which decreases the inactivation from MAGs allowing for cytoskeleton assembly. Increased cAMP also increases signal transducer and activator of transcription 3(STAT-3) through the upregulation of interleukin-6 (IL-6). This pathway plays an unclear role in axonal regeneration but appears important for the conditioning lesion paradigm, a concept that nerve regeneration is increased if preceded by a lesion within a given time frame (Cafferty *et al.*, 2004).

cAMP role is not confined to promoting axonal outgrowth. When cAMP is upregulated by neuregulin stimulus SC proliferation can be promoted via the MEK/ERK and PI3K/Akt

pathways (Monje *et al.*, 2013). The secondary messenger may also have a role in transforming the SC back to a promyelinating state when proliferation is complete through increasing Krox-20 expression (Topilko *et al.*, 1994).

Rho-ROK signaling

The role of Rho GTPase is to postively or negatively regulate the formation of the actin cytoskeleton within the growth cone (Govek *et al.*, 2005). Rho is upregulated in axonal injury and acts through its downstream mediator Rho associated kinase (ROK) to inhibit axon outgrowth inhibition(C. Cheng *et al.*, 2008). This inhibition can be overcome by cAMP dependent PKA inhibition. The reason for the inhibition is unclear but may play a role in preferential nerve regeneration. Preferential motor nerve regeneration refers to the ability of a motoneuron to selectively regenerate along a motoneuron pathway over a sensory pathway to reinnervate a motor end plate. Joshi et al showed through the use of Rho inhibitor that chondrotin sulfate polyglycans (CSPGs) inhibition of axonal outgrowth can be overcome in motoneurons but not sensory nerves (Joshi *et al.*, 2015).

Anatomy of Carpal Tunnel Syndrome

The carpal tunnel is a fibro-osseous tunnel in the wrist. The volar boundary of the carpal tunnel is the transverse carpal ligament (TCL) which attaches to the scaphoid and trapezium radially and the hamate and triquetrum on the ulnar side. The carpal bones form the dorsal boundary of the carpal tunnel. Through the carpal tunnel runs nine tendons along with the median nerve. The tunnel contains the flexor digitorum profundus and flexor digitorum superficialis to each digit along with the flexor pollicis longus. The median nerve lies superficial to all tendons (See Figure 1-8).

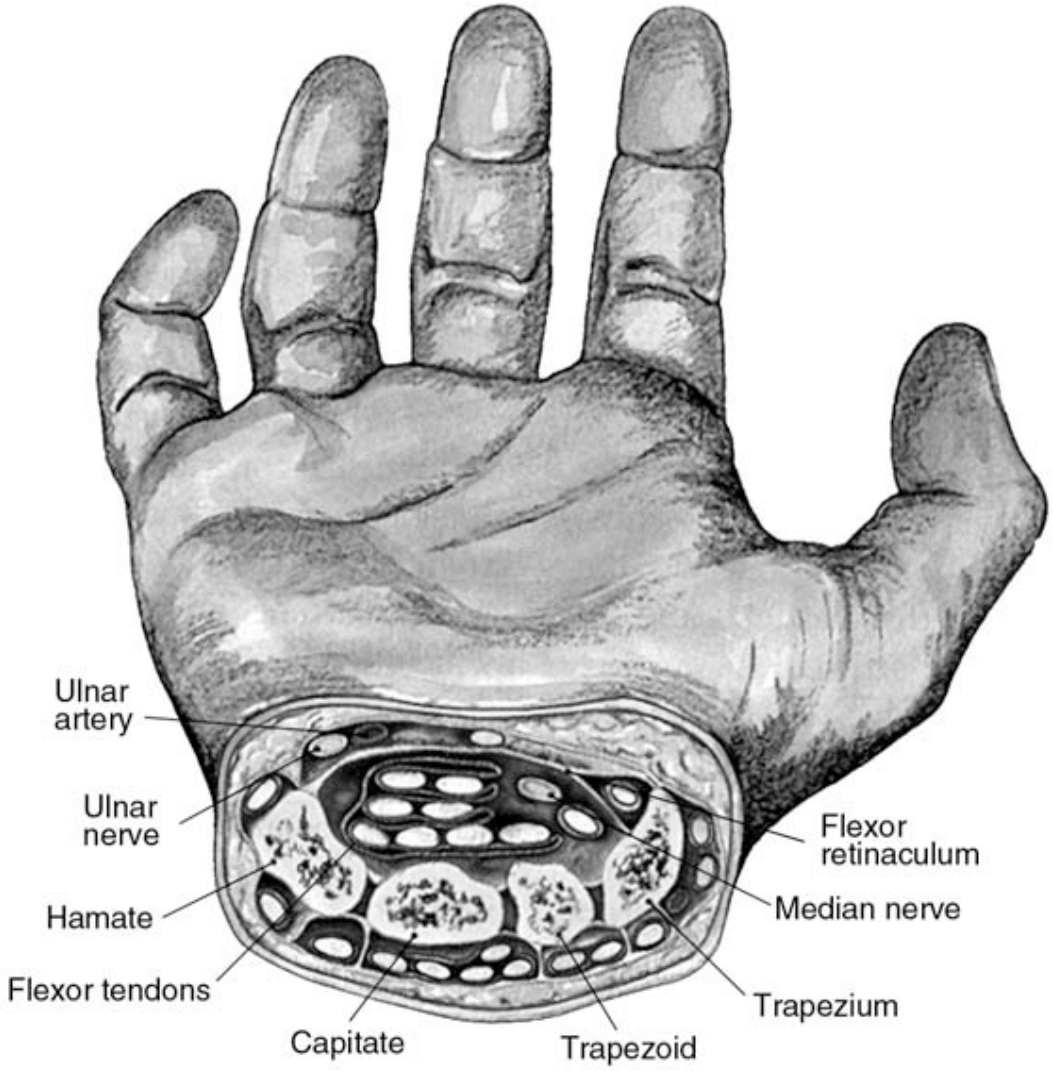


Figure 1-8 Cross sectional anatomy of the human wrist.

The carpal tunnel consists of the carpal bones linked by the transverse carpal ligament. Traversing the carpal tunnel are nine flexor tendons and the median nerve. Note the superficial location of the median nerve in relation to the tendons crossing the carpal tunnel and the close proximity to the transverse carpal ligament (Cranford *et al.*, 2007). Figure not to scale.

The carpal tunnel measures 2.5 cm x 1.2 cm at the proximal edge tapering down to 2.0 cm x 1.0 cm at the hook of the hamate. This represents the narrowest part of the carpal tunnel and may contribute to the development of the hourglass deformity seen in carpal tunnel syndrome. The carpal tunnel volume has been shown to be smaller in females in some studies (Papaioannou *et al.*, 1992) The transverse carpal ligament measures 24 to 36 mm in length and 1.6 to 2 mm in thickness. Cobb divided into three distinct but continuous areas;(Cobb *et al.*, 1993) Proximally the TCL is continuous with the deep investing fascia of the forearm representing the proximal area. The TCL proper, where the ligament is the thickest represents the middle area. A thin distal area that connects with the hypothenar and thenar aponeurosis represents the final distal area.

The median nerve travels laterally to the brachial artery in the upper arm crossing the brachial artery to enter the forearm on the arteries medial aspect. It does not give off any branches in arm. Once across the elbow the median nerve runs between the two heads of pronator teres. Here the median nerve gives off branches to innervate flexor carpi radialis (FCR), pronator teres, FDS, and palmaris longus (PL). At this level, the median nerve branches into the anterior interosseous nerve (AIN) which innervates the FDP to the index and long finger, FPL and pronator quadratus.

The median nerve continues distally, running between the FCR and PL until it enters the carpal tunnel ulnar to the FCR. Approximately 3 cm prior to the carpal tunnel the median nerve gives off the palmar cutaneous branch, which provides sensation to the radial aspect of the palm. This is an important anatomical caveat as in classic CTS the sensation to the palm is spared. If the palm is involved, it should point the clinician in the direction of a more proximal lesion.

After traversing the carpal tunnel the recurrent motor branch arises from the radial aspect of the median nerve before branching into seven digital nerves. The digital nerves provide sensation to the thumb, index, long and radial aspect of the ring finger. The recurrent motor branch supplies the thenar muscles; opponens pollicis, superficial head of the flexor pollicis brevis and abductor pollicis brevis. The recurrent motor branch which normally arises from the radial aspect of the median nerve, has been shown to have a number of variations on its branching pattern which is important clinically for surgeons during carpal tunnel release. Lanz characterized four groups of branching in the recurrent motor nerve. The most common was an extraligamentous branching (46-90%). Less common variations include the subligamentous (31%) and transligamentous (23%), while the recurrent motor branch overlying the TCL is rare. There have been reports of the recurrent motor branch originating on the ulnar aspect of the median nerve (Lanz, 1977) [See Figure 1-9]. Certainly, the branching patterns of the recurrent motor branch and the location of the palmar cutaneous branch has influenced the placement of incision for carpal tunnel release (CTR) (Taleisnik, 1973). Surgeons performing CTR need to be aware of anomalous branching patterns of the median nerve to avoid transection of a branch.

Diagnosis and Assessment of Outcomes in Carpal Tunnel Syndrome

Diagnosis

The gold standard diagnosis of CTS was described by Rempel in 1998 at a consensus conference. The result was defining CTS as a combination of symptoms (numbness, tingling, burning, pain and nocturnal symptoms) with confirmatory median neuropathy on NCS (Rempel *et al.*, 1998). While this definition has been largely accepted for epidemiological studies there remains some heterogeneity in clinical diagnosis (Werner & Andary, 2002). The diagnosis of carpal

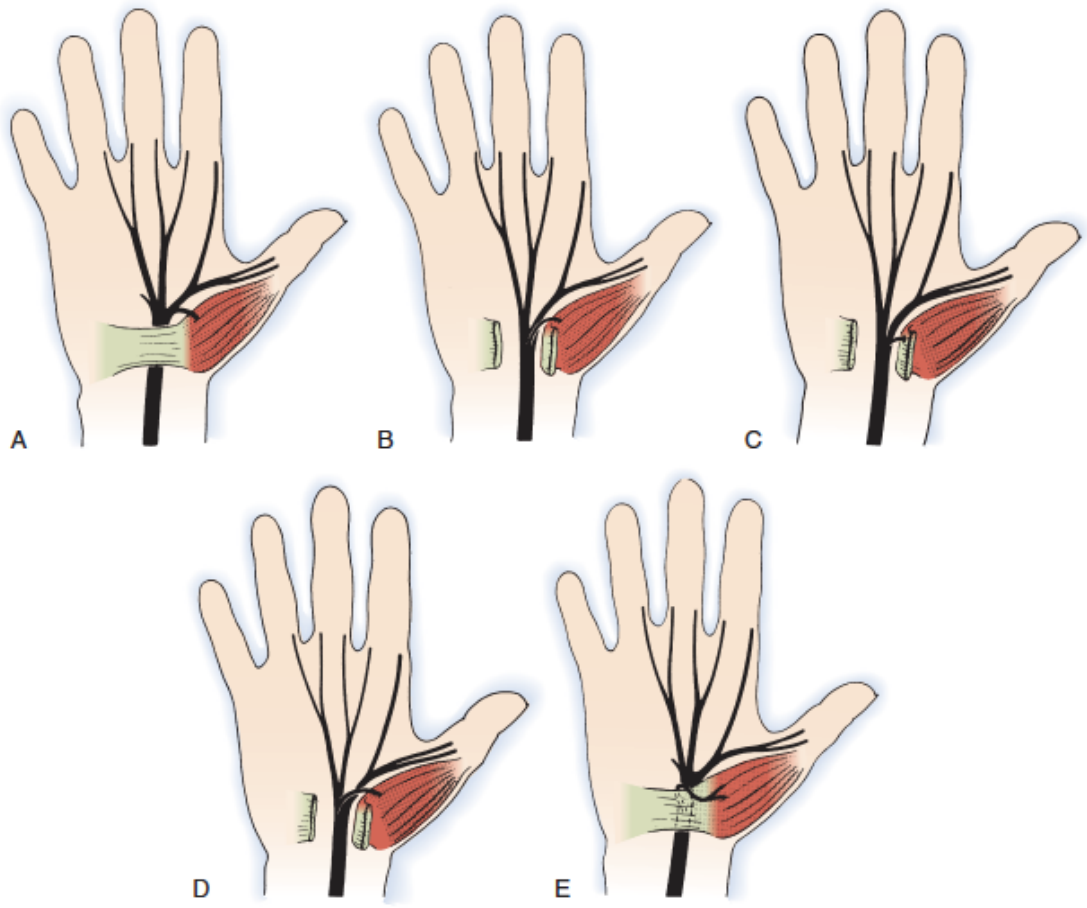


Figure 1-9 Branching patterns of the recurrent motor branch of the median nerve.

The main branching pattern for the recurrent motor branch occurs distal to the TCL, which injury to results in weak abduction and eventually thenar atrophy. Branching can be extraligamentous, subligamentous, intraligamentous or overlie the TCL (Lanz, 1977).

tunnel syndrome requires a precise history and physical exam combined with electrodiagnostics and other investigations to confirm the diagnosis.

History

The mainstay of diagnosis of CTS remains clinical with a good history being paramount. The initial descriptions of carpal tunnel formulated the important elements of the history (Phalen, 1966). Patients will complain of nocturnal paresthesias, numbness and pain in the median distribution. They may endorse having to shake their hands out at night to relieve symptomatology (the flick sign). As the disease progresses the patient may complain of weakness, dropping items and inability to do fine manual tasks. It is important to document any of the associated conditions with CTS as it could change management or predict outcomes from treatments. The distribution of numbness is also important to document. A validated hand diagram can be useful in determining the exact location of symptoms (Katz & Stirrat, 1990). While distributions of symptoms are rarely isolated to the median nerve distribution (Clark *et al.*, 2011) the pattern of symptoms may alert the clinician to an alternative diagnosis (pronator syndrome when palm is involved) or the presence of the double crush phenomena. The double crush phenomena refers to the susceptibility for a nerve to be compressed in a second location after an initial compression neuropathy (Upton & McComas, 1973).

Physical Exam and Provocative Tests

After a thorough history, the physical exam is the next most useful tool in diagnosing CTS. The physical exam focuses on a few key elements such as inspection for thenar atrophy, strength of thumb abduction and provocative tests. There are a large number of provocative tests

which can be seen in Table 1-1 and Table 1-2. The review will not examine all of the tests but mention a few pertinent tests.

Durkan's compression test has the highest sensitivity and specificity of any test. It consists of applying pressure over the carpal tunnel while the wrist is held in flexion (Durkan, 1991). A positive test is when the patient reports paresthesiae. Phalen's test is one of the oldest provocative tests described. It involves the forced flexion of the wrists bilaterally by pressing the dorsum of the hands together for 60 seconds (Phalen, 1966). Again, reproduction of paresthesias or pain represents a positive test. Tinel's sign uses percussion to activate nerves into sending electrical impulses along its distribution (Heller *et al.*, 1986). Unfortunately, there is a high false positive rate as up to 45% of the population can have a positive Tinel's with no other evidence of a neuropathy (D'Arcy & McGee, 2000). The presence of a positive Tinel's or Phalen's sign was often included on previous diagnostic criteria for carpal tunnel syndrome (Graham, 2008; Phalen *et al.*, 1950).

Syndrome*

Findings by Reference and Year	No. of Hands†	Sensitivity	Specificity	LR+ (95% CI)	LR– (95% CI)
Patient Interview					
Classic or probable hand diagram					
Katz et al,[53] 1990	145	0.64	0.73	2.4 (1.6-3.5)	0.5 (0.3-0.7)
Age >40 years					
Katz et al,[32] 1990	110*	0.80	0.41	1.3 (1.0-1.7)	0.5 (0.3-1.0)
Nocturnal paresthesia					
Buch-Jaeger and Foucher,[31] 1994	112*	0.51	0.68	1.6 (1.0-2.6)	0.7 (0.5-1.0)
Gupta and Benstead,[62] 1997	92	0.84	0.33	1.2 (1.0-1.6)	0.5 (0.2-1.1)
Katz et al,[32] 1990	110*	0.77	0.27	1.1 (0.9-1.3)	0.8 (0.4-1.6)
Pooled results	. . .	. . .	. . .	1.2 (1.0-1.4)	0.7 (0.5-0.9)
Bilateral Symptoms					
Katz et al,[32] 1990	110*	0.61	0.58	1.4 (1.0-2.1)	0.7 (0.4-1.0)
Motor Examination					
Weak thumb abduction					
Gerr et al,[33] 1995	115	0.63	0.62	1.7 (1.1-2.4)	0.6 (0.4-0.9)
Kuhlman and Hennessey,[30] 1997	228	0.66	0.66	2.0 (1.4-2.7)	0.5 (0.4-0.7)
Pooled results	. . .	. . .	. . .	1.8 (1.4-2.3)	0.5 (0.4-0.7)
Thenar atrophy					
Gerr et al,[33] 1995	115	0.28	0.82	1.6 (0.8-3.2)	0.9 (0.7-1.1)
Golding et al,[64] 1986	110	0.04	0.99	5.4 (0.2-129.5)	1.0 (0.9-1.0)
Katz et al,[32] 1990	110*	0.14	0.90	1.5 (0.5-4.1)	0.9 (0.8-1.1)
Pooled results	. . .	. . .	. . .	1.6 (0.9-2.8)	1.0 (0.9-1.0)
Sensory Examination					
Hypalgesia					
Golding et al,[64] 1986	110	0.15	0.93	2.2 (0.7-6.7)	0.9 (0.8-1.1)
Kuhlman and Hennessey,[30] 1997	228	0.51	0.85	3.4 (2.0-5.8)	0.6 (0.5-0.7)
Pooled results	. . .	. . .	. . .	3.1 (2.0-5.1)	0.7 (0.5-1.1)
2-Point discrimination					
Buch-Jaeger and Foucher,[31] 1994, 6 mm	167	0.06	0.99	4.5 (0.6-36.9)	1.0 (0.9-1.0)
Gerr et al,[33] 1995, 5 mm	115	0.28	0.64	0.8 (0.5-1.3)	1.1 (0.9-1.5)
Katz et al,[32] 1990, 4 mm	110*	0.32	0.80	1.6 (0.8-3.1)	0.8 (0.7-1.1)
Pooled results	. . .	. . .	. . .	1.3 (0.6-2.7)	1.0 (0.9-1.1)
Abnormal vibration					
Buch-Jaeger and Foucher,[31] 1994	172	0.20	0.81	1.1 (0.6-2.0)	1.0 (0.8-1.1)
Gerr et al,[33] 1995	115	0.61	0.71	2.1 (1.3-3.3)	0.5 (0.4-0.8)
Pooled results	. . .	. . .	. . .	1.6 (0.8-3.0)	0.8 (0.4-1.3)
Abnormal monofilament findings					
Buch-Jaeger and Foucher,[31] 1994	167	0.59	0.59	1.5 (1.1-2.0)	0.7 (0.5-0.9)
Buch-Jaeger and Foucher,[31] 1994	167	0.59	0.59	1.5 (1.1-2.0)	0.7 (0.5-0.9)

Table 1-1: Sensitivity and specificity of signs and symptoms for CTS.

Systematic review of signs and symptoms that are found in carpal tunnel syndrome. The calculated sensitivities, specificity and likelihood ratios for each sign or symptom are displayed. This JAMA diagnosis guideline provides useful information on the most effective way to diagnose CTS. Adapted from (D'Arcy & McGee, 2000).

Table 2. Diagnostic Accuracy of History and Physical Examination for Carpal Tunnel Syndrome (cont)*

Findings by Reference and Year	No. of Hands†	Sensitivity	Specificity	LR+ (95% CI)	LR− (95% CI)
Other Tests					
Square wrist sign					
Kuhlman and Hennessey,[30] 1997	228	0.69	0.73	2.6 (1.8-3.7)	0.4 (0.3-0.6)
Radecki,[27] 1994	665	0.47	0.83	2.8 (2.1-3.8)	0.6 (0.6-0.7)
Pooled results	. . .	. . .	. . .	2.7 (2.2-3.4)	0.5 (0.4-0.8)
Closed fist sign					
De Smet et al,[28] 1995	35	0.61	0.92	7.3 (1.1-49.1)	0.4 (0.2-0.7)
Flick sign					
Pryse-Phillips,[29] 1984	396	0.93	0.96	21.4 (10.8-42.1)	0.1 (0.0-0.1)
Tinel sign					
Gerr et al,[33] 1995	115	0.25	0.67	0.7 (0.4-1.3)	1.1 (0.9-1.4)
Golding et al,[64] 1986	110	0.26	0.80	1.3 (0.6-2.6)	0.9 (0.7-1.2)
Heller et al,[65] 1986	80	0.60	0.77	2.7 (1.2-5.9)	0.5 (0.3-0.8)
Katz et al,[32] 1990	110*	0.59	0.67	1.8 (1.2-2.7)	0.6 (0.4-0.9)
Kuhlman and Hennessey,[30] 1997	228	0.23	0.87	1.8 (1.0-3.4)	0.9 (0.8-1.0)
Buch-Jaeger and Foucher,[31] 1994	172	0.42	0.64	1.1 (0.8-1.7)	0.9 (0.7-1.2)
Pooled results	. . .	. . .	. . .	1.4 (1.0-1.9)	0.8 (0.7-1.0)
Phalen sign					
Buch-Jaeger and Foucher,[31] 1994	166	0.58	0.54	1.3 (0.9-1.7)	0.8 (0.6-1.1)
Gerr et al,[33] 1995	115	0.75	0.33	1.1 (0.9-1.4)	0.7 (0.4-1.3)
Heller et al,[65] 1986	80	0.67	0.59	1.6 (1.0-2.8)	0.6 (0.3-0.9)
Katz et al,[32] 1990	110*	0.75	0.47	1.4 (1.1-1.9)	0.5 (0.3-0.9)
Kuhlman and Hennessey,[30] 1997	228	0.51	0.76	2.1 (1.4-3.2)	0.6 (0.5-0.8)
Golding et al,[64] 1986	110	0.10	0.86	0.7 (0.2-2.2)	1.0 (0.9-1.2)
Burke et al,[66] 1999	200	0.51	0.54	1.1 (0.7-1.8)	0.9 (0.6-1.3)
De Smet et al,[28] 1995	66	0.91	0.33	1.4 (0.9-2.0)	0.3 (0.1-0.9)
Pooled results	. . .	. . .	. . .	1.3 (1.1-1.6)	0.7 (0.6-0.9)
Pressure provocation test					
Kuhlman and Hennessey,[30] 1997	228	0.28	0.74	1.1 (0.7-1.7)	1.0 (0.8-1.1)
Burke et al,[66] 1999	205	0.52	0.38	0.8 (0.6-1.2)	1.3 (0.7-2.2)
Buch-Jaeger and Foucher,[31] 1994	155	0.49	0.54	1.1 (0.8-1.5)	0.9 (0.7-1.3)
De Smet et al,[28] 1995	66	0.63	0.33	0.9 (0.6-1.5)	1.1 (0.5-2.7)
Pooled results	. . .	. . .	. . .	1.0 (0.8-1.3)	1.0 (0.9-1.1)
Tourniquet test					
Buch-Jaeger and Foucher,[31] 1994	145	0.52	0.36	0.8 (0.6-1.1)	1.3 (0.9-2.0)
Golding et al,[64] 1986	110	0.21	0.87	1.6 (0.7-3.9)	0.9 (0.8-1.1)
Pooled results	. . .	. . .	. . .	1.0 (0.5-1.9)	1.0 (0.7-1.5)

Table 1-2: Sensitivity and specificity of signs and symptoms for CTS.

Systematic review of signs and symptoms that are found in carpal tunnel syndrome. The calculated sensitivities, specificity and likelihood ratios for each sign or symptom are displayed. This JAMA diagnosis guideline provides useful information on the most effective way to diagnose CTS. Adapted from (D'Arcy & McGee, 2000).

A newer provocative test has been described by Cheng named the scratch collapse test (C. J. Cheng *et al.*, 2008). The test consists of two phases. First an examiner attempts internal rotation of the hand against resistance with the patient sitting with arms by their side, and elbows at 90 degrees. Next the examiner irritates the compressed nerve by scratching over the area of compression and repeats the internal rotation against resistance. A positive test is denoted by the arm giving way to the internal rotation. The sensitivity and specificity (64% and 99% respectively) were greater than or equal to other provocative tests with exception of the flick sign (D'Arcy & McGee, 2000). However, other studies have failed to reproduce the sensitivity and specificity of the initial study (Simon *et al.*, 2017).

Due to the relative simplicity of the tests and minimal cost Semmes-Weinstein Monofilament test and two point discrimination are often used for sensory testing. The issue with the additional tests of Semmes-Weinstein Monofilament test and aside from their sensitivity and specificity are that they only evaluate the function of large myelinated Aβ fibers and do not test Aδ or C fibres that have been shown to be involved by CTS (Clarke *et al.*, 2017).

Electrodiagnostics

Electrodiagnostics can be divided into two techniques: nerve conduction studies (NCS) and needle electromyography (EMG). Traditionally, NCS are more useful than needle EMG in the diagnosis of compression neuropathies. However, both have limitations. Firstly, both techniques only assess the activity of large myelinated fibers. Second, needle EMG is a purely motor technique and is unable to assess the sensory symptoms that are prominent in CTS. Careful electrophysiological studies require appropriately trained and certified technologists and physicians using established standards.

NCS involves the stimulation of a nerve and measuring the elicited response. In the case of motor axons, the compound motor action potential (CMAP) is measured while sensory nerve action potential (SNAP) evaluates sensory nerve fibers. Decreases in the amplitude of either SNAP or CMAP are usually markers of axonal damage. If a set distance is known, the conduction velocity (CV) can be measured. Decreases in CV may result from loss of myelin which is seen in demyelinating disorders. Often this is seen in compression neuropathies due to the demyelination. Other findings seen in compression neuropathies include increased latency and conduction blocks.

The diagnosis of CTS relies on the testing of sensory and motor amplitudes, conduction velocity and latency. The American Association for Electrodiagnostic Medicine (AAEM) published a practice guide on the testing that should be completed to diagnose CTS (American Association of Electrodiagnostic Medicine *et al.*, 2002). Sensory NCS across the wrist with comparison to an adjacent sensory nerve such as the ulnar or superficial branch of the radial nerve are considered standard tests. The inclusion of motor NCS are considered a guideline while all other tests are deemed optional.

Specific values for the diagnosis of CTS are not routinely quoted for two main reasons. The criteria used to define CTS is study dependent. Individual studies define abnormal levels for latency, amplitude, and conduction velocity based on the study protocol which generates a sensitivity and specificity for each study. The variability in each study provides a range rather than an absolute cut-off for each test. Secondly, measurements are dependent on the electrodiagnostic laboratory they are taken from, as variations in recording equipment, setup, patient age, BMI, and temperature will alter the values produced. However, normative data was recently published which may can be used as a reference, minimizing the lab to lab variations

(Chen *et al.*, 2016). Table 1-3 outlines recommendations for test to be performed to confirm diagnosis and a range of abnormal values

With a needle EMG electrode, microphysiological changes in the muscle can be detected. However, these changes are usually not present in mild and moderate CTS. With axonal loss, fibrillation potentials and positive sharp waves are generated by denervated muscle fibres. Collateral sprouting results in giant motor unit action potentials which can be used to identify reinnervation by needle EMG (Tapadia *et al.*, 2010). The AAEM considers needle EMG to be an optional test for CTS, most useful in detecting the presence of a confounding radiculopathy.

Assessment of Outcomes

While changes in the clinical picture and conventional diagnostic modalities are often used to follow progression of disease, they often provide an incomplete picture. First, symptom severity is largely subjective in nature. Second, collateral sprouting can falsely mask changes in CMAP amplitudes potentially leading to over estimation of recovery in instances of axonal damage. Repair of demyelination is straightforward to identify, providing identical techniques are utilized. However, additional research tools are valuable. These can be divided into three categories: motor, sensory, and functional.

Motor

A quantitative method to measure motor function is the electrophysiological technique of motor unit number estimation (MUNE). This electrophysiologic technique can provide an

Nerve Conduction Test	**AAEM Standard**	**Reference Value (Chen *et al.*, 2016)**	**Abnormal Range (American Association of Electrodiagnostic Medicine *et al.*, 2002)**
Sensory NCS	Standard		
Latencies Onset (wrist) Peak (wrist)		3.3 ms (97th percentile) 4 ms (97th percentile)	>2.72 to 3.4 ms >3.o to 3.48 ms
Conduction Velocity			D2 < 46 m/s D3 < 44 m/s
SNAP		13 μV (3rd percentile)	
Motor Studies	Guideline		
Distal Latency		4.5 ms (97th percentile)	> 3.2 to 4.3 ms
Conduction Velocity		49 m/s (3rd percentile)	< 35 to 38 m/s
CMAP		4.1 mV (3rd percentile)	< 5 mV

Table 1-3: Recommended testing for the confirmation of the diagnosis of CTS.

AAEM recommendations for standard testing required for confirmation. Motor NCS represent guideline testing to determine if motor nerve involvement is present. Optional tests include F-waves and EMG. Reference values are listed from Chen et.al. study with representative percentile in parenthesis. Abnormal ranges are taken from the 2002 AAEM Practice Parameters.

estimate of extent motor unit loss or conduction block of motor units. The fundamental principle which MUNE is based upon compares the average single motor unit action potential (MUAP) with the maximum CMAP which represents the summation of all motor units in the whole muscle. Division of these two terms then gives the resultant estimation of motor unit numbers as defined by the formula:

MUNE = Amplitude of the maximum CMAP/Average single MUAP

Multiple techniques have been described to identify the single MUAPs. McComas initially utilized the incremental stimulation method to determine the MUNE of the EDB muscle (McComas *et al.*, 1971). The incremental method manually increases stimulation to a nerve at a single site and detecting the resultant increase in CMAP. The incremental steps in CMAP are hypothesized to represent a recruited MUAP. However, the accuracy of the estimate is greatly impacted by alterations. While the incremental change in CMAP is meant to represent the recruitment of a single MUAP, it more closely represents the overlap of multiple MUAPs with similar excitability a electrophysiologic phenomenon known as alternations. These alterations can substantially underestimate the MUNE (Brown & Milner-Brown, 1976). Therefore, after the incremental technique was introduced, other MUNE methods were described to attempt to correct these shortcomings (See Table 1-4).

Methods of Motor Unit Number Estimation
Manual incremental stimulation
Automated incremental stimulation
Multiple point stimulation
F-response method
Spike-triggered averaging
Statistical methods

Table 1-4: Motor Unit Number Estimation (MUNE) techniques for identifying motor unit action potentials (MUAP) (Bromberg, 2007)

One widely used methodology is the multiple point stimulation technique. It involves stimulating the nerve at multiple points and taking only the lowest threshold MUAPs (Kadrie *et al.*, 1976). By doing so, it eliminates the issue of alternation as utilizing the lowest threshold MUAPs will be insufficient to activate multiple motor units. MUNE has previously been used to evaluate carpal tunnel syndrome both pre and post operatively and was found to be reliable (Bromberg, 2007).

Sensory

Sensory testing of peripheral nerves provides more of a challenge given the multiple fibre types that provides sensory feedback. In order to fully test sensory recovery multiple tests are required to test the full spectrum of sensory function. Two-point discrimination or Semmes-Weinstein Monofilament Test are often used to measure the function of Aβ fibres.

Measurement of cold and pain sensation served by small unmyelinated sensory fibres is more challenging. A reliable technology introduced in the past 2 decades is quantitative sensory testing (QST). Multiple methods have been described for the determination of thresholds in QST (Siao & Cros, 2003). An automated method termed the "4, 2, 1 with null stimuli" methodology developed by Dyck is one of the most widely used for cold detection threshold, warm detection threshold, and vibration detection threshold (Dyck *et al.*, 1993). In this psychophysical test, a set of thermal or vibrational stimuli is sent to the patient to elicit forced responses. Percentage of the temperature change is progressively fine-tuned until the detection threshold is captured. To prevent errors from guessing, null stimuli responses are interspersed in between real stimuli. While this method is effective for less noxious stimuli, it was felt to be unethical to subject patients to more intense stimulus. Therefore, to test pain, Dyck developed the non-repeating ascending algorithm (Dyck *et al.*, 1996). An ascending set of stimulus are applied to the patient, whom responds on a modified visual analog scale; 0 for non-painful

stimuli then 1 for least painful stimulus to 10 for maximal painful stimulus. Heat-pain threshold is defined by a quadratic equation of the halfway point between 0 and 1 or HPT 0.5. QST has been shown to be reproducible for both pain and temperature measurements (Peltier *et al.*, 2009)

Functional

Functional outcomes are often measured via patient reported outcome metrics and objective testing of functional movements. Patient reported outcomes allow for the patients to rate their ability to perform activities and symptom severity. Multiple hand and upper extremity questionnaires have been developed. Two most commonly used questionnaires are the Disabilities of Arm, Shoulder and Hand (DASH) questionnaire and the Boston CTS questionnaires. Both are well validated in CTS and are frequently used to gauge functional impairment and symptom severity (Greenslade *et al.*, 2004; Hudak *et al.*, 1996; Levine *et al.*, 1993).

While patient reported outcomes are a critical metric in longitudinal assessment they are subjective in nature. Therefore, having an objective measure of functional return is important. These hand performance tests can quantify the functional deficits of patients. Examples of validated hand performance tests include the Purdue Pegboard Test (Amirjani *et al.*, 2011b) and Moberg pick-up test (Amirjani *et al.*, 2011a).

Management of Carpal Tunnel Syndrome

"If steroid injections into the carpal tunnel give only transient relief, treatment should be by complete section of the transverse carpal ligament. This procedure will almost always relieve the patient's pain and numbness in the hand, and in many cases will also cure the paralysis of the thenar muscles, which may be present". GS Phalen 1966

The aforementioned quote formed the final thoughts of George Phalen in his abstract on his findings of CTS at the Cleveland Clinic. In the 50 years that have elapsed since his large series the foundations of treatment have not changed. Initially, conservative measures are attempted with steroid injection being a pillar of the conservative modalities. Other effective conservative measures included non-steroidal anti-inflammatories (NSAIDs), splinting or combinations of these therapies. For those that fail conservative measures or present with more advanced disease surgery is currently the only option. Surgical management is aimed at the release of the TCL to decompress the carpal tunnel. This can be done via an open approach or an endoscopic approach.

Conservative Therapies

Splinting

Splinting is often the first line treatment for symptomatic carpal tunnel syndrome. It is noninvasive and readily available. Despite the accessibility, the studies supporting the use of splints are heterogenous in terms of regime and outcomes (Page *et al.*, 2012). This can make deciphering meaningful results quite a challenge.

Two RCT have looked at the efficacy of splinting versus no treatment. Manette examined the role of nighttime splinting in CTS and found significantly improved scores on the Boston Carpal Tunnel Questionnaire (BCTQ) vs no treatment at 4 weeks (mean difference of -1.07 95%CI -1.29 to -0.85) (Manente *et al.*, 2001). The second RCT found similar results at 3 months (mean difference -0.94 95% CI-1.10 to -0.78) and 6 months (mean difference -0.90 95% CI -1.11 to -0.78) (Premoselli *et al.*, 2006). Both of these RCTs examined the use of splints at night time only. Celik further supported the use of night splinting by showing improved CMAP at 6 weeks and improved sensory conduction velocity at 12 weeks (Celik *et al.*, 2015). A further

RCT examined the role of continuous splinting versus night time only splinting using a neutral wrist splint in 17 patients. No difference was found between changes in symptom severity score. However, there was a considerable compliance issue with the trial. The night time only splinting group had a patient reported compliance of 85% compared to a reported compliance of 27% in the full-time group during the daytime. The compliance issues would skew the results of the trial as there would not be an adequate comparison group to compare night time splinting with (Walker *et al.*, 2000).

Splinting is often combined with other adjuvant therapy. In an RCT by Yildiz et al, they examined U/S and phonopheresis combined with splinting. They found improvement in all groups but there was no difference between groups indicating the efficacy of splinting alone (Yildiz *et al.*, 2011).

Corticosteroids

Corticosteroids are used to reduce inflammation surrounding the median nerve. It can be administered locally via blind or U/S guided injection. Local injection into the carpal tunnel provides the benefit of decreasing inflammation while not exposing the patient to the systemic complications of corticosteroids. It should also provide a higher concentration of corticosteroids to the affected area than systemic therapy. Multiple RCTs examined the use local steroid injections compared to placebo (Armstrong *et al.*, 2004; Atroshi *et al.*, 2013; Dammers *et al.*, 1999). They showed that local corticosteroids can provide symptomatic relief up to 10 weeks and fewer patients require surgery at 1 year. Studies on dosing have been unclear with one study showing no benefit to high dose steroids (O'Gradaigh & Merry, 2000). In the Atroshi RCT there were two doses of methylprednisolone (40 mg and 80 mg) that were compared to placebo. Both

showed symptomatic improvement at 10 weeks but there was no comparison between the two corticosteroid groups (Atroshi *et al.*, 2013).

Cortisone has also been administered systemically or topically using phonopheresis, an ultrasound technique used to increase the delivery of topical agents. Systemic corticosteroids have shown benefit in symptom relief over placebo. In a Cochrane review O'Connor pooled the results of three trials and found a significant improvement in symptoms at two weeks. Two of the trials continued follow-up until 8 weeks with both showing significant improvement at 4 weeks with one trial showing improved symptoms at the completion of the trial (O'Connor *et al.*, 2003).

Comparing the efficacy of local to systemic corticosteroids has found that the administration of corticosteroids locally is more efficacious in symptom reduction as measured by the Global Severity Scale (GSS) at up to 12 weeks (Ozdogan & Yazici, 1984). The GSS represents a validated subjective measure of symptom severity that can be applied to any disease. Interestingly, there was no difference found at 2 weeks (Wong *et al.*, 2002). However, administration of systemic steroids can have serious complications including hypertension, hyperglycemia, cataracts, weight gain, peptic ulcers, menstrual abnormalities, adrenal insufficiency and osteoporosis. Given the risk profile and the efficacy of local steroids, local injection should be the preferred treatment.

The use of corticosteroids has been compared with other treatment modalities. In Chang 1998 RCT, oral prednisolone was found to be superior in reducing symptom severity using the GSS when compared to diuretics (mean difference 11.60 95% CI 7.25 to 15.95) and NSAIDs (mean difference 14 95% CI8.57 to 19.43) (Chang *et al.*, 1998). Oral corticosteroids were found to be no more effective at reducing symptoms than splinting (Mishra *et al.*, 2006). The use of a

neutral wrist splint and NSAID was found to have a similar efficacy as local steroid injection (Celiker *et al.*, 2002).

Non-Steroidal Anti-inflammatories (NSAIDs)

Despite their widespread use, the evidence for NSAIDs is quite sparse in CTS. In one RCT the use of the NSAID tenoxicam in 22 patients was compared with, placebo, oral prednisolone, and the diuretic trichlormethiazide in mild to moderate CTS (Chang *et al.*, 1998). The study found no difference in GSS between NSAIDs versus placebo at 4 weeks. A second RCT compared the use of splinting with the combination of a splint and adjuvant therapies including ketoprofen, phonophoresis and therapeutic U/S. With the exception that the splint and ketoprofen phonophoresis had less pain at 8 weeks, there was no significant difference between the three groups (Yildiz *et al.*, 2011). This has led to the recommendation that NSAIDs may play a role in treating tendonitis associated with CTS but its role in symptom management of CTS is limited (Ashworth, 2010).

Other therapies

Many other treatments have been described for CTS. These include systemic treatments, local treatments, nerve gliding, and ultrasound. A complete review of all of these treatments is outside the scope of this review. Hormonal therapies have been an interest in CTS for multiple reasons. The predisposition of women at age 50-65 has led to the postulation of a hormonal contribution to CTS (Bjorkqvist *et al.*, 1977; Song *et al.*, 2014). Additionally, progesterone has been shown to increased neural regeneration potentially via the formation of new myelin sheaths (Schumacher *et al.*, 2007). Bahrami compared the injection of progesterone to triamcinolone in a randomized control trial and found no difference in function as measured by the Boston

questionnaire or NCS. However, patients satisfaction was higher in the corticosteroid group (Bahrami *et al.*, 2015).

Diuretics have been examined as a treatment of CTS aimed at decreasing the edema associated with compression. The effectiveness of this therapy has been explored in two RCTs. Chang et.al. studied the use of multiple systemic agents for the treatment of CTS including the diuretic trichlormethiazide, finding no difference in GSS at 4 weeks between placebo and diuretics (Chang *et al.*, 1998) A second RCT compared the use of bendroflumethiazide to placebo in 48 patients. It was found that at 6 months there was no difference in symptom severity between the bendroflumethiazide groups (74%) vs placebo (75%) with a relative risk of 0.98 (Pal *et al.*, 1988). There appears to be little benefit for diuretics in the treatment of CTS.

Pyridoxine (vitamin B6) acts as a co-factor in the synthesis of many proteins important in heme synthesis, biosyntheses of lipids and glycogenolysis. Its use in CTS stems from patients with pyridoxine deficiency suffering from neuropathies along with dermatits, anemia, and glossitis. Trials comparing the use of pyridoxine to placebo in the treatment of CTS failed to show any symptomatic improvement. However, they did show a significant improvement in finger swelling. There is only low level evidence to support the use of pyridoxine and it likely is ineffective (Ashworth, 2010; O'Connor *et al.*, 2003).

Surgical Therapy

Open Carpal Tunnel Release

There have been no randomized control trials to show that carpal tunnel release (CTR) is effective against placebo. However, in case series, CTR has shown good efficacy at 85% recovery. There have been trials to compare the efficacy of simple decompression to

decompression with internal neurolysis. There was no additional benefits with internal neurolysis (Mackinnon *et al.*, 1991)[See Figure 1-10].

The efficacy of open CTR has been evaluated in various subsets of patients. Multiple studies have examined the efficacy of CTR in patients with DM and found no difference compared to non-diabetic controls (Thomsen *et al.*, 2014). In patients with CTS undergoing hemodialysis, improvement is seen on the BCTQ though the hemodialysis patients do not return to the same level as idiopathic patients indicating some residual impairment (Kang *et al.*, 2012) It has been shown that recovery is suboptimal in elderly patients (age >70) when compared to younger patients (Mondelli *et al.*, 2004). Kronlage et.al. showed that CTR can still be beneficial in patients with severe CTS. Unfortunately, no comparison was made to an age matched cohort of less severe patients to eliminate any confounding by age on the significance of this recovery (Kronlage & Menendez, 2015).

While no trials have been conducted to compare the efficacy of CTR vs placebo, there have been comparison trials to conservative therapies. Gerritsen *et.al.* found that surgery was superior in reducing symptom severity when compared to splinting for up to 18 months (Gerritsen *et al.*, 2002). Contradictory results have been found when surgery is compared to local corticosteroid injection. A 2005 trial by Hui found that surgery had superior improvement in the Global Symptom Score (GSS) at 20 weeks compared to methylprednisolone injection (Hui *et al.*, 2005). However, a second study published in 2005 showed that steroid injection was superior to surgery at three months and with no difference up to 1 year (Ly-Pen *et al.*, 2005). However, over 80% of patients required repeat steroid injection over the year to remain symptomatic free which can skew the results of the trial.

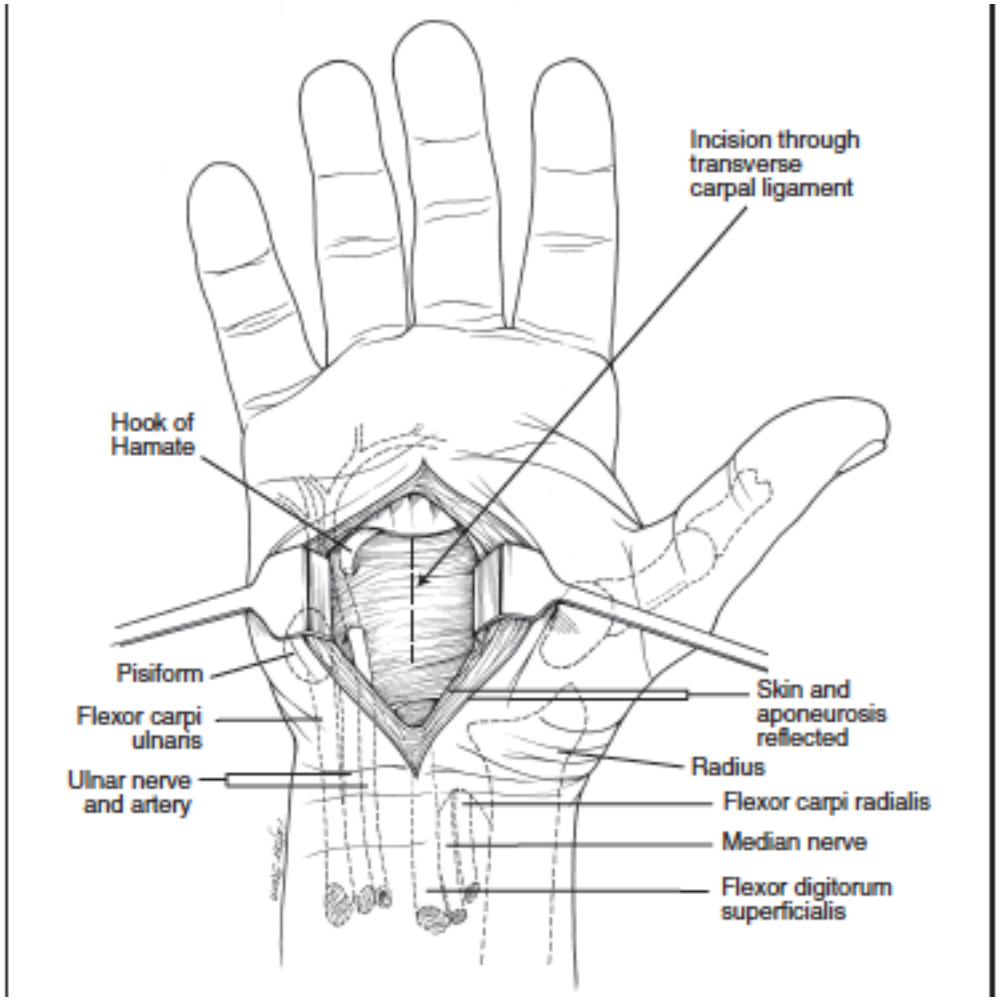
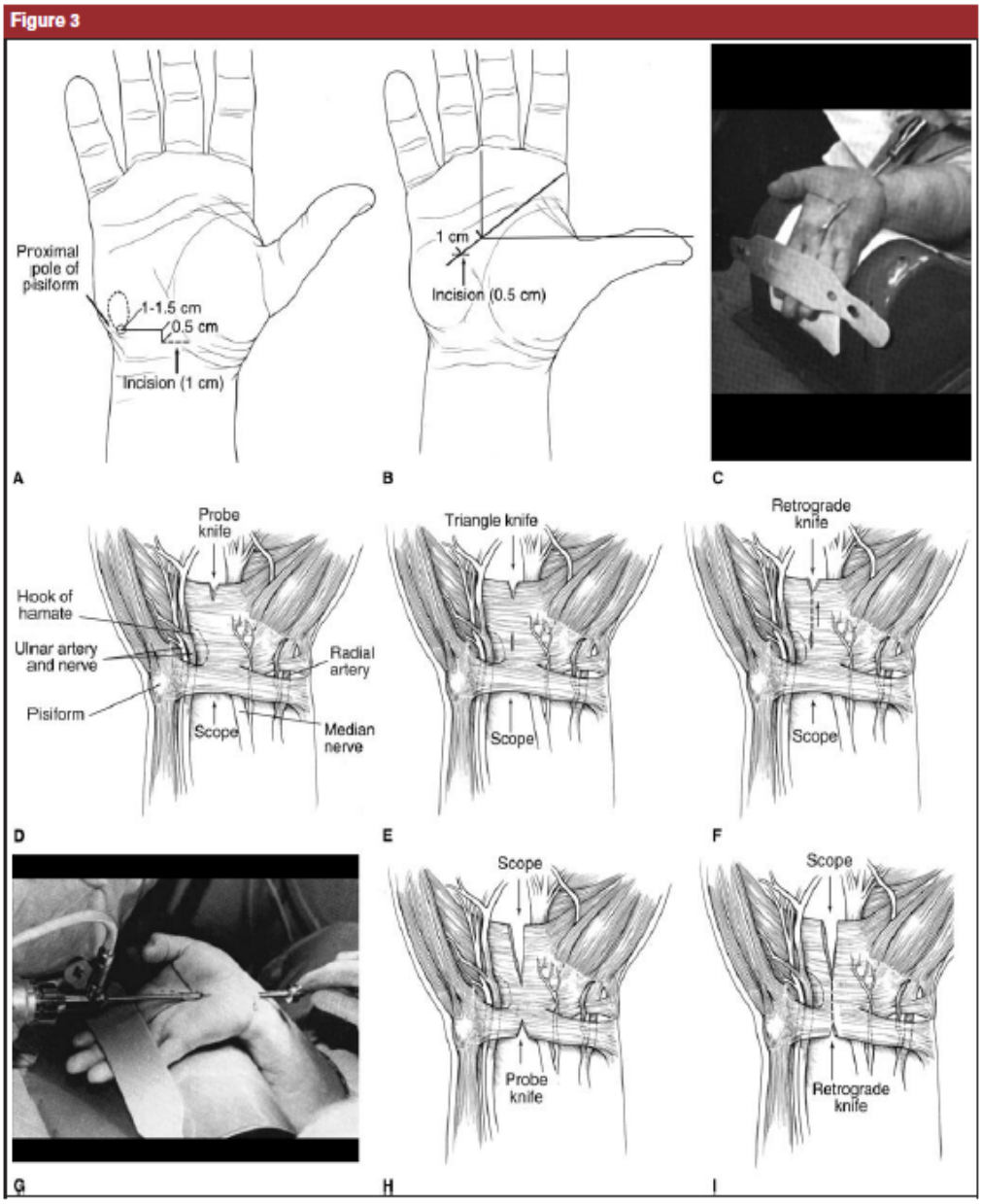


Figure 1-10 Schematic of open carpal tunnel release.

Longitudinal incision is made in the skin from the distal wrist crease to Kaplans cardinal line. Sharp dissection continues until the TCL is identified. The TCL is sharply divided to expose the median nerve. The division continues proximally, releasing the antebrachial fascia. Distally, the division of the TCL finished at the palmar fat pad (Cranford *et al.*, 2007).

Endoscopic Carpal Tunnel Release

Endoscopic CTR was first described in 1989 by Chow et.al. who described a two port technique (Chow, 1989). Agee would later augment the technique to a one port system (Agee *et al.*, 1992) [See Figure 1-11]. With the small ports providing access for the operation, the hope was that this technique would significantly decrease complications from the incision. This has been examined in a Cochrane review that shows no difference in terms of symptomatic improvement. Although there were fewer minor complications in the endoscopic cohort, there was a higher rate of nerve injury, particularly neurapraxia in this population. One of the most important findings was that return to work was quicker in the endoscopic group by 8 days which is clinically important (Vasiliadis *et al.*, 2014).



Chow two-portal endoscopic technique. **A,** Entry portal. **B,** Exit portal. **C,** The endoscope and blade assembly are passed from the proximal incision through the distal incision, deep to the transverse carpal ligament (TCL). **D,** The distal edge of the TCL is released using a probe knife. **E,** A second cut is made in the midsection of the TCL with a triangular knife. **F,** The first and second cuts are connected with a retrograde knife. **G,** The endoscope is repositioned beneath the TCL through the distal portal. **H,** A probe knife is inserted to release the proximal edge of the TCL. **I,** A retrograde knife is inserted into the midsection of the TCL and drawn proximally to complete the release. (Panels A, B, D, E, F, H, and I are adapted with permission from Chow JCY: Endoscopic carpal tunnel release: Two portal technique. *Hand Clin* 1994;10:637-646.)

Figure 1-11 Schematic of two port endoscopic carpal tunnel release.

See above legend for step by step procedure (Cranford *et al.*, 2007).

Conventional Treatment of Choice

Research has yet to provide the ideal treatment for all CTS. Certainly, surgery has been shown to have superior outcomes when compared with non-operative measures. However, surgery also has more significant adverse events compared to conservative measures. While surgery has superior outcomes relative to conservative measures the increase in complications can be a difficult solution to accept, especially in milder disease.

Given the lower side effect profile of non-operative treatments it should be considered the first-line treatment in mild to moderate disease (Duncan *et al.*, 1987). Non-operative treatments also can act as a bridge for patients requiring surgery while they wait. Because of their minimal side-effect profile, splint can be worn but local corticosteroids should be the principal treatment. It is important to remember that non-operative treatments do not relieve the compression of the median nerve. Therefore, the risk of ongoing damage to the median nerve is possible. Patients treated non-operatively should be followed to ensure resolution of symptoms.

Surgery should be performed in all patients with severe CTS or who have failed a trial of conservative measures. The procedure to be performed still remains controversial. Both open and endoscopic CTR are adequate for release of the TCL with similar outcomes. Given the evidence choice should be based on surgeon abilities and availability of resources. Open CTR can be completed under local anesthetic in a minor clinic, whereas the endoscopic CTR requires a general anesthetic and the main operating theatre. Despite surgery the functional recovery can be incomplete. Therefore, having an additional treatment that can increase nerve recovery is an attractive therapeutic option.

Novel adjunct therapies for nerve regeneration

Multiple different therapeutics have been described to increase nerve regeneration; See (Chan *et al.*, 2014) for a review and Figure 1-12. One that has shown the most promise is increasing neural regeneration through the ERK 1/2 pathway. Agents that enhance this pathway include geldanamycin and tacrolimus (Sun *et al.*, 2012). While both can substantially increase neurite outgrowth both have significant side effects. Geldanamycin is a chemotherapeutic agent that causes hepatotoxicity, while tacrolimus is a potent immunosuppresion drug used in transplant patients with substantial risks.

Similar to geldanamycin and tacrolimus ALCAR works to upregulate the ERK1/2 pathway to increase nerve regeneration. However, compared to the previous two compounds ALCAR has a substantially better side effect profile with the major side effect being nausea in 1% of patients (De Grandis, 1998).

Evidence for ALCAR

Basic Science

ALCAR is a peptide derivative critical in fatty acid transport into mitochondria. It has been found to have a role in sperm motility (Zhou *et al.*, 2007), hepatic encephalopathy (Jiang *et al.*, 2013), and dysthymia treatment (Wang *et al.*, 2014). The role of ALCAR in nerve injury was first elucidated in the central nervous system (CNS). It was found that ALCAR could improve

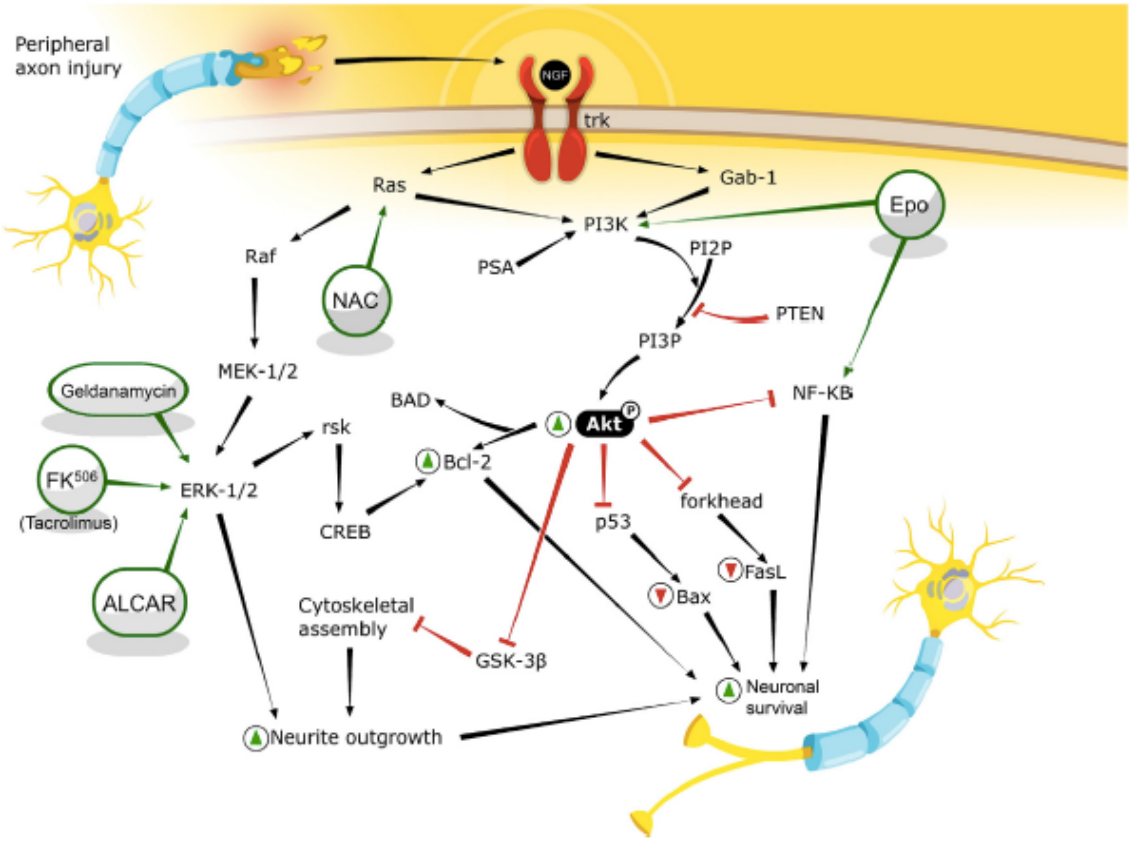


Figure 1-12 Molecular mechanisms of peripheral nerve regeneration and pharmacologic targets.

Multiple pathways are involved in peripheral nerve regeneration. A few of the key pathways are outlined here and potential therapeutic targets that act on each pathway are included (Chan *et al.*, 2014).

memory and behaviour in aged rats. Badiali de Giorgi et.al demonstrate histological maintenance in rat hippocampal cells when treated with ALCAR (Badiali de Giorgi *et al.*, 1987). Similarly, Ricci showed increased mossy fibre area in rats treated with ALCAR (Ricci *et al.*, 1989). Angelucci attributed this to decreases in nerve growth factor (NGF) binding in specific areas including the hippocampus of aged rats that could be ameliorated with ALCAR (Angelucci *et al.*, 1988). Attention was later turned to peripheral nerves where multiple studies have shown that ALCAR prevents degeneration and causes regeneration in transected peripheral nerves. Taglialaltela et.al. showed that neuronal preservation can be attributed to increases in NGF receptors (Taglialatela *et al.*, 1992). Barhwal et.al. later demonstrated that this increase in NGF receptors led to increased ERK1/2 phosphorylation that in turn resulted in upregulation of CREB and neuroprotective factors (Barhwal *et al.*, 2008). Clinical translation of this work has been applied to diabetic neuropathy, HIV antiretroviral toxic neuropathy (ARN) and chemotherapy induced peripheral neuropathy (CIPN).

Diabetic Neuropathy

The efficacy of ALCAR in the treatment of diabetic neuropathy has been examined in three studies. Sima et.al. combined the results of a North American and a European randomized control trials (Sima *et al.*, 2005) A total of 1257 patients were randomized into placebo, ALCAR 500 mg and ALCAR 1000 mg orally three times per day. The study demonstrated an improvement in pain scores but only in the ALCAR 1000 mg tid group. However, the primary endpoints of improved NCS was not reached and further clinical use of the drug was not pursued. Sural nerve biopsies were available from 245 patients from one cohort demonstrating increased fiber numbers in patients taking ALCAR 500 mg tid.

De Grandis completed a multicenter RCT using ALCAR 2000 mg/d to placebo in 333 patients (De Grandis & Minardi, 2002). At 52 weeks, the trial showed a statistically significant improvement in both NCV and SNAP/CMAP amplitude when compared to placebo. The effect was seen in multiple nerves including the sural, ulnar and median nerve. The trial also demonstrated an improvement in VAS scores indicating neurophysiological and pain improvement.

A third trial compared the use of ALCAR with methylcobalamin (Li *et al.*, 2016). Both groups showed improvement in the primary outcome of neuropathic disability and symptom score but there was no significant difference between the groups. However, the study lacked a placebo controlled group for comparison making the effect difficult to interpret.

ALCAR may have to have a role in improving pain associated with diabetic peripheral neuropathy but overall it has not been shown to have a more substantial impact to date. Nerve regeneration has been found in sural nerve biopsies but only one of the two RCT showed improvement in neurophysiological outcomes.

Chemotherapy Induced Neuropathy (CIPN)

The incidence of CIPN is quite variable. Development depends on the type of chemotherapeutic used, cumulative dose, and duration of treatment. Platinum compounds, taxanes, vinca alkaloids among others have all been shown to cause neurotoxicity (Argyriou *et al.*, 2012; Wolf *et al.*, 2008). In animal models, it has been shown that ALCAR can decrease the severity of the neurotoxic effects of paclitaxel, cisplatin and oxaliplatin. It has even been shown to have a preventative effect on the development of oxaliplatin and paclitaxel induced neuropathy (Ghirardi *et al.*, 2005; Pisano *et al.*, 2003);(Flatters *et al.*, 2006)

There have been clinical studies to examine ALCARs preventative and therapeutic role. A well-powered RCT examined the preventative role of ALCAR in patients receiving taxane therapy. Hershman et.al. found that ALCAR demonstrated no improvement at 12 weeks and at 24 weeks ALCAR actually worsened FACT-NTX scores, a validated questionnaire for measuring the impact of side effects from taxanes (Hershman *et al.*, 2013). While the study was well constructed, a subjective measure alone of CIPN may not represent the best outcome to assess the prevalence and severity of CIPN. However, a second trial examined the use of ALCAR to prevent sagopilone-induced PN, finding a similar incidence of CIPN in the ALCAR and placebo group though with a milder severity in the ALCAR groups (Campone *et al.*, 2013). Similar results were found when ALCAR was used as a preventative strategy for the prevention of bortezomib (Callander *et al.*, 2014). Taken together the role of ALCAR as a preventative drug seems limited.

ALCAR has also been explored as a therapeutic agent for patients with previously diagnosed CIPN. An Italian prospective case series was able to show an improvement in total neuropathy score in 92% of patients with cisplatin and paclitaxel induced CIPN (Bianchi *et al.*, 2005). In addition, Bianchi et.al. showed a statistically significant improvement in SNAP ($p < 0.03$) and a trend towards improvements in CMAP. While the mean changes are not presented nor are the results compared to a control this does provide objective evidence of neural regeneration in this patient population. A larger RCT has recently been completed on a similar group of CIPN patients showing significant improvement in National Cancer Institute – Common Terminology Criteria (NCI-CTC) grades of neuropathy, (Sun *et al.*, 2016). The NCI-CTC represents a standardized set of toxicity criteria for chemotherapy agents measuring the severity of adverse event on a Likert scale. Both peripheral motor and sensory neuropathies are

defined in the criteria. The Bianchi et.al. series also looked at graded improvement in NCI-CTC and found improvement in 60% of patients with sensory neuropathy and 79% of patients with motor neuropathy. Masestri et al treated 27 patients with paclitaxel and cisplatin induced neuropathy with ALCAR 1g/d IV for 10 days and found a greater than or equal to one stage decrease in WHO classification in 73% of patients (Maestri *et al.*, 2005). All studies indicate that ALCAR may have a potential role as a therapeutic for CIPN, but studies are nto definitive to date..

The inclusion of different treatment strategies (prevention vs. therapeutic), differing chemotherapeutics and outcome assessments makes the combining these studies for analysis difficult. The studies in the prevention paradigm have shown no improvement in incidence indicating a limited role for ALCAR.

Anti-retroviral Induced Neuropathy

Approximately 10-35% of patients treated with antiretrovirals, particularly nucleoside reverse transcriptase inhibitors, experience a distal sensory polyneuropathy (Youle, 2007). Patients with ARN have lower levels of systemic ALCAR when compared to HIV patients on antiretrovirals without neuropathies (Famularo *et al.*, 1997)

A recent systematic review examined the effects of ALCAR on ARN. Due to the inclusion criteria 6 studies were identified but only included one in its analysis concluding that the evidence is currently inconclusive (Phillips *et al.*, 2010). The included study was a RCT of 90 patients randomized to ALCAR or placebo. The trial found a statistically significant change in the VAS for the ALCAR group (-0.89[-1.64,-0.14]; p=0.02). However, this effect was only found in the efficacy evaluable population. There was no significance found in Total Symptom Score, McGill Pain Questionnaire and number or rescue analgesics required (Youle & Osio,

2007). The trial continued as an open-label study for 56 days showing trends of improvement in VAS, McGill Pain Questionnaire, and Total Symptom Severity score. While the trial was able to demonstrate improvements in pain, the trial design with a follow-up period of 14 days was insufficient for the assessment of peripheral nerve regeneration which can take significantly longer.

In the excluded trials from Phillips meta-analysis, we find evidence to support ALCAR ability to promote peripheral nerve regeneration. Initial evidence resulted from an Italian open-label study that demonstrated symptom improvement in 62.5% of patients (Scarpini *et al.*, 1997). A further open label study showed improvement in the McGill Pain Questionnaire after 3 weeks of ALCAR treatment. However, there was no evidence of electrodiagnostic improvement in this cohort of patients (Osio *et al.*, 2006). Hart *et.al.* demonstrated increased epidermal and dermal nerve fibres on biopsy in patients treated with ALCAR for 6 months or greater (Hart *et al.*, 2004). A long-term follow-up study on these patients showed the majority of patients (15 of 16 available) are asymptomatic or not requiring analgesia with a mean 4.3 year follow up (Herzmann *et al.*, 2005).

Role of ALCAR in the Management of Neuropathic Pain

Pain represents a common symptom of most peripheral neuropathies. While no formal trials have been aimed at treating patients with neuropathic pain, improvement in pain scores is an often-included outcome in the effects of ALCAR on specific neuropathies. Li et.al. attempted to summarize these results in a meta-analysis of currently available RCTs showing improved VAS in DPN and ARN (S. Li *et al.*, 2015). In an open label study, De Grandis et.al. found significant improvements in VAS in a study of 1097 patients (De Grandis, 1998). While the study was primarily focused on the tolerability of ALCAR, it showed a reduction in VAS,

with 60% of patients reporting mild pain at 30 days. This study contained multiple etiologies including compression neuropathies.

Thesis Formulation

Given the need for new therapeutics to increase the peripheral nerve regeneration in severe CTS cases we have chosen to examine the role of ALCAR in CTS. Although one animal study did show increased soleus weights after treatments with ALCAR in a rat model, there have been no direct human studies examining the role of ALCAR in compression neuropathy (De Grandis, 1998; Kotil *et al.*, 2007). The De Grandis open label of trial of ALCAR on tolerability did contain compressions neuropathies; there was an improvement in conduction velocity in both motor and sensory nerves. However, these results are pooled with all other neuropathies included in the study precluding individual analysis.

Given some promising pre-clinical data and data in other neuropathies we sought oout to examine the effects of ALCAR treatment on nerve regeneration in CTS. We hypothesized that treatment with ALCAR will increase nerve regeneration and functional outcomes when compared to placebo.

To examine the effect of ALCAR we constructed a randomized-double blind trial where 3g/d of ALCAR or placebo was given after surgical decompression. Only patients with severe CTS were recruited to ensure patients have both demyelination and axonal loss. Patients took the drug or a matched placebo for 2 months and were followed for 1 year.

The primary outcome was motor recovery as determined by MUNE. This allowed for the quantification of motor recovery relative to placebo. For the purposes of our primary outcome we sought to have a single quantifiable outcome. While sensory abnormalities are primary in CTS there is no single test that examines the breadth of sensory function making it difficult to

choose one as a primary outcome. Functional outcomes are also critical in the evaluation but most outcomes are subjective in nature. Therefore, it was decided upon MUNE as our primary outcome to give us an objective measure of recovery.

Our secondary outcomes will consist of sensory and functional outcomes. Sensory testing will be designed to examine all sensory fiber types. Functional recovery will be measured by subjective patient reported outcome and objective measures of function.

Chapter 2

Protocol for a randomized control trial to assess peripheral nerve regeneration

Basic Trial Design

This double blinded placebo controlled trial received ethics approval from the University of Alberta Health Ethics Research Board (Pro00045538). The study protocol has been reviewed by Health Canada and a No Objection Letter (NOL) was issued for the use of ALCAR. Subjects were recruited from plastic surgery clinics at the University of Alberta Hospital and Royal Alexandra Hospital as well as the electromyography (EMG) clinic at the Glenrose Rehabilitation Hospital.

Subjects

All patients referred to either the plastic surgery clinics or the EMG lab were eligible to be screened if CTS was confirmed by electrodiagnosis. Inclusion into the study was based upon the criteria listed in Table 2-1. Patients meeting eligibility criteria were recruited and informed consent was obtained. The eligible patents then underwent motor unit number estimation (MUNE) to quantify the severity of motor axonal loss. Only those with motor axonal loss of > 2 SD below the mean for the age group were included in the study.

Randomization

After enrollment, patients were randomized to placebo or ALCAR in a 1:1 manner. A randomization sequence was generated electronically and kept by a statistician not directly involved in patient care. Randomization codes were kept on a computer under password encryption. Randomization would only be broken if a severe adverse event occurred to a patient. At the end of the study the statistician would provide the research team with the randomization codes.

Inclusion Criteria	Exclusion Criteria
1) Age > 18 years 2) One or more of the following symptoms of CTS a) numbness and paresthesias in the median nerve distributions b) precipitation of those symptoms by repetitive motions that are relieved by rubbing and/or shaking c) nocturnal awakening of the above symptoms d) weakness of thumb abduction and thenar atrophy	1) Motor unit loss in the median nerve less than 2 standard deviations below the mean for the age group 2) Presence of other neurologic conditions 3) Previous carpal tunnel release 4) Cognitive impairment that renders the patient incapable of providing consent 5) History of seizures 6) Kidney disease/renal impairment 7) Sensitivity to any of the drug components of the formulation or are 8) Pregnant/breast feeding 9) For women of child-bearing potential, those that are not willing to use adequate contraceptive prevention methods for at least 30 days after the last dose of the medication.

Table 2-0-1: Inclusion and exclusion criteria for the randomized control trial.

Blinding

The study was conducted in a double-blind manner. The randomization sequence was kept confidential and only accessible to authorized personnel. Pharmacy distributed identical pills labelled in boxes with a study specific code. The research team was unaware of which contained the study drug or placebo. Blinding was maintained until the final follow-up has occurred, at which point the research team was un-blinded.

Primary Outcome

The primary endpoint was motor axon reinnervation in the thenar muscles. Motor recovery was analyzed by MUNE, a non-invasive electrophysiological technique. Multipoint stimulation, the most commonly used MUNE technique, has been shown to be a reliable test for measuring motor axon reinnervation. MUNE provides an objective assessment of large nerve fibres. Eligibility for the trial was determined in part by MUNE at a screening visit. Primary outcome were measured at 3, 6, and 12 months post-operatively.

Secondary Outcomes

Secondary outcomes of the study focus on sensory function restoration and functional outcomes. Measures included:

1. Two point discrimination (Aβ fibers) using Dellon-MacKinnon Disk
2. Pressure sensitivity (Aβ fibers) using Semmes-Weinstein Monofilament
3. Cold threshold (Aδ fibers) and pain threshold (C fibers) using CASE IV Quantitative Sensory Testing (QST) equipment
4. Boston Questionnaire for CTS to monitor symptom severity
5. Disability for Arm, Shoulder, and Hand (DASH) Questionnaire for functional outcomes

6. Moberg Pick-up Test to measure hand dexterity and functional performance

All outcome measures were monitored at baseline, 3 months, 6 months, and 12 months. Sensory testing of peripheral nerves provides more of a challenge given the multiple fibre types that provides sensory feedback. The above list of tests was designed to capture the full range of sensory fiber functions. One major advantage of QST is the provision of an objective means of measuring the function of different classes of nerve fibers with good reliability. The DASH questionnaire and the Boston questionnaire used to evaluate symptom severity are both well validated in CTS(Gay *et al.*, 2003)Greenslade, 2004 #882}. Finally, hand function was measured objectively with the Moberg Test, a validated tool for CTS with good reliability (Amirjani *et al.*, 2011a). All primary and secondary outcome measures were performed by the same investigators.

Interventions

Patients were assigned to either the placebo or ALCAR arm of the study. Placebo and ALCAR was provided by the same company (Sigma Tau). Both pills were identical tablets of a similar content. Upon arrival, the tablets were sent to pharmacy to package the tablets in identical boxes containing a random sequential code. After screening patients underwent open carpal tunnel release without epineurolysis performed by experienced hand surgeons. Post-operatively patients took either placebo or ALCAR for 2 months. Patients randomized to the ALCAR arm took 3000 mg orally divided in three times per day dosing while the control group took a matched placebo. Dosing for the study drug was chosen based upon previous work from Bianchi et.al. which showed good tolerability and results. As our study used the same formulation we elected to follow the previously described treatment course.

Sample Size

Initially, a pilot study of 20 patients was carried out. Evaluation of the effect size will be based on the primary outcome MUNE. A pilot study would allow for the determination of the effect size of ALCAR treatment. While sample size could be based on previous works that requires the assumption that the effect size will be similar. For example, the Gordon *et.al*. trial of electrical stimulation uses similar outcomes to measure the effect size(Gordon *et al*., 2010). However, there are some issues with using this methodology. This trial based its sample size on previous animal data using similar outcomes(Al Majed *et al*., 2000). There was no available data to estimate ALCAR effect size on MUNE. In addition, the proposed mechanisms of increasing nerve regeneration are different for LACAR and electrical stimulation. The effect size of each could vary significantly leading to under or over prediction of the sample size for a full scale trial. Using 20 patients as a pilot study would provide a more accurate means of determining the effect size. The results could be used to provide the necessary data for the sample size calculation for a fully powered large scale study.

Statistical Analysis

Demographic data was analyzed for differences using students T-test and Fishers exact test. The primary and secondary outcome measures were analyzed using two-way ANOVA with the treatment groups and time points being the independent factors. When significant associations were identified, post-hoc analysis was completed for pairwise analysis. Due to the exploratory nature of the study sample size calculations and formal analysis of missing variables was not undertaken until the full-scale trial.

Safety and Potential Risks

To monitor any potential adverse events, rigorous safety measures were put in place to ensure patient safety. Initial baseline testing included:

1. Physical examination
2. Vitals
3. EKG
4. Urine pregnancy tests
5. Complete blood count with differential, electrolytes (Na, K, Cl, CO2), creatinine, urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin
6. serum ALCAR

Vital signs (blood pressure, temperature, pulse, respiratory rate, oxygen saturation) were recorded at every visit. Safety lab tests including blood chemistries were done at screening, and periodically during follow up visits. In addition, an electrocardiogram was completed at screening. The serum ALCAR level was monitored. In healthy subjects, the serum ALCAR levels had a range of 10-70 μmol/l with a mean of 39 μmol/l. In a large study of over 333 patients with diabetic neuropathy taking 3 g of ALCAR/day (the same dose used in this study), the rise in serum level was only around 50% compared to baseline. Since no sign of ALCAR toxicity was observed in any of the subjects, the chances of toxic effects in this study will be very small.

All adverse events will be documented at follow-up visits. Further laboratory investigations were completed depending on the nature and severity of the adverse events reported.

Chapter 3

Acetyl-l-carnitine does not increase nerve regeneration nor improve functional outcomes in carpal tunnel syndrome.

Study Recruitment and Demographics

Twenty-six patients were screened between October 2015 and May 2016. Twenty-five patients were recruited based on the severity of motor unit loss. Of those, 5 patients were excluded for the following reasons: one due to coexisting neurologic condition, one due to inability to complete follow-up after enrollment and 3 due to undiagnosed renal dysfunction. The 20 remaining patients were randomized in a 1:1 fashion to receive either placebo or ALCAR post carpal tunnel decompression. One patient was lost to follow-up (inability to contact patient) from each arm. Three patients, 2 from the ALCAR and 1 from the placebo group, are awaiting their final follow-up appointment, leaving 8 patients in the placebo group and 7 patients in the ALCAR group used for analysis in this thesis (See Figure 3-1). Due to the time constraints placed by the academic schedule of residency, prior decision was made to unblind the candidate to ensure the data analysis could be complete in time. Given this decision, three patients fell outside this window and were not included in analysis. Due to the exploratory nature of the trial the exclusion of these patients should not substantial impact our analysis. To manage the missing patients the data was analyzed as per protocol analysis.

The patient demographics are depicted in Table 3-1. Analysis demonstrated that the ALCAR group had significantly more diabetic patients ($p=0.04$). Patients with diabetic neuropathy were excluded from the trial so the presence of neuropathy was controlled. It has also been proven that diabetics have similar results after CTR to non-diabetics so this difference should not impact the outcome (Thomsen *et al.*, 2014). No other differences between the placebo and ALCAR treatment groups. Baseline outcomes were also similar between both groups (See Table 3-2) with the exception of the cold detection threshold in the ulnar control

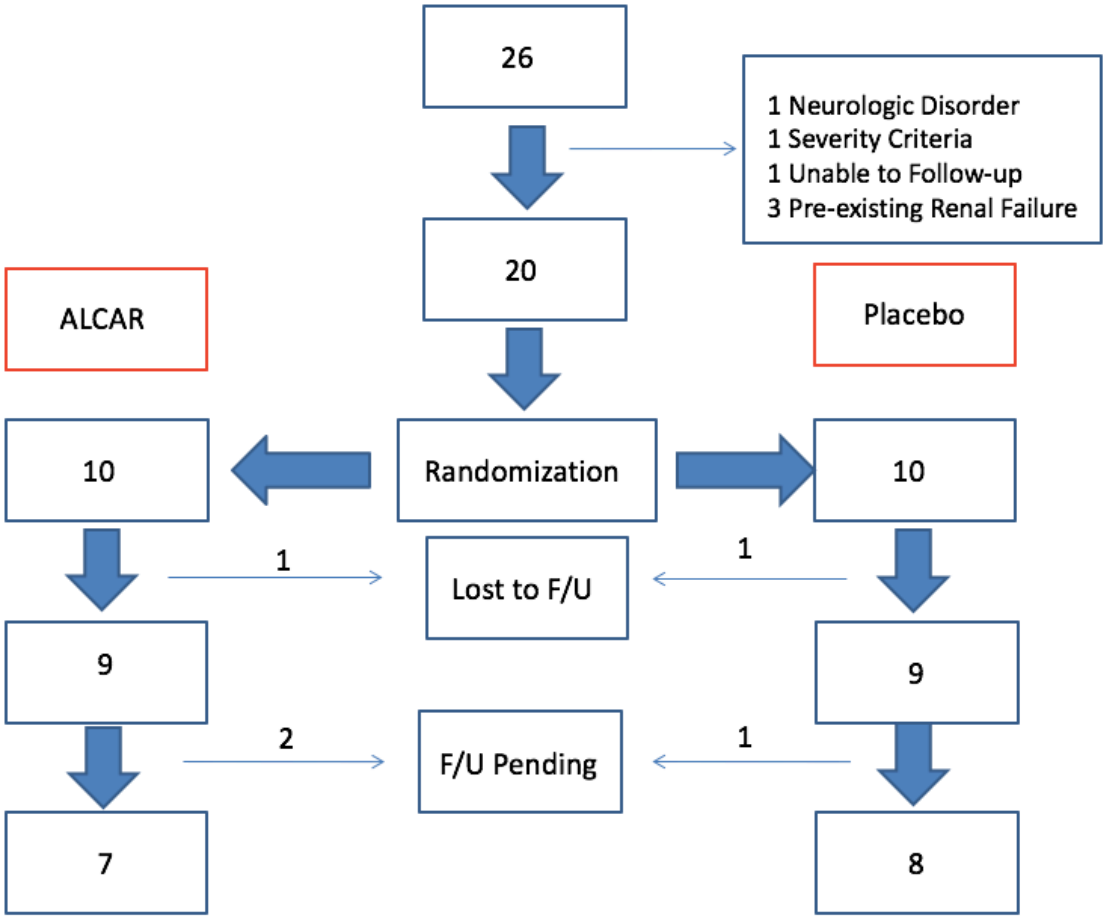


Figure 3-1: CONSORT trial diagram

Two patients were lost to follow-up due to inability to contact patients. Three patients are awaiting follow-up.

Demographic	Total (n=15)	ALCAR (n=7)	Placebo (n=8)	p-value
Age	59.9+/-2.8	60.2+/-4.4	59.6+/-4.0	0.91
Gender (F:M)	9:6 (60% F)	5:2 (71% F)	4:4 (50% F)	0.61
Hand Dominance (R:L)	13:2 (87% R)	6:1 (86% R)	7:1 (88% R)	1.0
BMI	31.6+/-1.5	31.9+/-2.1	31.3+/-2.3	0.85
Co-morbidities	5.8+/-0.81	5.4+/-1.1	6.1+/-1.3	0.68
Diabetes	12:3 (75% Non-diabetic)	4:3 (Non-diabetic)	8:0 (Non-diabetic)	0.038
Medications	4.4+/-0.8	4+/-1.0	4.9+/-1.2	0.59

Table 3-1: Demographic data

Data is presented as mean +/- SD for continuous data and frequency for categorical data. There was more diabetics in the treatment arm of the study. However, any patient with evidence of diabetic neuropathy was excluded from the study. There was no statistical difference between the two groups for the remaining variables with α=0.05.

Outcome	ALCAR (n=7)	Placebo (n=8)	p-value
MUNE	46 +/-10	50 +/-11	0.78
2PD (mm)	14+/-3	11+/-2	0.35
SWMT (g)	4.68+/-0.25	4.69+/-0.33	0.98
CDT (JND)	18.0+/-1.3	20.4+/-1.4	0.23
HP (JND)	17.7+/-1.9	19.5+/-3.2	0.66
Moberg (s)	77+/-22	119+/-44	0.43
DASH	32.9+/-6.6	41.1+/-6.4	0.38
Levine's Symptom	3.44+/-0.17	3.27+/-0.39	0.71
Levine's Function	2.75+/-0.19	2.61+/-0.38	0.76
Levine's Total	6.19+/-0.22	6.01+/-0.81	0.84

Table 3-2: Baseline outcome measures

No statistical significance was found between the two groups using the Student's t-test with α=0.05.

digit ($p<0.01$). As the ulnar digit was only used for control it likely has no major impact on the key outcome measures.

Control testing for variability

To control for intersubject variability, for the two-point discrimination, Semmes-Weinstein monofilament test and QST measures, the results in the median innervated 3rd digit were compared to the ulnar innervated 5th digit. Given the nature of the Moberg pick-up test the contralateral hand was used as a control. While bilateral CTS may impact the results of the Moberg pick-up test, there was no statistically significant difference in the presence of bilateral CTS between the treatment and control group ($p=0.44$).

None of these secondary outcome measures in the ulnar innervated 5th digit showed any statistically significant change over the course of the study with the exception of cold detection threshold. The control digit displayed a significant improvement from treatment ($p<0.01$) and time ($p=0.04$). This could indicate a learning effect that can be seen with psychosocial testing affecting the reliability of this test result.

Outcome Assessment

ALCAR does not increase motor reinnervation after CTR.

Two-way ANOVA analysis was carried out using time (4 time points) and treatment (2 arms) as independent factors. At the 12 month follow-up, MUNE of both placebo (133±120 from 50 ± 32) and ALCAR groups (113±48 from 46±26) had improved from baseline ($p=0.02$). Post hoc analysis utilizing the Bonferroni method revealed that MUNE was significantly higher at 12 months relative to baseline ($p=0.02$). However, there was no difference between the placebo and ALCAR groups in the extent of motor reinnervation ($p=0.22$) [See Figure 3-2].

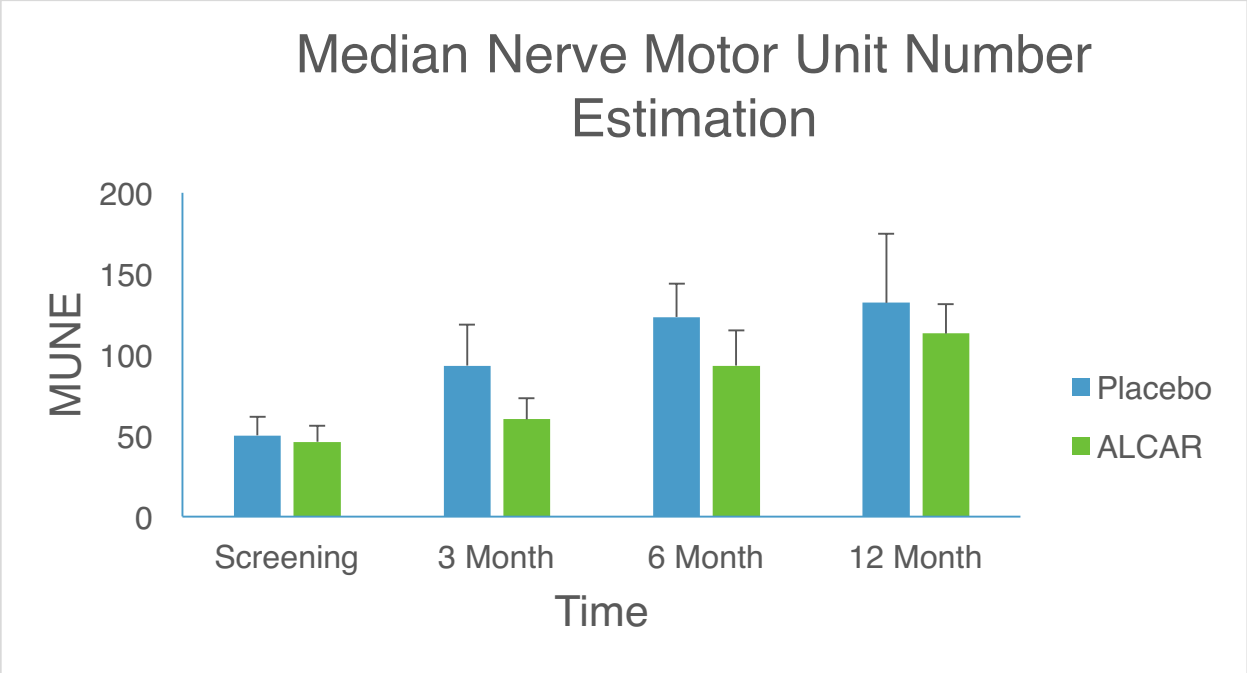


Figure 3-2: Median nerve MUNE

Median nerve MUNE as determined by the multipoint stimulation method. There was no difference between placebo and ALCAR treatment (p=0.22). MUNE did significantly increase over the 12month follow-up period (p=0.02).

No other NCS measures had a significant interaction. Sensory measures of SNAP and conduction velocity improved with time. dML approached significance (p=0.06) however there was a wide varience in starting dML. Results are summarized in Table 3

Secondary Outcomes

Sensory and functional measures were utilized as secondary outcomes to further evaluate nerve regeneration and the impact it would have functionally. Two-point discrimination and Semmes-Weinstein Monofilament test were used to evaluate large fiber nerve function. The quantitative sensory tests cold detection threshold and heat-pain threshold were used for Aδ and C fiber function, respectively. Functional outcomes were measured subjectively by two patient reported outcomes through the DASH and Levine's CTS questionnaires and objectively by the Dellon-modified Moberg Pickup Test.

Large Fiber Sensory Nerve Function

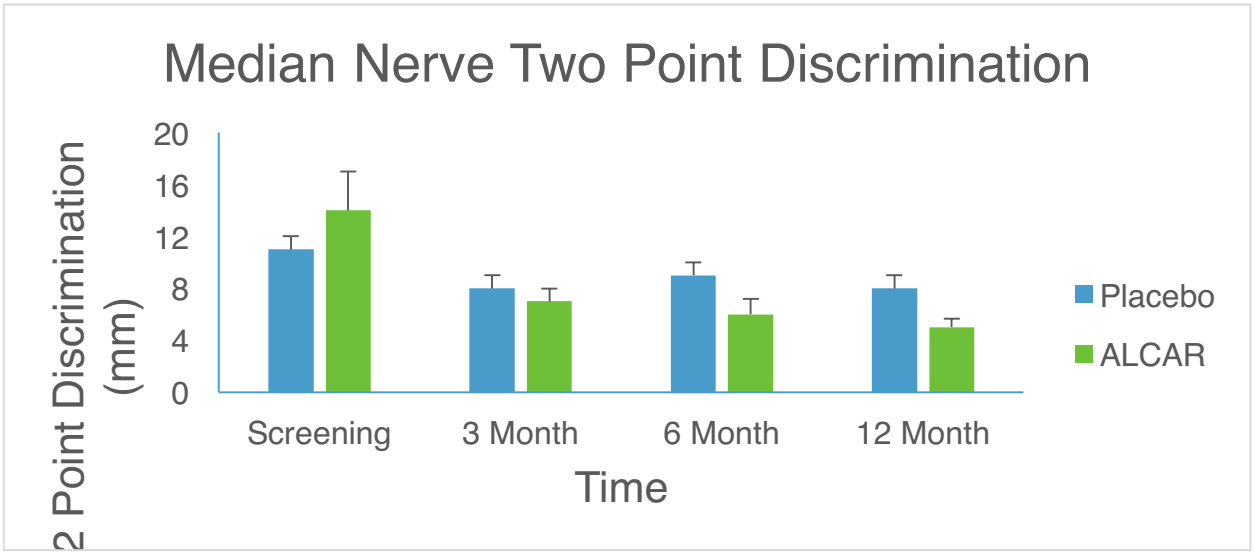
Large fiber sensory nerve function improved over the 12 month follow-up period after CTR. Two-point discrimination improved from baseline to 8 ±4 mm in the placebo group and 5±2 mm in the treatment group. Two-way ANOVA showed that time had a significant effect on 2PD (p=0.02) while treatment type did not (p=0.61). There was no interaction between drug and treatment (p=0.36) [See Figure 3-3]. Similarly, Semmes-Weinstein monofilament test improved in the placebo group from 3.93±0.72 g to 4.70±0.93 g and in the ALCAR group from 3.54±0.34 g to 4.68±0.66 g. Two-way ANOVA showed time had a significant effect on Semmes-Weinstein monofilament test ($p<0.01$) while treatment did not (p=0.21), with no interaction between the two variables (p=0.29) [See Figure 3-4]. These results are in concordance with two-

Outcome	p-value
Sensory conduction velocity	0.63
Distal sensory latency	0.79
SNAP	0.92
Distal motor latency	0.06
CMAP	0.89

Table 3-3: Nerve conduction studies outcomes.

Outcomes of sensory and motor nerve conduction parameters. P-values were deemed significant if < 0.05.

A.



B.

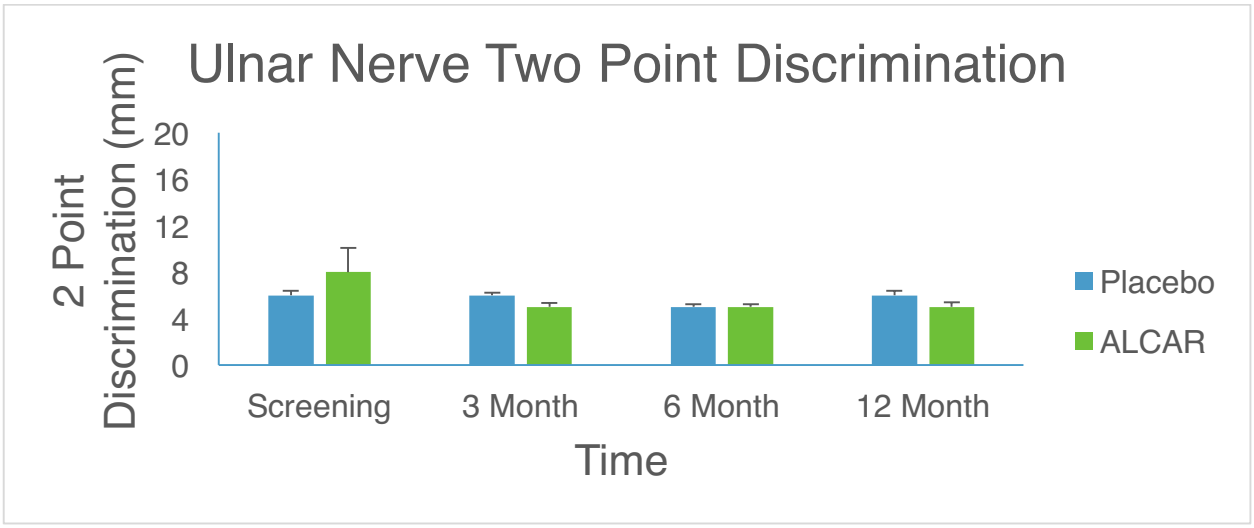
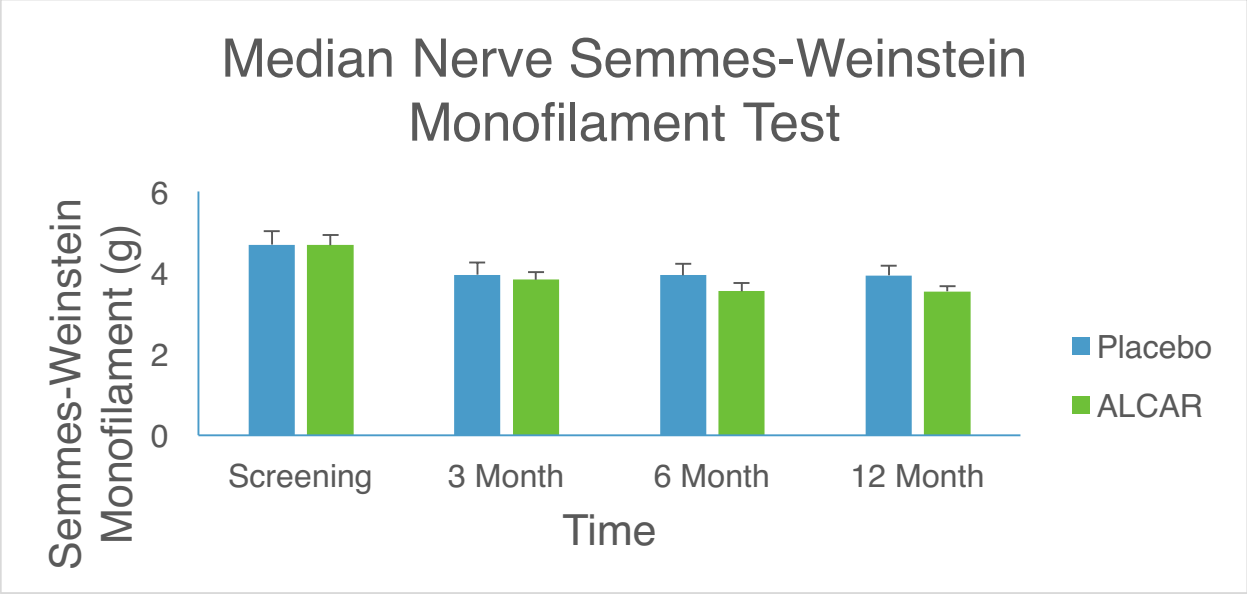


Figure 3-3: Two Point Discrimination

Two point discrimination changes in A.) median innervated 3rd digit and B.) ulnar innervated 5th digit. Two-point discrimination improved with time ($p<0.01$) but treatment had no effect ($p=0.61$). There was no interaction between each variable ($p=0.36$).

A.



B.

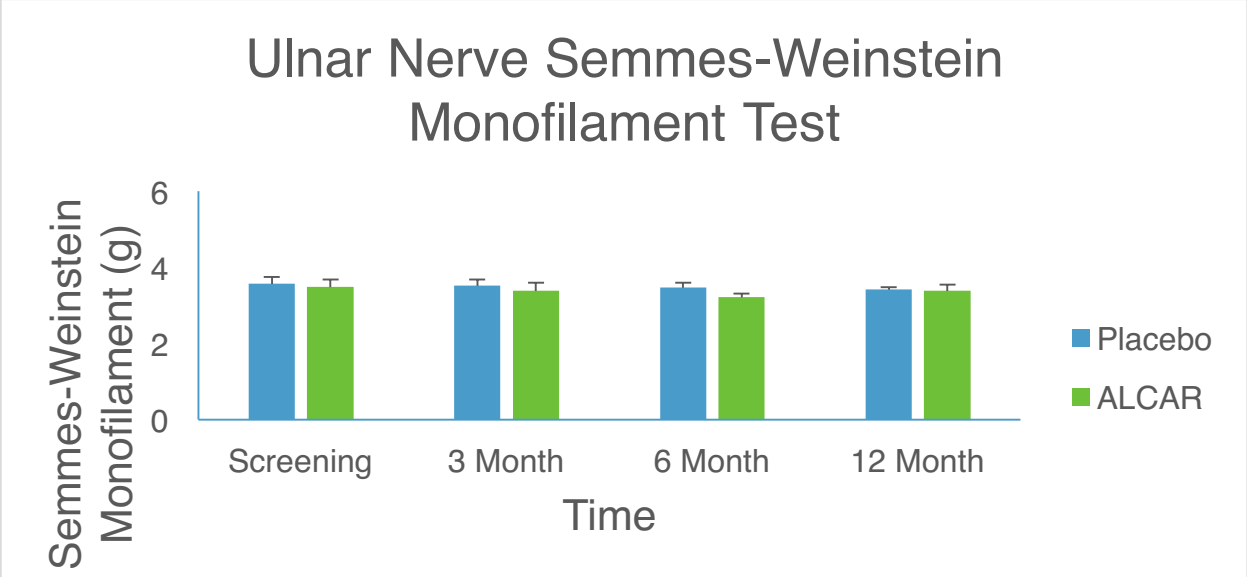


Figure 3-4: Semmes Weinstein Monofilament Test

Semmes-Weinstein monofilament changes in A.) median innervated 3rd digit and B.) the ulnar innervated 5th digit. SWMT improved with time ($p<0.01$) but treatment had no effect ($p=0.22$). There was no interaction between both variables ($p=0.83$)

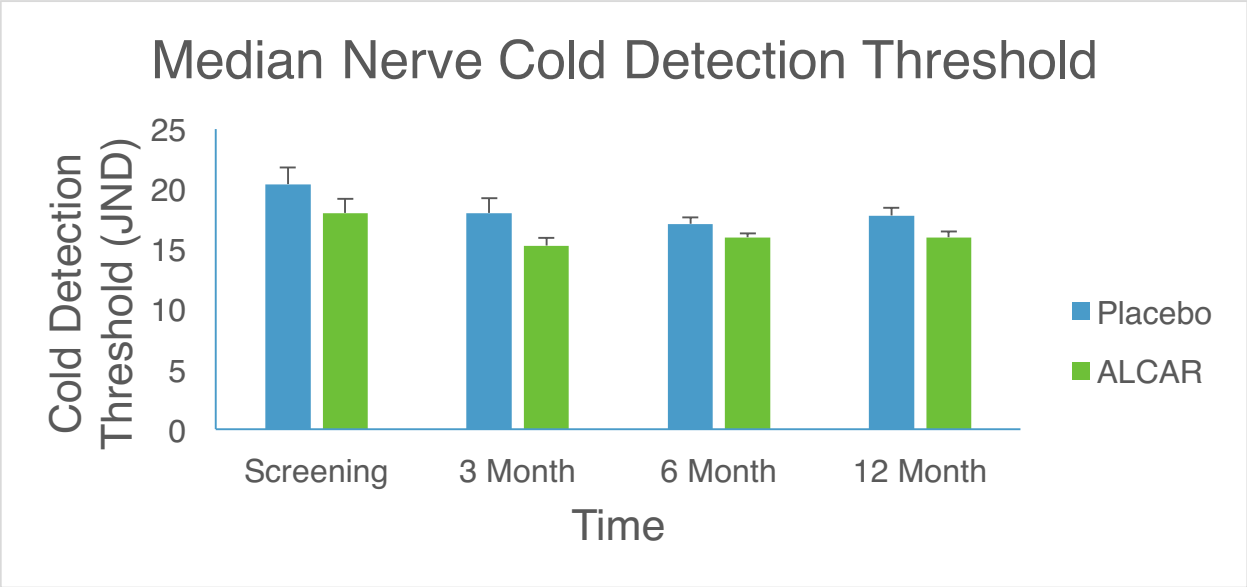
point discrimination indicating that large fiber sensory nerve fiber improves over time after CTR but treatment with ALCAR does not improve large fiber sensory nerve regeneration.

Small Fiber Sensory Nerve Function

Cold detection threshold was determined using a Case IV system with Dyck's 4, 2, 1 algorithm. The ALCAR group showed an improvement in cold detection threshold from 20.4±3.9 JND at baseline to 17.8±1.9 JND while placebo improved from 18±3.5 JND to 18±3.5 JND respectively. Two-way ANOVA showed that there was significant improvement in both groups ($p<0.01$) over time ($p=0.02$) (See Figure 3-5). However, there was no interaction between the two groups ($p=0.84$) indicating that the type of treatment did not have a significant influence. When the analysis for the control data was examined a significant main effect is found for the ulnar control digit for the treatment group ($p<0.01$) with no interaction with time ($p=0.91$). It is possible that cold detection threshold improved over time with the decompression. Cold detection threshold is an automated psychophysical test, so the possibility of learning over subsequent follow-ups could explain the improvement in time. Learning does not explain the significant relationship found between the treatment groups.

A potential explanation for this finding can be found when Figure 3-5 is examined. Both the median innervated 3rd digit and the ulnar innervated 5th digit are lower for the treatment group than the placebo group. While this did not reach statistical significance at baseline, the additional time points incorporated into the ANOVA could have increased the sample size to a point where statistical significance could be met. The effect seen by ALCAR on cold detection result is likely a spurious result given there was no interaction between the two variable nor an additive effect seen between the two variables.

A.



B.

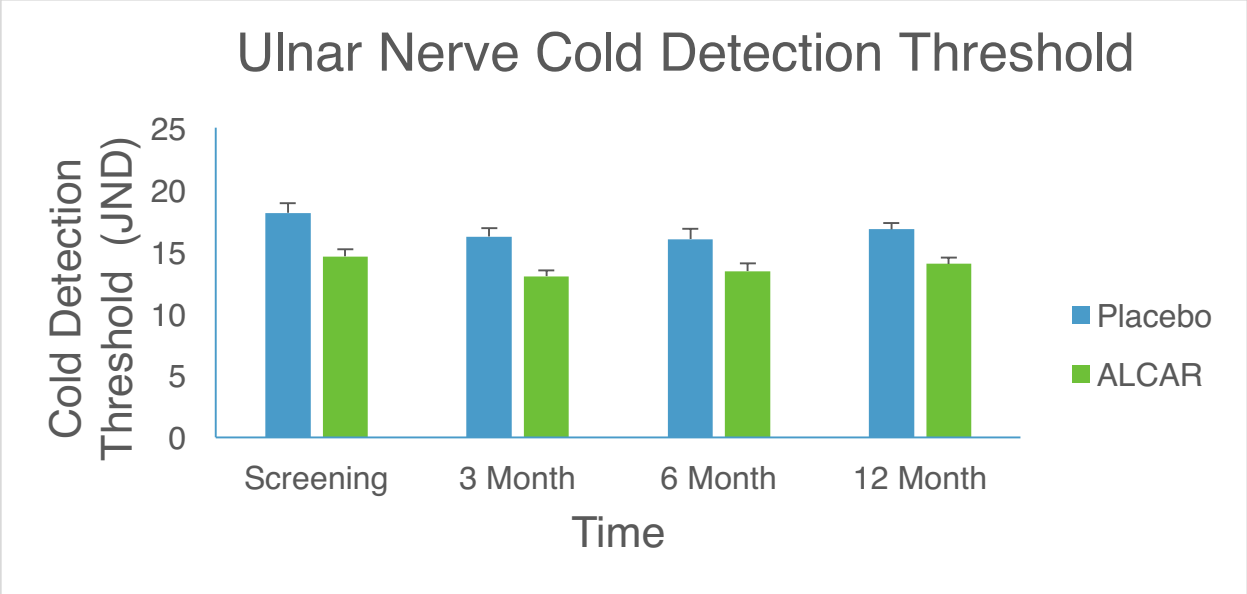


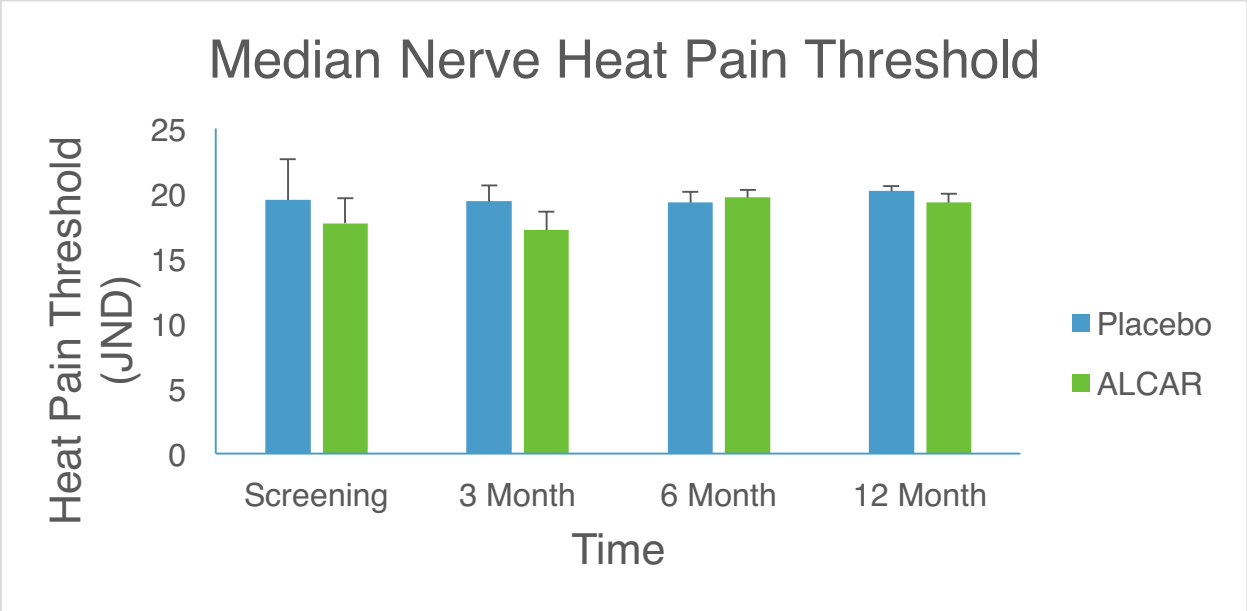
Figure 3-5: Cold detection threshold

Cold detection threshold results for A.) median innervated 3rd digit and B). ulnar innervated 5th digit.

Heat-pain threshold was also determined by the CASE IV system using the nonrepeating algorithm described by Dyck *et al*. The method uses a modified VAS scale where the patient choses an answer on a scale of 0-10 where 0 is a non-painful stimulus, 1 the minimal painful threshold and 10 the maximal painful threshold. The point between 0 and 1 is defined as the heat detection threshold or HP 0.5. This point was used for analysis. There was no effect of time ($p=0.78$) or treatment ($p=0.33$) on HP scores without a significant interaction between the two ($p=0.87$). Examining Figure 3-6 we see that the placebo group remained unchanged over the course of the follow-up while the ALCAR group showed a slight trend towards improvement.

Given the results of the QST tests on return of small nerve fiber function it appears that ALCAR plays no role in the regeneration of small nerve fibers in CTS. While a significant effect was found for CDT a similar result was found in the control digit over the same time. One explanation for these results would be increased intersubject variability through test learning. The remainder of the control data did not display a significant relationship.

A.


Median Nerve Heat Pain Threshold
Heat Pain Threshold (JND)
25
20
15
10
5
0
Screening
3 Month
6 Month
12 Month
Time
Placebo
ALCAR

B.

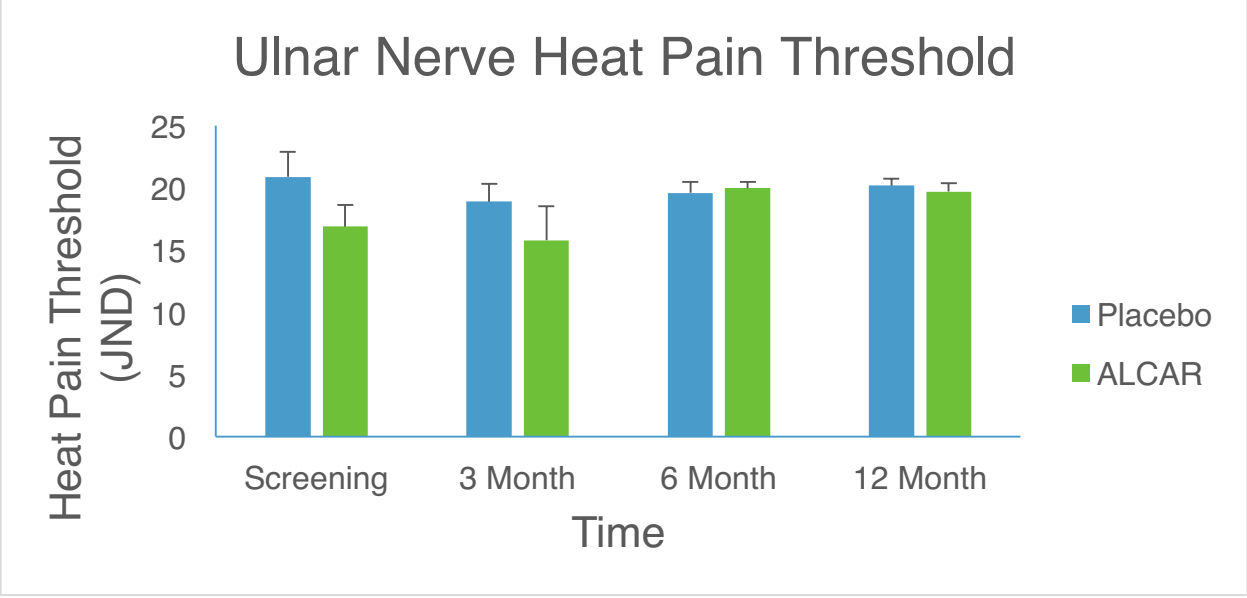

Ulnar Nerve Heat Pain Threshold
Heat Pain Threshold (JND)
25
20
15
10
5
0
Screening
3 Month
6 Month
12 Month
Time
Placebo
ALCAR

Figure 3-6: Heat-Pain detection threshold

Heat-pain detection threshold for A.) median innervated 3rd digit and B.) ulnar innervated 5th digit. There was no significant effect of time (p=0.78) nor treatment (p=0.33) on HP threshold.

Functional Outcomes

Patient reported outcomes are a vital part of patient assessment as it provides the only measure of patients' perception of the effects of treatments. The Disabilities of the Arm, Shoulder and Hand (DASH) and Levine's CTS Questionnaire were used. Due to incomplete answers in the optional sections of the DASH only the main portion of the questionnaire was analyzed.

Both the placebo (20.8±20.1 vs 41.1±18) and ALCAR (13.2±7.6 vs 32.9±17.5) improved over the duration of the study. Two-way ANOVA showed that time had a significant effect of DASH score. Treatment had no effect on DASH score with no interaction between time and treatment (Figure 3-7).

Levine's questionnaire is separated into two parts; the first examining symptom severity and the second examining patients function. Scores are reported as the average score of a 5 point Likert scale and can be combined to give a total score. For the purpose of the analysis mean symptom, function and total scores were all analyzed using two-way ANOVA as shown in Figure 3-6. Analysis revealed that time had a significant effect on all three of the scores (Symptom $p<0.01$; Function $p=0.04$; Total $p<0.01$). However, the treatment the patient received had no effect on score of any of the tests (Symptom $p=0.80$; Function $p=0.75$; Total $p=0.68$).

Figure 3-7: Disabilities of Arm, Shoulder and Hand (DASH) scores

DASH scores for placebo and ALCAR treatment groups. Both groups showed a significant effect from time ($p=0.02$) while treatment showed no effect ($p=0.18$).

There was no interaction between time and treatment or any of the Levine's score (Symptom p=0.73; Function p=0.87; Total p=0.93). Data is summarized in Figure 3-8 and 3-9.

While patient reported outcomes are imported for functional outcomes they do not provide an objective measure of function. To incorporate an objective measure of functional return, the Moberg pick-up test was chosen. Consisting of two parts, the Moberg pick-up test gets the patient to pick up 12 household objects while be timed. This is carried out with and without visual help. Given the sensory disturbances associated with CTS, the Moberg without vision was chosen for analysis. There was no significant effect of time (p=0.41) or treatment (p=0.07) on Moberg times with no significant interaction between the two (p=0.98) [See Figure 3-10].

Safety and Adverse Events

Safety of ALCAR treatment has been well documented previously. To monitor safety in our trial, assessments consisting of history and physical exam were carried out at each visit. There was no difference in vital signs over the course of the trial. In addition, a panel of blood work was completed at baseline, 1 month and 3 months post-operatively. There was no statistically significant change over the course of the trial in any of the blood tests performed. See Table 3-4 for the comprehensive results of the blood work over the trial. A final panel of blood work was completed at the 12 month visit to ensure no long-term effects of treatment. This set of blood work was not included in the analysis due to missing values at the time of thesis preparation.

There were 4 adverse events documented in the trial. Of these 4 events, only 1 occurred in the treatment group. There was no difference in the adverse outcomes between treatment

groups (p=0.47). All adverse events resolved without intervention. See Table 3-5 for breakdown of adverse events.

Serum ALCAR levels were drawn at baseline, 1 months and 2 months. There was no difference found in baseline levels between the two groups (10.26 ± 8.78 μM for placebo; 8.04 ± 3.12 μM; p= 0.54 for ALCAR). There was no significant effects of time or treatment on ALCAR levels (See Figure 3-11 for results). Despite no significant difference between the treatment group and the placebo group examining the change in Figure 3-11 it can be seen that ALCAR group doubled during the treatment phase while placebo only improved slightly, with a large variability. Increasing the sample size may lead to statistical significance for the serum ALCAR levels. However, it remains possible that the patients were underdosed. This could result from lack of patient compliance, which was accounted for by monthly drug counts. It could be an inherent flaw in the study design as well. Further work with the group whom analyzed the serum ALCAR levels is planned.

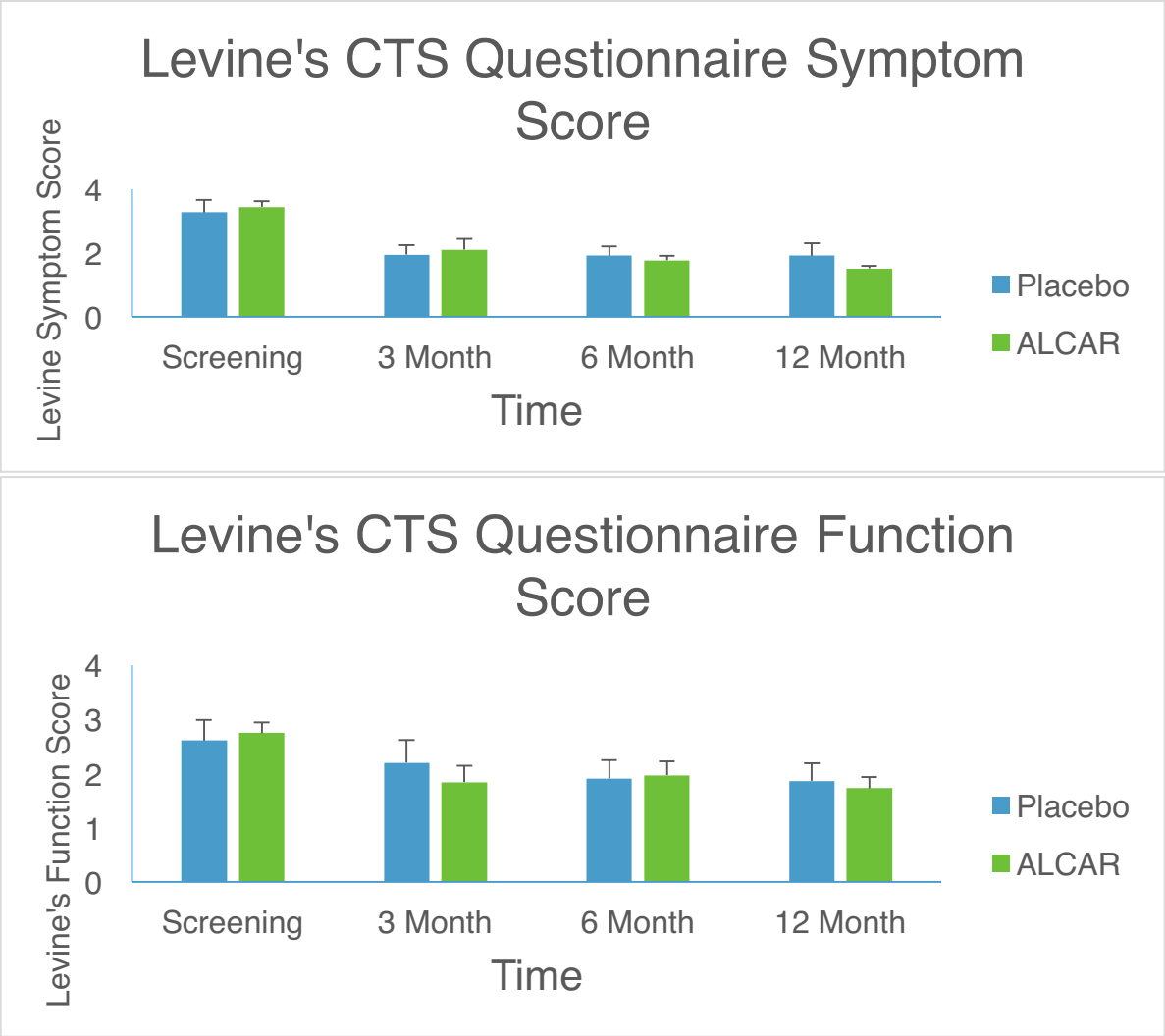


Figure 3-8: Levine symptom and function scores

Levine symptom and function scores. Both ALCAR and placebo groups improved over the course of the study for both scores ($p<0.01$ and $p=0.04$). There was no difference between treatment groups ($p=0.80$ and $p=0.75$).

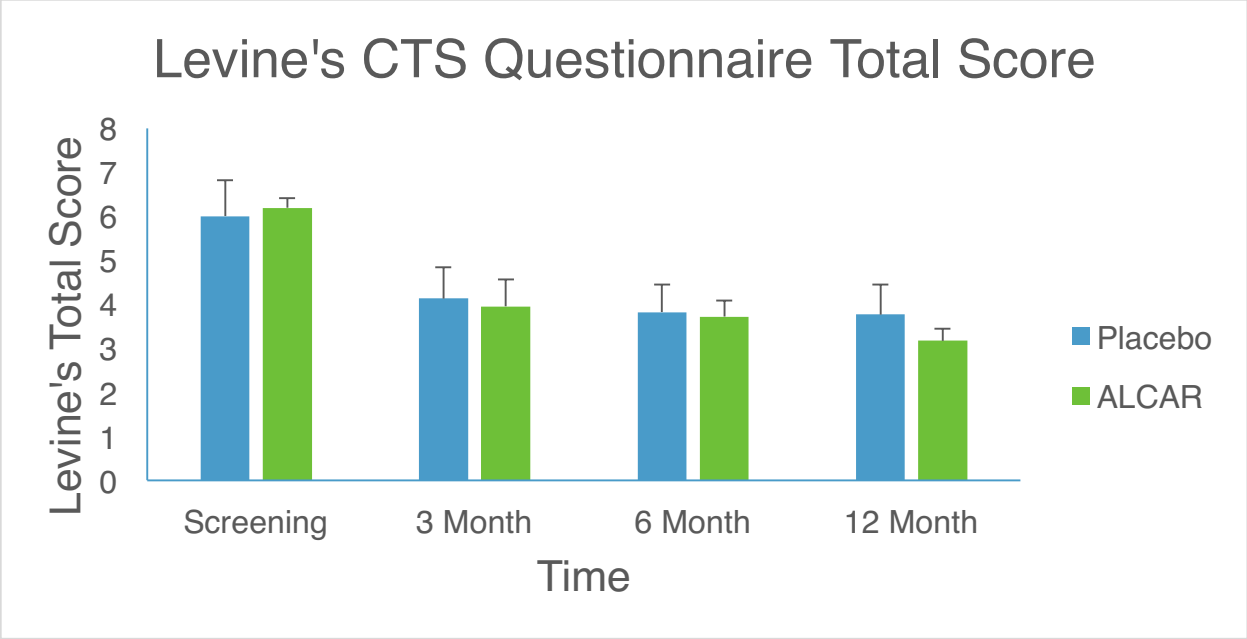
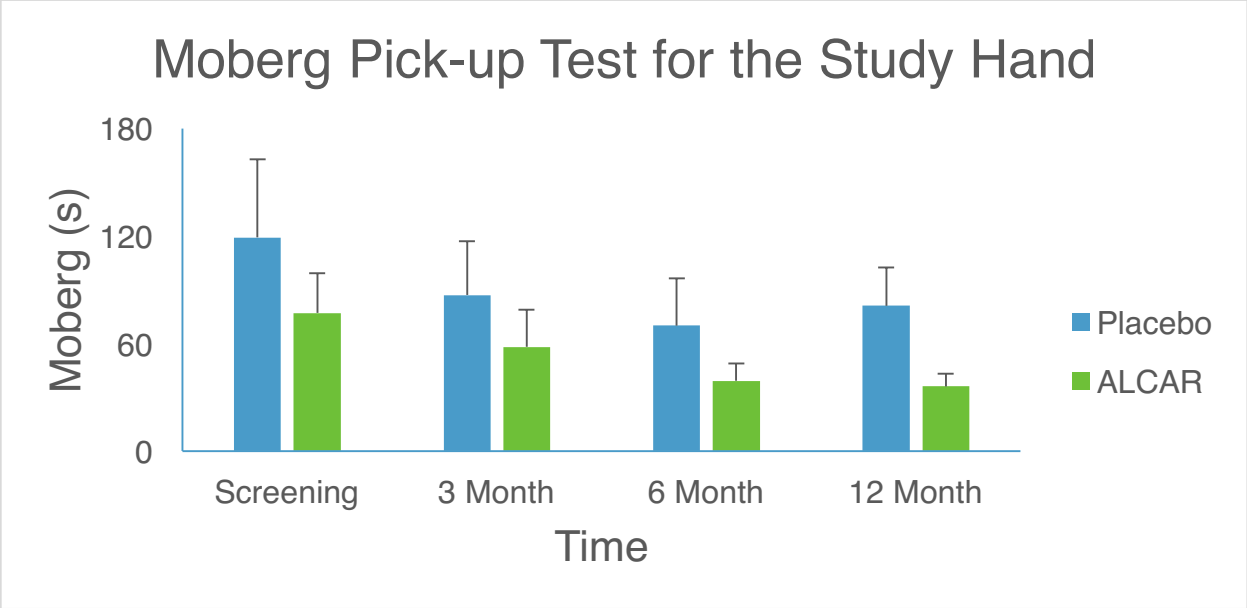


Figure 3-9: Levine total score

Levine total score (symptom + function scores) improved over time ($p<0.01$). There was no difference in the treatment groups ($p=0.93$).

A.



B.

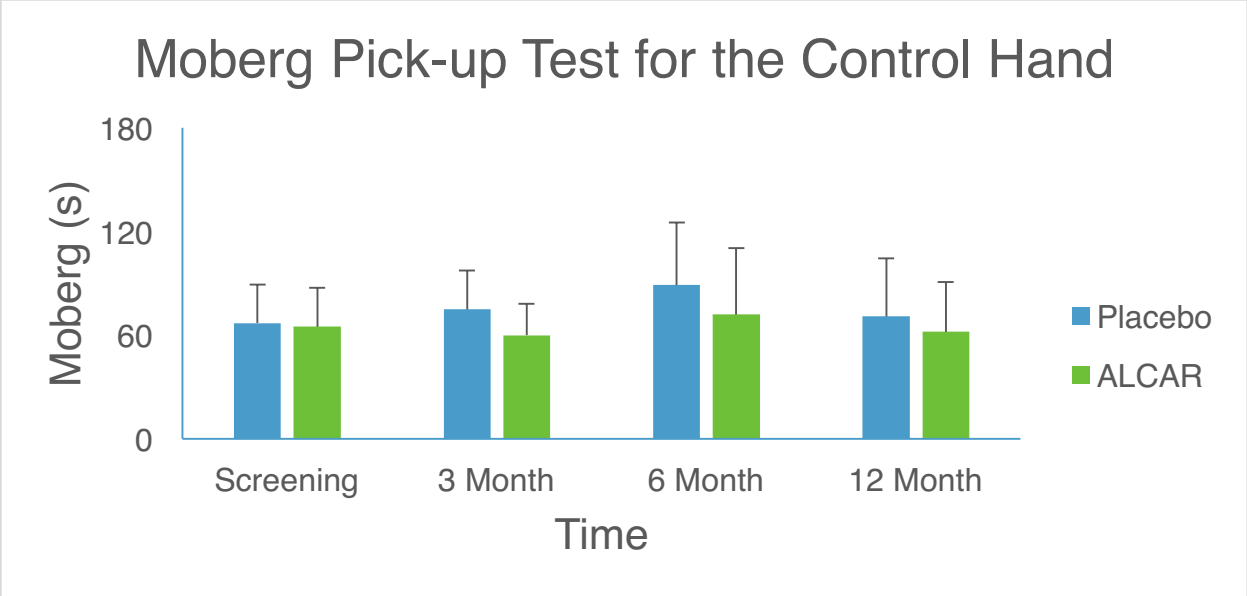


Figure 3-10: Moberg pick-up test

Moberg pick-up test of A.) the study hand and B.) the contralateral control hand. There was no effect of time (p=0.41) or treatment (p=0.07). Lower times represent improved scores.

Blood Test	ALCAR	Placebo	p-value
Hgb (g/L)	143+/- 12.4	139+/-20	0.80
Plt (10**9/L)	238+/-54	262+/-69	0.87
WBC (10**9/L)	7.1+/-2.4	6.6+/-2.1	0.05
Alkaline Phosphatase (U/L)	83+/-14	90+/-16	0.92
Alanine Aminotransferase (U/L)	32+/-25	28+/-17	0.77
Aspartate Aminotransferase (U/L)	26+/-12	28+/-11	0.93
Total Bilirubin (μmol/L)	10+/-6	9+/-2	0.06
Creatinine (μmol/L)	66+/-15	82 +/-20	0.19
Urea (mmol/L)	5.2+/-1.0	6.3+/-1.7	0.25
Sodium (mmol/L)	139 +/-3	140+/-2	0.79
Potassium (mmol/L)	4.3+/-0.42	4.2+/-0.64	0.14
Chloride (mmol/L)	102+/-3	105+/-3	0.09
Total CO2 (mmol/L)	28+/-3	26+/-4	0.15

Table 3-4: Laboratory values of study patients

Lab values for both treatment arms. There was no significant difference in either groups at α = 0.05. Values are represented as mean +/- SD.

ALCAR (n=1)	Placebo (n=3)
Nausea	Transverse Process Fracture Distal Radius Fracture Sinusitis

Table 3-5: Adverse events

Breakdown of adverse events for both groups. Only 1 patient in the treatment group had nausea associated with taking the study drug. 3 patients in the placebo group had adverse events; two fractures in two patients resulting from falls and an episode of sinusitis. There was no significant difference between the two group (p=0.47).

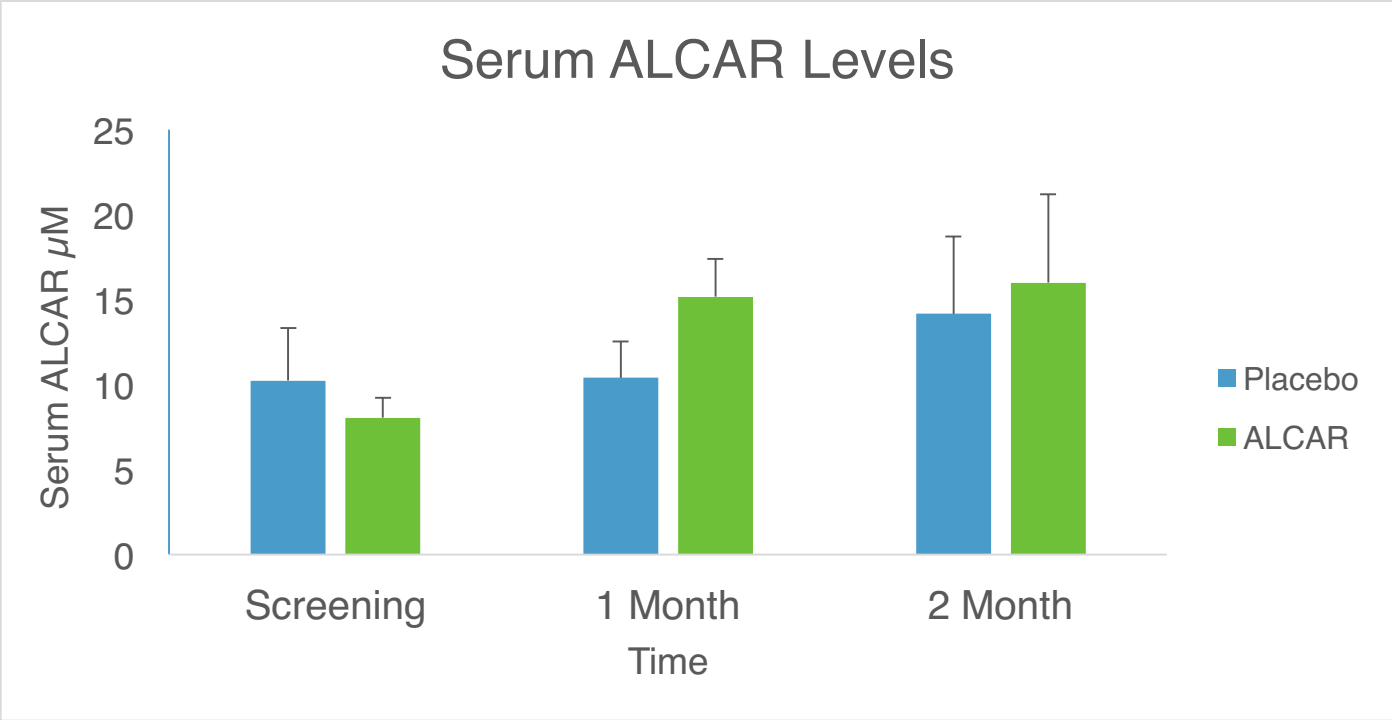


Figure 3-11: Serum ALCAR levels

Serum ALCAR levels as determined by LC-MS/MS. There were no significant changes with time p=0.21) or treatment (p=0.59).

Chapter 4

Discussion of findings in the ALCAR study to enhance peripheral nerve regeneration in carpal tunnel syndrome.

In this double blind randomized controlled trial, we did not find any beneficial effect of ALCAR in enhancing nerve regeneration, symptom or functional recovery. To explore the potential reasons for these findings, we will start with a discussion of the dosage regime used and how it compares with previous studies. Taking the dosage differences into account, we will compare our findings to those reported in the literature to explore potential reasons for the similarities and differences. Finally, the ERK1/2 pathway should be revisited to review its role in nerve regeneration and other potential agents that can target this pathway.

ALCAR Dosing for Neuropathy

A potential explanation of the negative results could be inadequate or incorrect dosing. Unfortunately, systematic evaluation on dose response has been sparse. There has been an animal study that looked at dose response curves in a sciatic nerve transection model (Wilson *et al.*, 2003). However, this study only looked at neuroprotection rather than nerve regeneration as the ends of the sciatic nerve were capped in silastic tubes. They found that high dose of ALCAR at 50 mg/kg/d and 10 mg/kg/d were able to protect against sensory neuronal loss when compared to contralateral controls. Lower doses (≤ 5mg/kg/d) were not able to protect against sensory neuron loss. While this study provided information on the dosage required for neuroprotection, there are some limitations impacting its generalizability. First, since only sensory neurons in the DRGs were evaluated, we cannot draw conclusion about motoneuron survival. Also, since the transected nerve was capped to prevent regeneration, it precludes the ability to infer the dosage effect of ALCAR on nerve regeneration. Further work from this group provides contradictory evidence of ALCAR role in regeneration (Hart *et al.*, 2002; Wilson *et al.*, 2010). Wilson *et.al.* were able to demonstrate improved axon counts in a rat sciatic nerve immediate repair model.

While this indicates ALCARs potential role in axonal outgrowth, the sham surgery was also found to have a higher axon count than normal though this relationship failed to meet statistical significance. In the Hart el.al. study there was no difference in axon regeneration distances nor soleus weights. There was a higher amount of pan axonal neurofilament staining and S100 staining indicating increased numbers of axons and Schwann cells, respectively in the distal stump. Although not directly applicable, this study nevertheless represents the best dose response data available for ALCAR in nerve injury.

In the absence of other dose ranging studies, we will compare the dosage used in our study to trials with positive results. These trials can provide a useful template for dosing regimen. Unfortunately, both animal and human studies have utilized highly variable dosing regimens.

Using animal models, ALCAR has been tested in CIPN (Flatters *et al.*, 2006; Ghirardi *et al.*, 2005)}, DPN (Cotter *et al.*, 1995; Lowitt *et al.*, 1995; Malone *et al.*, 1996; Pop-Busui *et al.*, 2002; Sima *et al.*, 1996; Soneru *et al.*, 1997)}, neuropathic pain(Chiechio *et al.*, 2006; Di Cesare Mannelli *et al.*, 2010), compression neuropathy (Kotil *et al.*, 2007), spinal cord injury (Karalija *et al.*, 2014), and after peripheral nerve repair (Farahpour & Ghayour, 2014; Hart *et al.*, 2002; Kokkalis *et al.*, 2009). Reported dosing concentrations are heterogeneously reported with, the most commonly reported dosing in mg/kg. Dosing ranged from 0.5 mg/kg (Wilson *et al.*, 2003) up to 500 mg/kg (Cotter *et al.*, 1995) with 50 mg/kg or 100 mg/kg the most frequent dosing. These would be the equivalence of 3500 mg/day to 7000 mg/day in a 70 kg person. It is worth noting that the one study on compression neuropathy used a 20 mg/kg/d dosing. This dosing strategy falls within the 10-50 mg/kg dosing Wilson *et.al.* found to be effective for sensory

neuroprotection. While this provides a guide to selecting a dose, the differences in metabolism between animal models and humans may make direct translation difficult.

Human studies tend to report the regime as mg/d dosing with no studies titrating to weight. Similarly, there is a large variability in the dosing ranging from 500 to 3000 mg/d (See Table 4-1 for review). The route and duration also highly varied. Routes cover most of the major forms of drug administration and often are used in combination. Durations range from 10 days (Maestri *et al.*, 2005) to 5.4 years (Herzmann *et al.*, 2005). Extrapolating from the animal studies positive results with the dose response analysis were found at 10mg/kg and 50 mg/kg (Wilson *et al.*, 2003). Given that our average weight for patients was 88±3.6kg; 50 mg/kg dosing would be 4400 mg/d and 10 mg/kg would be 880 mg/d. Our dose of 3000 mg falls well within this range so we would anticipate it to be effective. Unfortunately, we do not have data on doses higher than 50 mg/kg in the Wilson *et.al.* study. It therefore, could be reasoned that the optimal dose is higher than 50 mg/kg meaning our study population would be underdosed.

Indication	Dosing	Route	Duration	Reference
DPN				
Sima *et.al.*	500 mg tid 1000 mg tid	Oral	52 weeks	(Sima *et al.*, 2005)
De Grandis *et.al.*	1000 mg daily 2000 mg daily	IM oral	10 days 355 days	(De Grandis & Minardi, 2002)
Li *et.al.*	500 mg t.i.d.	oral	24 weeks	(S. Li *et al.*, 2015)
CIPN (Preventative)				
Campone *et.al.*	1000 mg q3d	IV	N/A	(Campone *et al.*, 2013)
Hershman *et.al.*	3000 mg daily	oral	24 weeks	(Hershman *et al.*, 2013)
Callander *et.al.*	1500 mg b.i.d	oral		(Callander *et al.*, 2014)
CIPN (Therapeutic)				
Bianchi *et.al.*	1000 mg t.i.d.	oral	8 weeks	(Bianchi *et al.*, 2005)
Sun *et.al.*	1000 mg t.i.d	oral	8 weeks	(Sun *et al.*, 2016)
Maestri *et,al*	1000 mg t.i.d.	IV	10 days	(Maestri *et al.*, 2005)
ARN				
Hart *et.al*	1500 mg b.i.d.	oral	5.4 years	(Hart *et al.*, 2004)
Youle *et.al.*	500 mg b.i.d.	IM oral	14 days 42 days	(Youle & Osio, 2007)
Osio *et.al.*	1000 mg b.i.d	oral	4 weeks	(Osio *et al.*, 2006)
Valcour *et.al.*	1500 mg b.i.d	oral	24 weeks	(Valcour *et al.*, 2009)
Scarpini *et.al.*	500-1000 mg daily	IV/IM	3 weeks	(Scarpini *et al.*, 1997)
Neuropathic Pain				
De Grandis	1000 mg daily 2000 mg daily	IM oral	10 days 355 days	(De Grandis, 1998)

Table 4-1: Overview of ALCAR dosing in previous human studies

ALCAR dose, route and duration used in previous human studies. There is significant variation in all of dose, route and duration. These dosing regimes are compared to the 1000 mg po TID dosing used in the trial.

The dosing regimen used in this study was based on the Bianchi *et.al.* study. Though an uncontrolled cohort study, the trial was able to show improvement in neurophysiologic parameters. Damage in CIPN is limited to the time period the patient is taking chemotherapy. When the chemotherapy is discontinued, the axonal damage ceases and the neuropathy no longer progresses. In CTS, once the TCL is divided the damage to the nerve also presumably ceases. Given the similarities in the non-progressive nature of the neuropathies it was felt that this dosing paradigm should be effective. In addition, the trial used a short duration and an oral dosage which would be more convenient for patients making it an attractive option to ensure drug compliance. We felt this was important as CTR was the main intervention in the study with ALCAR being an adjunct therapy. It was felt that it would be difficult to recruit patients to take one years' worth of drug. Finally, the pharmaceutical company Sigma Tau recommended this dosing regimen based on their previous work in other neuropathies.

A previous pharmokinetics study in HIV patients demonstrated no difference in plasma ALCAR levels over multiple dosing regimens after an induction period (Herzmann *et al.*, 2008). The induction period consisted of 1.5 g in twice daily dosing for three months. Patients where then switched to 1, 2, or 3 g/d dosing. The levels of ALCAR in the serum did not change between these three groups. This study indicates that once this level has been reached variations in dosing should not impact the plasma level of patients. These results indicate that variability with dosing such as inappropriate dose or missed doses should have little effect on the plasma level.

While knowing that variability should not impact the dose level, this does not imply that the level is therapeutic. In one of the CIPN trials serum levels of ALCAR were drawn at the 3 month time point and found serum levels of 7.2 μg/mL which was found to be significantly

higher than placebo (Hershman *et al.*, 2013). However, the trial was a negative study, designed to prevent neuropathy and utilized outcome measures that would not be able to follow nerve regeneration. Cumulatively, this makes the serum level difficult to analyze. The level could be underdosed for the desired effect meaning a serum level higher than 7.2 μg/mL is required. However, this serum level may not represent the ideal level in our study due to the heterogeneity of indications and outcomes.

As a part of our safety analysis serum ALCAR levels were drawn at baseline, 1 month, and 2 months post-operatively. Our analysis failed to show any difference between the treatment group and placebo group. Analyzing the raw data, we see a doubling in the levels of the treatment group whereas the increase in the placebo group was more modest with a high variability. Despite the lack of significance, it does appear there was a larger change in the ALCAR group. When compared to the Hershman trial both the treatment and placebo groups are higher than the reported levels. Given both are negative trials, the dose level would have to be higher than 15 μM for ALCAR to be effective. While a trend towards the treatment increasing the ALCAR levels more than placebo exists, it remains a possibility that the negative results were due to inadequate dosing. If no difference was found between the placebo and the ALCAR groups then the dose may be insufficient. Without an increase in serum ALCAR levels above that found in placebo, there should be no benefit to the ALCAR treatments.

Assessment of outcomes in ALCAR trials

The outcomes of previous ALCAR trials centered mainly around two objectives: neuropathic pain or nerve regeneration. Each should be considered separately as the mechanisms with which ALCAR affects these outcomes are different. As the outcomes of our study was centered on nerve regeneration we will focus on studies aimed at promoting similar outcomes.

To determine neurophysiological recovery many techniques can be used. In animal models the nerve can be harvested and counted through different measures. Nerves can be cut longitudinally to measure the distance axons have grown from a suture repair or crush. Transverse sections of nerves can provide axon counts, axon diameter, g ratio among others. Using retrograde labelling the motor and sensory axons can be labelled and counted in the ventral horn and DRG, respectively. An indirect measure of motor recovery is the preservation of muscle weights. This can be combined with electrophysiological techniques similar to the techniques used in humans to monitor regeneration.

Evidence from animal studies

There has been evidence of improved nerve regeneration with ALCAR in animal models of diabetic neuropathy (Cotter *et al.*, 1995; Lowitt *et al.*, 1995; Sima *et al.*, 1996) and improved sensory recovery in CIPN (Ghirardi *et al.*, 2005). No animal model of antiretroviral neuropathy has been developed to date with all work being completed in humans. In animal models of nerve repair, the results are mixed (Hart *et al.*, 2002; Karsidag *et al.*, 2012; Kostopoulos *et al.*, 2009; Wilson *et al.*, 2010).

In the Kotil *et.al.* study of compression neuropathy, soleus weights were compared between 5 study groups (Kotil *et al.*, 2007): 1) control group; 2) compression group; 3) surgical decompression; 4) surgical decompression + ALCAR; 5) ALCAR with no decompression. ALCAR treatment improved soleus weights when compared to compression and control. ALCAR treatment also improved soleus weights in the no decompression treatment. However, these results should be interpreted with caution. There was no difference between group significance between the compression and decompression groups which may indicate an inadequate animal model. In the study Kotil et.al. use perineural tightening on light microscopy

to determine the presence of compression neuropathy but was not standardized among groups. In addition, soleus weights are an indirect measure of nerve regeneration that can be confounded by multiple variables (Beer *et al.*, 2008). Use of axon counts, myelin thickness, or retrograde labeling would have been a more direct measure of nerve regeneration.

Comparisons with human studies

In human studies, some studies have used electrophysiologic methods to assess nerve regeneration. Skin biopsies are also commonly used to quantify reinnervation in the skin (See Table 4-2 for review of ALCAR outcomes).

|However, in none of the CIPN studies was nerve regeneration used as the primary end point. Instead, they predominantly use toxicity scales as a surrogate measure for ALCARs efficacy, with the NCI-CTC being the most common. This is a modified functional Likert scale where grade 1) asymptomatic; 2) moderate symptoms, limiting instrumental ADL; 3) severe symptoms, limited self ADL; 4) life-threatening illness, requiring urgent intervention and 5) death. These indirect outcomes are neither sensitive nor specific and therefore are not meaningful indicators of nerve regeneration.

Two trials did use nerve conduction studies as a secondary outcome in CIPN. Bianchi *et.al.* demonstrated improvements in SNAP and sensory conduction velocity in the sural and peroneal nerves. However, there was no significant difference in CMAP or motor conduction velocity (Bianchi *et al.*, 2005). More importantly, this is an uncontrolled study, making it difficult to attribute the change to ALCAR alone. The Sun *et.al.* study did utilize a RCT design. They

Indication	Primary Outcome	Secondary Outcomes	Reference
DPN			
Sima *et.al.*	NCS	Skin Biopsies Vibration VAS	(Sima *et al.*, 2005)
De Grandis *et.al.*	NCS	VAS	(De Grandis & Minardi, 2002)
Li *et.al.*	Neuropathy Symptom Score	NCS	(S. Li *et al.*, 2015)
CIPN (Preventative)			
Campone *et.al.*	Incidence of CIPN	N/A	(Campone *et al.*, 2013)
Hershman *et.al.*	FACT-Ta	FACIT Neurotoxicity	(Hershman *et al.*, 2013)
Callander *et.al.*	FACT-GOG-NTX	FACIT-Fatigue Neuropathic Pain Index Grooved Pegboard	(Callander *et al.*, 2014)
CIPN (Therapeutic)			
Bianchi *et.al.*	NCI-CTC	NCS TNS	(Bianchi *et al.*, 2005)
Sun *et.al.*	NCI-CTC	NCS	(Sun *et al.*, 2016)
Maestri *et,al*	WHO Grade	N/A	(Maestri *et al.*, 2005)
ARN			
Hart *et.al*	Small Nerve Fibre Number	N/A	(Hart *et al.*, 2004)
Youle *et.al.*	VAS	Total Symptom Score McGill Pain Questionnaire	(Youle & Osio, 2007)
Osio *et.al.*	McGill Pain Questionnaire	NCS	(Osio *et al.*, 2006)
Valcour *et.al.*	Intraepidermal Nerve Fibres	Mitochondrial DNA Gracely Pain Score	(Valcour *et al.*, 2009)
Scarpini *et.al.*	Huskissons Analogic Scale	N/A	(Scarpini *et al.*, 1997)

Table 4-2: Comparison of outcomes used to assess efficacy of ALCAR

Review of the primary and secondary outcomes used to examine ALCAR efficacy. There outcomes used are heterogenous in nature but focus on analgesic effects and nerve regeneration. NCS = Nerve Conduction Studies; VAS = Visual Analogue Scale; FACT-Ta = Functional Assessment of Cancer Therapy/Taxanes; FACT-GOG-NTX = Functional Assessment of Cancer Therapy/Gynecologic Oncology

Group/Neurotoxicity; NCI-CTC = National Cancer Institute Common Terminology Criteria; WHO = World Health Organization; FACIT = Functional Assessment of Chronic Illness Therapy; TNS = Total Neuropathy Score

showed a significant difference in sensory nerve conduction velocity in the ALCAR and placebo group. Though the trial grouped the CV into a "changed group" or invalid "unchanged group" to calculate the significance as binomial data. This grouping placed any patients with an improvement in CV in the changed group and anyone that remained in the unchanged group, while it is unclear how any patient that worsened was dealt with. While we can say that more patients had a changed CV, this is misrepresentation of the data. The CV should be presented as raw data calculating the means. This allows for a numerical comparison of improvement. The clinical significance of a "changed" CV change can be a significant improvement or a clinically insignificant change. While both studies were able to show improvement in sensory function, each have limitations. Both studies do not support motor nerve regeneration.

Conflicting results are found in analysis of the use of ALCAR in diabetic neuropathy. Sima *et.al.* was unable to find any improvement in NCS, though did find increased fiber numbers on biopsy in the North American cohort (Sima *et al.*, 2005). The second large RCT was able to show improvement in both CV and amplitude for motor and sensory nerves (De Grandis & Minardi, 2002). This demonstrates the ability of ALCAR to promote both axonal regeneration and remyelination. It is also the first study to demonstrate ALCAR can promote motoneuron regeneration. The final trial examining ALCAR in DPN again found improvement in CV and amplitude of sensory and motor nerves (S. Li *et al.*, 2015). This trial lacked a control though so the results, while supportive of the role of ALCAR in nerve regeneration should be taken with caution.

No study on ARN used electrophysiology as an outcome. However, two studies did take biopsies from patients to look at reinnervation. Hart *et.al.* found increased small nerve fibre

reinnervation in the dermis after treatment with ALCAR (Hart *et al.*, 2004). However, a second study was not able to show that ALCAR increased intraepidermal nerve fibers (Valcour *et al.*, 2009).

Taken together, there is some evidence that ALCAR increase reinnervation for sensory nerves. However, evidence for its ability to promote motor regeneration has been less consistent. Also, the measure of CMAP/SNAP amplitude and conduction velocity are relatively indirect measures. CMAP can overestimate the amount of regeneration due to the presence of enlarged motor units from collateral sprouting. SNAP amplitude is a poor reflection of nerve regeneration as it can be affected by temporal dispersion and phase cancellation.

ERK 1/2 Pathway and Alternative Agents for Future Studies

The ERK 1/2 signalling is the downstream mediator of NGF binding to TrkA and other growth factor - Trk interactions. Its role in nerve regeneration has been reviewed elsewhere (Kaplan & Miller, 2000; Skaper, 2008). Briefly, ERK 1/2 has been shown to play a role in neuronal survival (Bonni *et al.*, 1999) and neurite outgrowth (Tsuda *et al.*, 2011) through CREB and Bcl-2 (Riccio *et al.*, 1999).

Multiple therapeutic agents have been found to act through the ERK1/2 pathway. These include geldanamycin (Sun *et al.*, 2012), methylcobalamin (Liao *et al.*, 2010; Okada *et al.*, 2010), liraglutide (M. Li *et al.*, 2015) among others (Liu *et al.*, 2015). All have been shown to upregulate ERK ½ activity. However, the one that has been studied the most is the immunosuppressant tacrolimus (FK506).

Tacrolimus (FK506)

Initially isolated from a Japanese soil sample in 1984, tacrolimus has become one of the standard immunosuppressants used in solid organ transplantation (Starzl *et al.*, 1989). Shortly after its use as an immunosuppressant was elucidated, interest shifted to its properties in the nervous system. Interest stemmed from co-localization of calcinurein and FK506 binding protein (FKBP), the family of chaperone proteins that are the target of tacrolimus, to neural tissues (Liu & Storm, 1989). In addition, increases in mRNA of FKBP with regenerating nerves appear to be temporally related to increases in GAP-43, a regeneration associated gene (Lyons *et al.*, 1994; Lyons *et al.*, 1992). Two early groups demonstrated that tacrolimus enhanced neurite outgrowth. Lyons *et al.* showed that tacrolimus enhanced neurite outgrowth in the PC12 cell line (Lyons *et al.*, 1994). Gold *et al.* demonstrated similar results *in vivo*, showing improved toe spread in a rat sciatic nerve crush model (Gold *et al.*, 1994). These behavioural improvements were supported by further work that found an increased rate and distance of regeneration in the same rat crush model (Gold *et al.*, 1995). These initial findings were later applied to various techniques of nerve repair (Chen *et al.*, 2009; Konofaos *et al.*, 2010; Navarro *et al.*, 2001; Snyder *et al.*, 2006).

The mechanisms for tacrolimus and other calcineurin inhibitors such as cyclosporine are carried out through a group of chaperone proteins known as the immunophillins. Tacrolimus effects are mediated through the immunophilin FKBP family. Tacrolimus' immunosuppressive effect acts through the FKBP-12 and its interaction with calcineurin in T-cells (Liu *et al.*, 1992). It appears that tacrolimus nerve pro-regenerative effects act through a separate mechanism from its immunosuppressive actions. In an FKBP-12 knockdown mouse, tacrolimus was able to

increase neurite outgrowth in human neuroblastoma cells (Gold *et al.*, 1999). This action was completely blocked by an antibody to FKBP-52 another member of the FKBP family. Additional work in the facial nerve supports this mechanism (Yeh *et al.*, 2007). These results indicate that the neurotrophic effects of tacrolimus are mediated through FKBP-52 rather than the immunosuppressive FKBP-12. FKBP-52 forms the mature steroid receptor complex along with HSP-90 and p23. The dissociation of p23 from this complex appears to be an important step in the signalling cascade (Gold *et al.*, 1999). Both Price *et al.* and Gold *et al.* demonstrated that this complex activates the ERK1/2 pathway to promote neurite outgrowth (Gold & Zhong, 2004; Price *et al.*, 2003).

A major hurdle to overcome the use of tacrolimus for nerve regeneration in clinical work are its side effects. Major side effects from tacrolimus including nephrotoxicity, hyperglycemia and immunosuppression (Scheenberger *et al.*, 2013). While the immunosuppression is critical for transplantation, the risk of opportunistic infection would be high. Ethically, it may be difficult to justify subjecting nerve injury patients to these potential side effects.

One strategy to limit the amount of toxicity from tacrolimus would be to utilize lower concentrations of tacrolimus. Toxicity from tacrolimus occurs in a dose dependent manner. Wang *et al.* found that maximal nerve regeneration occurred at 5 mg/kg (Wang *et al.*, 1997). However, a study by Yang *et al.* showed that tacrolimus could promote nerve regeneration at doses lower than required for immunosuppression (Yang *et al.*, 2003). Other studies have found that low-dose tacrolimus could also be used to successfully increase nerve regeneration (Brenner *et al.*, 2004; Sulaiman *et al.*, 2002)}. Udina *et al.* found a bimodal distribution to doses that promoted nerve regeneration with doses as low as 0.2 mg/kg (Udina *et al.*, 2002). Utilizing sub-

immunosuppression doses could potentially represent one strategy to minimize the risks of side effects.

A second strategy focuses on administration of the drug locally. Local administration prevents the systemic effects of the tacrolimus. This may be achieved in the form of chitosan conduit (Zhao *et al.*, 2014), PLGA nerve guide (Labroo *et al.*, 2016), and PLGA microparticles (Tajdaran *et al.*, 2015). Although promising, these systems need further studies before being deemed safe and appropriate for clinical applications.

A final option would be to use therapeutic agents that are similar to tacrolimus, but without immunosuppressive activity. A small molecule, FK1706 may represent one potential solution. FK1706 binds to both FKBP-12 and FKBP-52 but has less immunosuppressive effects than tacrolimus, while still has potent neuroregenerative effects. It has been used in spinal cord hemi transection injuries, resulting in increased motor axonal growth in the red nucleus (Yamaji *et al.*, 2008).

To date only one pilot study of 6 patients has looked at the effects of tacrolimus on nerve regeneration in humans (Phan & Schuind, 2012). Tacrolimus was given systematically within 7 days of repair. The study included both nerve graft and nerve repair making the study heterogeneous. Outcome measures used include the Tinel's sign, CV, EMG, DASH, and two-point discrimination. There was no improvement in any outcome after 40 months of follow up. Patients tolerated the tacrolimus well with no undue side-effects, though one patient did drop out due to non-compliance.

Often quoted to support the use of tacrolimus for nerve regeneration are the unexpectedly good functional outcomes in hand transplant literature. Functional outcomes are based on the Carroll score, DASH and the Hand Transplant Score System (Bernardon *et al.*, 2015; Shores *et*

al., 2017; Singh *et al.*, 2016). While these scores have shown improvement, they are indirect measures of nerve regeneration. A French group did look at two-point discrimination and Semmes-Weinstein monofilament showing return of protective sensation in all patients (Bernardon *et al.*, 2015). Despite this, much more detailed analysis of nerve regeneration is required in hand transplantation patients, though growing evidence in animal models is supportive (Yan *et al.*, 2016). Without that, its use in compression neuropathy such as carpal tunnel syndrome would be hard to justify.

Limitations and Futures

Limitations

The dosing of serum ALCAR represents a limitation of the study. While the trial was formed using the best available data to determine the difference between study group and placebo group. Without a dose response curve in humans it would be difficult to extrapolate the required dose from regeneration. The dose response curve would also have to be aimed at morot recovery to be able to be applicable to use for a trial.

The use of MUNE as the primary outcome for the study may be considered a limitation. Though our lab has previously demonstrated success with MUNE as a primary outcome (Gordon *et al.*, 2010), other outcomes could have been utilized. Patients with CTS predominately complain of sensory symptoms which stands to reason that a measure of sensory recovery should be the primary outcome. The main issue with having a sensory measure as the primary outcome becomes which indicator to use. No singular measure represents a quantifiable that reflects all types of nerve fibres. A potential measure that can be used to measure sensory recovery is skin biopsies. The ingrowth of either intradermal or epidermal fibres can be used to track

regeneration. The downside of this measure revolves around the invasive nature of the sampling and the requirement for multiple samples.

NCS indexes such as distal motor latency and conduction velocity could have been used to determine recovery. Both of these parameters are indicators of myelination which represents the primary pathology of compression neuropathy. Amplitudes have also been used in previous studies, however issue of collateral sprouting may limit the amount of CMAP to demonstrate motor recovery.

The low number of patients included in this study represents another limitation of the study. Given the exploratory nature of the study design, the possibility of demonstrating non-significant relationships was considered. Therefore, trends in difference of effect would be the important parameter to be considered, as this form the foundation of a sample calculation for a well powered RCT. Despite this consideration, the small sample size persists as a limitation of the study to demonstrate a significant difference between the two groups.

Futures

The aim of the study was to determine an effect size to facilitate a power calculation to conduct a full-scale trial. Unfortunately, there was no difference in the primary outcome thus preventing us to calculate the sample size required to power the study. This may stem from the inability to find a difference in serum ALCAR levels between the two groups. Further work will be re-analyzing the previous samples to see if any methodological errors were present in sample size. If no errors were present then dose response curves would be required to guide whether future work is warranted.

Conclusions

Acetyl-L-carnitine was not able to improve motor or sensory nerve regeneration in carpal tunnel syndrome nor did it improve functional outcomes. It appears that ALCAR is unable to increase regeneration after decompression in median nerve compression neuropathy. These findings add to a body of literature that is quite varied about the role of ALCAR in peripheral nerve regeneration. In a critical analysis of this literature it appears that ALCARs effects on peripheral nerve regeneration is limited. Other agents acting through the ERK1/2 pathway offer more potential as therapeutic targets for peripheral nerve regeneration.

Bibliography

Agee, J. M., McCarroll, H. R. J., Tortosa, R. D., Berry, D. A., Szabo, R. M., & Peimer, C. A. (1992). Endoscopic release of the carpal tunnel: a randomized prospective multicenter study. *J Hand Surg Am, 17*, 987-995.

Ahn, D. S., Yoon, E. S., Koo, S. H., & Park, S. H. (2000). A prospective study of the anatomical variations of the median nerve in the carpal tunnel in Asians. *Ann Plast Surg, 44*, 282-287.

Al Majed, A. A., Neumann, C. M., Brushart, T. M., & Gordon, T. (2000). Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. *J Neurosci, 20*, 2602-2608.

American Association of Electrodiagnostic Medicine, A. A. o. N., American Academy of Physical, M., & Rehabilitation. (2002). Practice parameter for electrodiagnostic studies in carpal tunnel syndrome: summary statement. *Muscle Nerve, 25*, 918-922.

Amirjani, N., Ashworth, N. L., Olson, J. L., Morhart, M., & Chan, K. M. (2011a). Discriminative validity and test-retest relaibility of the Dellon-modified Moberg pick up test in carpal tunnel syndrome. *J Peripher Nerv Syst, 223*, 192-202.

Amirjani, N., Ashworth, N. L., Olson, J. L., Morhart, M., & Chan, K. M. (2011b). Validity and reliability of the Purdue Pegboard test in carpal tunnel syndrome. *Muscle Nerve, 43*, 171-177.

Angelucci, L., Ramacci, M. T., Taglialatela, G., Hulsebosch, C., Morgan, B., Werrbach-Perez, K., & Perez-Polo, J. R. (1988). Nerve growth factor binding in aged rat central nervous system: Effect of acetly-l-carnitine *J Neurol Sci, 20*, 491-496.

Argyriou, A. A., Bruna, J., Marmiroli, P., & Cavaletti, G. (2012). Chemotherapy-induced peripheral neurotoxicity (CIPN): an update. *Crit Rev Oncol Hematol, 82*, 51-77.

Armstrong, T., Devor, W., Borschel, L., & Contreras, R. (2004). Intracarpal steroid injection is safe and effective for short-term management of carpal tunnel syndrome. *Muscle Nerve, 29*, 82-88.

Arthur-Farraj, P. J., Latouche, M., Wilton, D. K., Quintes, S., Chabrol, E., Banerjee, A., . . . Jessen, K. R. (2012). c-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration. *Neuron, 75*, 633-647.

Ashworth, N. (2010). Carpal Tunnel Syndrome. *Clin Evid, 3*, 1114-1142.

Atroshi, I., Flondell, M., Hofer, M., & Ranstam, J. (2013). Methylprednisolone injections for the carpal tunnel syndrome: A randomized, placebo-controlled trial. *Ann Int Med, 159*, 309-317.

Atroshi, I., Gummesson, C., Johnsson, R., Ornstein, E., Ranstam, J., & Rosén, I. (1999). Prevalence of carpal tunnel syndrome in a general population. *JAMA, 282*, 153-158.

Avellino, A. M., Dailey, A. T., Harlan, J. M., Sharar, S. R., Winn, R. K., McNutt, L. D., & Kliot, M. (2004). Blocking of up-regulated ICAM-1 does not prevent macrophage infiltration during Wallerian degeneration of peripheral nerve. *Exp Neurol, 187*, 430-444.

Badiali de Giorgi, L., Bonvicini, F., Bianchi, D., Bossoni, G., & Laschi, R. (1987). Ultrastructural aspects of ageing rat hippocampus and effects of L-acetyl-carnitine treatment. *Drugs Exp Clin Res, 13*, 185-189.

Bahrami, M. H., Shahreeni, S., & Raeissadat, S. A. (2015). Comparison between the effects of progesterone versus corticosteroid local injections in mild and moderate carpal tunnel syndrome: a randomized clinical trial. *BMC Musculoskelet Disord, 16*, 322.

Barhwal, K., Hota, S. K., Prasad, D., Singh, S. B., & Ilavazhagan, G. (2008). Hypoxia-induced deactivation of NGF-mediated ERK1/2 signaling in hippocampal cells: neuroprotection by acetyl-L-carnitine. *J Neurosci Res, 86*, 2705-2721.

Becker, J., Nora, D. B., Gomes, I., Stringari, F. F., Seitensus, R., Panosso, J. S., & Ehlers, J. A. C. (2002). An evaluation of gender, obestiy, age and diabetes mellitus as risk factors for carpal tunnel syndrome. *Clin Neurophysiol, 113*, 1429-1434.

Beer, G. M., Selfert, B., Schneller, M., Iscru, G., & Schmitz, H. C. (2008). Relevance of muscle weight as a functional parameter for the regeneration of the peroneal nerve in rabbits. *J Reconstr Microsurg, 24*, 11-19.

Bernardon, L., Gazarian, A., Petruzzo, P., Packham, T., Guillot, M., Guigal, V., . . . Badet, L. (2015). Bilateral hand transplantation: Functional benefits assessment in five patients with a mean follow-up of 7.6 years (range 4-13 years). *J Plast Reconstr Aesthet Surg, 68*, 1171-1183.

Bianchi, G., Vitali, G., Caraceni, A., Ravaglia, S., Capri, G., Cundari, S., . . . Gianni, L. (2005). Symptomatic and neurophysiological responses of paclitaxel- or cisplatin-induced neuropathy to oral acetyl-L-carnitine. *Eur J Cancer, 41*, 1746-1750.

Birch, R., Bonney, G., & Wynn Parry, C. B. (1998). *Surgical Disorders of Peripheral Nerves* (3rd ed.). New York: Churchill Livingstone.

Bitar, G., Alexandrides, J., Missirian, R., Sotereanos, D., & Nystrom, A. (2002). Carpal tunnel release in the United States and Sweden: reimbursement patterns, cost of treatment, and return to work. *Plast Reconstr Surg, 109*, 1574-1578.

Bjorkqvist, S. E., Lang, A. H., Punnonen, R., & Rauramo, L. (1977). Carpal tunnel syndrome in ovariectomized women. *Acta Obstet Gynecol Scand, 56*, 127-130.

Bland, J. D., & Rudolfer, S. M. (2003). Clinical surveillance of carpal tunnel syndrome in two areas of the United Kingdom, 1991-2001. *J Neurol Neurosurg Psychiatry, 74*, 1674-1679.

Bongers, F. J. M., Schellevis, F. G., Van Den Bosch, W. J. H. M., & Van Der Zee, J. (2007). Carpal tunnel syndrome in general practice (1987 and 2001): incidence and the role of occupational and non-occupational factors. *Br J Gen Pract, 57*, 36-39.

Boniface, S. J., Morris, I., & Macleod, A. (1994). How does neurophysiological assessment influence the management and outcome of patients with carpal tunnel syndrome? *Br J Rheumatol, 33*, 1169-1170.

Bonni, A., Brunet, A., West, A. E., Datta, S. R., Takasu, M. A., & Greenberg, M. A. (1999). Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. *Science, 286*, 1358-1362.

Boyd, J. G., & Gordon, T. (2003). Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Mol Neurobiol, 27*, 277-324.

Brenner, M. J., Fox, I. K., Kawamura, D. H., Yu, V. M., Lowe, J. B., 3rd, Hunter, D. A., & Mackinnon, S. E. (2004). Delayed nerve repair is associated with diminished neuroenhancement by FK506. *Laryngoscope, 114*, 570-576.

Bromberg, M. B. (2007). Updating motor unit number estimation (MUNE). *Clin Neurophysiol, 118*, 1-8.

Brown, W. F., & Milner-Brown, H. S. (1976). Some electrical properties of motor units and their effects on the methods of estimation of motor unit numbers. *J Neurol Neurosurg Psychiatry, 39*, 249-257.

Busti, A. J., & Kellogg, D. (2015). Homonculus:Somatosensory and somatomotorcortex.

Cafferty, W. B. J., Gardiner, N. J., Das, P., Qiu, J., McMahon, S. B., & Thompson, S. W. N. (2004). Conditioning-injury induced spinal axon regeneration fails in interleukin-6 knockout mice. *J Neurosci, 24*, 4432-4443.

Cai, D., Deng, K., Mellado, W., Lee, J., Ratan, R. R., & Filbin, M. T. (2002). Arginase I and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth, MAG and myelin in vitro. *Neuron, 35*, 711-716.

Callander, N., Markovina, S., Eickhoff, J., Hutson, P., Campbell, T., Hematti, P., . . . Miyamoto, S. (2014). Acetyl-L-carnitine (ALCAR) for the prevention of chemotherapy-induced peripheral neuropathy in patients with relapsed or refractory multiple myeloma treated with bortezomib, doxorubicin and low-dose dexamethasone: a study from the Wisconsin Oncology Network. *Cancer Chemother Pharmacol, 74*, 875-882.

Campone, M., Berton-Rigaud, D., Joly-Lobbedez, F., Baurain, J. F., Rolland, F., Stenzl, A., . . . Pautier, P. (2013). A double-blind, randomized phase II study to evaluate the safety and efficacy of acetyl-L-carnitine in the prevention of sagopilone-induced peripheral neuropathy. *Oncologist, 18*, 1190-1191.

Celik, B., Paker, N., Celik, E. C., Bugdayci, D. S., Ones, K., & Ince, N. (2015). The effects of orthotic intervention on nerve conduction and functional outcome in carpal tunnel syndrome: A prospective follow-up study. *J Hand Ther, 28*, 34-37; quiz 38.

Celiker, R., Arslan, S., & Inanici, F. (2002). Corticosteroid injection vs nonsteroidal antiinflammatory drug and splinting in carpal tunnel syndrome. *Am J Phys Med Rehabil, 81*, 182-186.

Chan, K. M., Gordon, T., Zochodne, D. W., & Power, H. A. (2014). Improving peripheral nerve regeneration: from molecular mechanisms to potential therapeutic targets. *Exp Neurol, 261*, 826-835.

Chang, M. H., Chiang, H. T., Lee, S. S., Ger, L. P., & Lo, Y. K. (1998). Oral drug of choice in carpal tunnel syndrome. *Neurology, 51*, 390-393.

Chen, B., Song, Y., & Liu, Z. (2009). Promotion of nerve regeneration in peripheral nerve by short-course FK506 after end-to-side neurorrhaphy. *J Surg Res, 152*, 303-310.

Chen, S., Andary, M., Buschbacher, R., Del Toro, D., Smith, B., So, Y., . . . Dillingham, T. R. (2016). Electrodiagnostic reference values for upper and lower limb nerve conduction studies in adult populations. *Muscle Nerve, 54*, 371-377.

Cheng, C., Webber, C. A., Wang, J., Xu, Y., Martinez, J. A., Liu, W. Q., . . . Zochodne, D. W. (2008). Activated RHOA and peripheral axon regeneration. *Exp Neurol, 212*, 358-369.

Cheng, C. J., Mackinnon-Patterson, B., Beck, J. L., & Mackinnon, S. E. (2008). Scratch collapse test for the evaluation of carpal and cubital tunnel syndrome. . *J Hand Surg Am, 33A*, 1518-1524.

Chiechio, S., Copani, A., De Petris, L., Morales, M. E., Nicoletti, F., & Gereau, R. W. t. (2006). Transcriptional regulation of metabotropic glutamate receptor 2/3 expression by the NF-kappaB pathway in primary dorsal root ganglia neurons: a possible mechanism for the analgesic effect of L-acetylcarnitine. *Mol Pain, 2*, 20.

Chow, J. C. (1989). Endoscopic release of the carpal tunnel ligament: a new technique for carpal tunnel syndrome. . *Arthroscopy, 6*, 288-296.

Christie, K. J., Webber, C. A., Martinez, J. A., Singh, B., & Zochonde, D. W. (2010). PTEN inhibition to facilitate intrinsic regenerative outgrowth of adult peripheral nerves. *J Neurosci, 30*, 9306-9315.

Clark, D., Amirfeyz, R., Leslie, I., & Bannister, G. (2011). Often atypical? The distribution of sensory disturbance in carpal tunnel syndrome. *Ann R Coll Surg Engl, 93*, 470-473.

Clarke, C., Christensen, C., Curran, M. W., & Chan, K. M. (2017). Assessment of small sensory fiber function across the spectrum of severity in carpal tunnel syndrome patients. *Muscle Nerve*.

Cobb, T., Dalley, B., Posteraro, R., & Lewis, R. (1993). Anatomy of the flexor retinaculum. *J Hand Surg Am, 18*, 91-99.

Coleman, M. P., & Freeman, M. R. (2010). Wallerian degeneration, wld(s), and nmnat. *Ann Rev Neurosci, 33*, 245-267.

Cotter, M. A., Cameron, N. E., Keegan, A., & Dines, K. C. (1995). Effects of acetyl- and proprionyl-l-carnitine on peripheral nerve function and vascular supply in experimental diabetes. *Metabolism, 44*, 1209-1214.

Cranford, C. S., Ho, J. Y., Kalainov, D. M., & Hartigan, B. J. (2007). Carpal Tunnel Syndrome. *J Am Acad Ortho Surg, 15*, 537-548.

D'Arcy, C. A., & McGee, S. (2000). Does this patient have carpal tunnel syndrome? *JAMA, 283*, 3110-3117.

Dahlin, L. B., & McLean, W. G. (1986). Effects of graded experimental compression on slow and fast axonal transport in rabbit vagus nerve. *J Neurol Sci, 72*, 19-30.

Dammers, P. M., Veering, M. M., & Vermeulen, M. (1999). Injection with methylprednisolone proximal to the carpal tunnel: a randomised double blind trial. *BMJ, 319*, 884-886.
De Grandis, D. (1998). Tolerability and efficacy of L-acetylcarnitine in patients with peripheral neuropathies: A short-term, open multicentre study. *Clin Drug Investig, 15*, 73-79.
De Grandis, D., & Minardi, C. (2002). Acetyl-L-carnitine (Levacecarnitine) in the treatment of diabetic neuropathy: A long-term randomised double blinded placebo-control trial. *Drugs R D, 3*, 223-231.
de Krom, M. C. T. F. M., Knipschild, P. G., Kester, A. D. M., Thus, C. T., & Boekkooi Spaans, F. (1992). Carpal Tunnel Syndrome: Prevalence in the general population. *J Clin Epidemiol, 45*, 373-376.
Di Cesare Mannelli, L., Ghelardini, C., Toscano, A., Pacini, A., & Bartolini, A. (2010). The neuropathy-protective agent acetyl-L-carnitine activates protein kinase C-gamma and MAPKs in a rat model of neuropathic pain. *Neuroscience, 165*, 1345-1352.
Duncan, K. H., Lewis, R. C. J., Foreman, K. A., & Nordyke, M. D. (1987). Treatment of carpal tunnel syndrome by members of the American Society for Surgery of the Hand: results of a questionnaire. *J Hand Surg Am, 12*, 384-391.
Durkan, J. (1991). A new diagnostic test for carpal tunnel syndrome. *J Bone Joint Surg, 73-A*, 535-538.
Dyck, P. J., O'Brien, P. C., Kosanke, J. L., Gillen, D. A., & Karnes, J. L. (1993). A 4, 2, and 1 stepping algorithm for quick and accurate estimation of cutaneous sensation threshold. *Neurology, 43*, 1508-1512.
Dyck, P. J., Zimmerman, I. R., Johnson, D. M., Gillen, D., Hokanson, J. L., Karnes, J. L., . . . O'Brien, P. C. (1996). A standard test of heat-pain responses using CASE IV. *J Neurol Sci, 136*, 54-63.
English, J. W., & Gwynne-Jones, D. P. (2015). Incidence of carpal tunnel syndrome requiring surgical decompression: A 10.5 year review of 2309 patients. *J Hand Surg Am, 40*, 2417-2434.
Famularo, G., Moretti, S., Marcellini, S., Trinchieri, V., Tzantzoglou, S., Santini, G., . . . De Simone, C. (1997). ALCAR defieciency in AIDS patients with neurotoxicity on treatment. *AIDS, 11*, 185-190.
Farahpour, M. R., & Ghayour, S. J. (2014). Effect of in situ delivery of acetyl-L-carnitine on peripheral nerve regeneration and functional recovery in transected sciatic nerve in rat. *Int J Surg, 12*, 1409-1415.
Feinstein, P. A. (1993). Endoscopic carpal tunnel release in a community based series. *J Hand Surg Am, 18*, 451-454.
Fisher. B, Gorsche, R., & Leake, P. (2004). Diagnosis, causation, and treatment of carpal tunnel syndrome: an evidence-based assessment.
Flatters, S. J., Xiao, W. H., & Bennett, G. J. (2006). Acetyl-L-carnitine prevents and reduces paclitaxel-induced painful peripheral neuropathy. *Neurosci Lett, 397*, 219-223.
Foley, M., Silverstein, B., & Polissar, N. (2007). The economic burden of carpal tunnel syndrome: long term earnings of CTS claimants in Washington State. *Am J Ind Med, 50*, 155-172.
Fu, S. Y., & Gordon, T. (1997). The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol, 14*, 67-116.
Gaudet, A. D., Popovich, P. G., & Ramer, M. S. (2011). Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *J Neuroinflammation, 8*, 110-123.
Gay, R. E., Amadio, P. C., & Johnson, J. C. (2003). Comparative responsiveness of the Disabilities of the Arm, Shoulder, and Hand, the Carpal Tunnel Questionnaire, and the SF-36 to clinical change after carpal tunnel release. *J Hand Surg Am, 28*, 250-254.
Gerritsen, A. A., H.C., d. V., Scholten, R. J., Bertelsmann, F. W., M.C., d. K., & L.M., B. (2002). Splinting vs surgery in the treatment of carpal tunnel syndrome: a randomized controlled trial. *JAMA, 288*, 1245-1251.

Ghirardi, O., Lo Giudice, P., Pisano, C., Vertechy, M., Bellucci, A., Vesci, L., . . . Carminati, P. (2005). Acetyl-L-carnitine prevents and reverts experimental chronic neurotoxicity induced by oxalaplatin, without altering its antitumor properties. *Anticancer Res, 25*, 2681-2688.

Gold, B. G., Densmore, V., Shou, W., Matzuk, M. M., & Gordon, H. S. (1999). Immunophilin FK506-Binding Protein 52 (Not FK506-Binding Protein 12) Mediates the Neurotrophic Action of FK506. *J Pharmacol Exp Ther, 289*, 1202-1210.

Gold, B. G., Katoh, K., & Storm-Dickerson, T. (1995). The immunosuppressant FK506 increases the rate of axonal regeneration in rat sciatic nerve. *J Neurosci, 15*, 7509-7516.

Gold, B. G., Storm-Dickerson, T., & Austin, D. R. (1994). The immunosuppressant FK506 increases functional recovery and nerve regeneration following peripheral nerve injury. *Restor Neurol Neurosci, 1994*, 287-296.

Gold, B. G., & Zhong, Y. P. (2004). FK506 requires stimulation of the extracellular signal-regulated kinase 1/2 and the steroid receptor chaperone protein p23 for neurite elongation. *Neurosignals, 13*, 122-129.

Gordon, T., Amirjani, N., Edwards, D. C., & Chan, K. M. (2010). Brief post-surgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients. *Exp Neurol, 223*, 192-202.

Govek, E. E., Newey, S. E., & Van, A. L. (2005). The role of the Rho GTPases in neuronal development. *Genes Dev, 19*, 1-49.

Graham, B. (2008). The value added by electrodiagnostic testing in the diagnosis of carpal tunnel syndrome. *J Bone Joint Surg Am, 90*, 2587-2593.

Gray, M., Palispis, W., Popovich, P. G., van Rooijen, N., & Gupta, R. (2007). Macrophage depletion alters the blood-nerve barrier without affecting Schwann cell function after neural injury. . *J Neurosci Res, 85*, 766-777.

Greenslade, J. R., Mehta, R. L., Belward, P., & Warwick, D. J. (2004). DASH and Boston questionnaire assessment of carpal tunnel syndrome outcome: what is the responsiveness of an outcome questionnaire. *J Hand Surg Br, 29*, 159-164.

Gupta, R., & Steward, O. (2003). Chronic nerve compression induces concurrent apoptosis and proliferation of Schwann cells. *J Comp Neurol, 461*, 174-186.

Gupta, R., Truong, L., Bear, D., Chafik, D., Modafferi, E., & Hung, C. T. (2005). Shear stress alters the expression of myelin-associated glycoprotein (MAG) and myelin basic protein (MBP) in Schwann cells. *J Orthop Res, 23*, 1232-1239.

Hart, A., Wiberg, M., & Terenghi, G. (2002). Pharmacological enhancement of peripheral nerve repair in the rat by systemic acetyl-L-carnitine treatment. *Neurosci Lett, 334*, 181-185.

Hart, A., Wilson, A. D., Montovani, C., Smith, C., Johnson, M., Terenghi, G., & Youle, M. (2004). Acetyl-l-carnitine: a pathogenesis based treatment for HIV-associated antiretroviral toxic neuropathy. *AIDS, 18*, 1549-1560.

Heller, L., Ring, H., Costeff, H., & Solzl, P. (1986). Evaluation of Tinel and Phalen signs in the diagnosis of carpal tunnel syndrome. *Eur Neurol, 25*, 40-42.

Hershman, D. L., Unger, J. M., Crew, K. D., Minasian, L. M., Awad, D., Moinpour, C. M., . . . Albain, K. S. (2013). Randomized double-blind placebo-controlled trial of acetyl-L-carnitine for the prevention of taxane-induced neuropathy in women undergoing adjuvant breast cancer therapy. *J Clin Oncol, 31*, 2627-2633.

Herzmann, C., Johnson, M., & Youle, M. (2005). Long-term effect of acetyl-L-carnitine for antiretroviral toxic neuropathy. *HIV Clin Trials, 6*, 344-350.

Herzmann, C., Whiting, S. J., Thomas, M., Byrne, P., Johnson, M., & Youle, M. (2008). Pharmokinietics of acetyl-l-carnitne given a single or multiple doses to HIV-1 infected patients with toxic peripheral polyneuropathy. *The Open Aids J, 2*.

Hudak, P. L., Amadio, P. C., Bombardier, C., & (UECG), a. t. U. E. C. G. (1996). Development of an upper extremity outcome measure: the DASH (disabilities of the arm, shoulder and hand). *Am J Ind Med, 29*, 602-608.

Hui, A. C. F., Wong, S., Leung, C. H., Tung, P., Mok, V., Poon, D., . . . Boet, R. (2005). A randomized controlled trial of surgery vs steroid injection for carpal tunnel syndrome. *Neurology, 64*, 2074-2078.

Jenkins, P. J., Watts, A. C., Duckworth, A. D., & McEachan, J. E. (2012). Socioeconomic deprivation and the epidemiology of carpal tunnel syndrome. *J Hand Surg Eur Vol, 37*, 123-129.

Jerosch-Herold, C., Shepstone, L., Wilson, E. C. F., Dyer, T., & Blake, J. (2014). Clinical course, cost and predictive factors for response to treatment in carpal tunnel syndrome: the PALMS study protocol. . *BMC Musculoskelet Disord, 15*, 35.

Jiang, Q., Jiang, G., Shi, K. Q., Cai, H., Wang, Y. X., & Zheng, M. H. (2013). Oral acetyl-L-carnitine treatment in hepatic encephalopathy: view of evidence-based medicine. *Ann Hepatol, 12*, 803-809.

Joseph, R. (2000). Parietal Lobes: Senior Executive of the Body in Physical and Visual Space. *Neuropsychiatry, Neuropsychology, Clinical Neuroscience*: Academic Press.

Joshi, A. R., Bobylec, I., Zhang, G., Sheikh, K. A., & Lehmann, H. C. (2015). Inhibition of Rho-kinase differentially affects axon regeneration of peripheral motor and sensory nerves. *Exp Neurol, 263*, 28-38.

Kadrie, H. A., Yates, S. A., Milner-Brown, H. S., & Brown, W. F. (1976). Multiple point electrical stimulation of the ulnar and median nerve. *J Neurol Neurosurg Psychiatry, 39*, 973-985.

Kang, H. J., Koh, I. H., Lee, W. Y., Choi, Y. R., & Lee, J. J. (2012). Does carpal tunnel release provide long-term relief in patients with hemodialysis-associated carpal tunnel syndrome? *Clin Orthop Relat Res, 470*, 2561-2565.

Kaplan, D. R., & Miller, F. D. (2000). Neurotrophin signal transduction in the nervous system. . *Curr Opin Neurobiol, 10*, 381-391.

Karadag, O., Kalyoncu, U., Akdogan, A., Karadag, Y. S., Bilgen, S. A., Ozbakir, S., . . . Caluneri, M. (2012). Sonographic assessment of carpal tunnel syndrome in rheumatoid arthritis: prevalence and correlation with disease activity. *Rheumatol Int, 32*, 2313-2319.

Karalija, A., Novikova, L. N., Kingham, P. J., Wiberg, M., & Novikov, L. N. (2014). The effects of N-acetyl-cysteine and acetyl-L-carnitine on neural survival, neuroinflammation and regeneration following spinal cord injury. *Neuroscience, 269*, 143-151.

Karpitskaya, Y., Novak, C. B., & Mackinnon, S. E. (2002). Prevalence of smoking, obesity, diabetes mellitus, and thyroid disease in patients with carpal tunnel syndrome. *Ann Plast Surg, 48*, 269-273.

Karsidag, S., Akcal, A., Sahin, S., Karsidag, S., Kabukcuoglu, F., & Ugurlu, K. (2012). Neurophysiological and morphological responses to treatment with acetyl-L-carnitine in a sciatic nerve injury model: preliminary data. *J Hand Surg Eur Vol, 37*, 529-536.

Katz, J. N., Keller, R. B., Fossel, A. H., Punnett, L., Bessette, L., Simmons, B. P., & Mooney, N. (1997). Predictors of return to work following carpal tunnel syndrome. . *Am J Ind Med, 31*, 85-91.

Katz, J. N., & Stirrat, C. R. (1990). A self-administered hand diagram for the diagnosis of carpal tunnel syndrome. *J Hand Surg Am, 15*, 360-363.

Kokkalis, Z. T., Soucacos, P. N., & Terzis, J. K. (2009). Effect of acetyl-L-carnitine on axonal sprouting following donor nerve injury distal to an end-to-side neurorrhaphy model. *J Reconstr Microsurg, 25*, 483-495.

Konofaos, P., Burns, J., & Terzis, J. K. (2010). Effect of low-dose FK506 after contralateral C7 transfer to the musculocutaneous nerve: a study in rats. *J Reconstr Microsurg, 26*, 225-233.

Kostopoulos, V. K., Davis, C. L., & Terzis, J. K. (2009). Effects of acetylo-L-carnitine in end-to-side neurorrhaphy: a pilot study. *Microsurgery, 29*, 456-463.

Kotil, K., Kirali, M., Eras, M., Bilge, T., & Uzun, H. (2007). Neuroprotective effects of acetyl-l-carnitine in experimental chronic compression neuropathy. A prosepctive randomized and placebo-controlled trial. *Turk Neurosurg, 2*, 67-77.

Kozak, A., Schedibauer, G., Wirth, T., Euler, U., Westermann, C., & Nienhaus, A. (2015). Association between work-related biomechanical risk factors and the occurrence of carpal tunnel syndrome: an overview of systematic reviews and meta-analysis of current research. *BMC Musculoskelet Disord, 16*, 231.

Kronlage, S. C., & Menendez, M. E. (2015). The benefit of carpal tunnel release in patients with electrophysiologically moderate and severe disease. *J Hand Surg Am, 40*, 438-444.

Labroo, P., Ho, S., Sant, H., Shea, J., B.K., G., & Agarwal, J. (2016). Controlled delivery of FK506 to improve nerve regeneration. *Shock, 46*, 154-159.

Lanz, U. (1977). Anatomical variations of the median nerve in the carpal tunnel. *J Hand Surg Am, 1*.

Learmonth, J. R. (1933). The principles of decompression in treatment of certain diseases of peripheral nerves. *Surg Clin N Am, 13*, 905-913.

Leigh, J. P., & Miller, T. R. (1998). Job-related diseases and occupations within a large workers compensation data set. *Am J Ind Med, 33*, 197-211.

Levine, D. W., Simmons, B. P., Koris, M. J., Daltroy, L. H., Hohl, G. G., Fossel, A. H., & Katz, J. N. (1993). A self-administered quesstionnaire for the assessment of severity of symptoms and functional status in carpal tunnel syndrome. *J Bone Joint Surg, 75A*, 1585-1592.

Li, M., Li, S., & Li, Y. (2015). Liraglutide Promotes Cortical Neurite Outgrowth via the MEK-ERK Pathway. *Cell Mol Neurobiol, 35*, 987-993.

Li, S., Chen, X., Li, Q., Du, J., Liu, Z., Peng, Y., . . . Tian, H. (2016). Effects of acetyl-L-carnitine and methylcobalamin for diabetic peripheral neuropathy: A multicenter, randomized, double-blind, controlled trial. *J Diabetes Investig, 7*, 777-785.

Li, S., Li, Q., Li, Y., Li, L., Tian, H., & Sun, X. (2015). Acetyl-L-carnitine in the treatment of peripheral neuropathic pain: a systematic review and meta-analysis of randomized controlled trials. *PLoS One, 10*, e0119479.

Liao, W. C., Chen, J. R., Wang, Y. J., & Tseng, G. F. (2010). Methylcobalamin, but not methylprednisolone or pleiotrophin, accelerates the recovery of rat biceps after ulnar to musculocutaneous nerve transfer. *Neuroscience, 171*, 934-949.

Liss, G. M., Armstrong, C., Kusiak, R. A., & Gailitis, M. M. (1992). Use of provincial health insurance plan billing data to estimate carpal tunnel syndrome morbidity and surgery rates. . *Am J Ind Med, 22*, 395-403.

Liu, J., Albers, M. W., Wandless, T. J., Luan, S., Alberg, D. G., Belshaw, P. J., . . . Schreiber, S. L. (1992). Inhibition of T-cell signaling by immunophilin ligand complexes correlates with loss of calcineurin phosphatase activity. *Biochemistry, 31*, 3896-3901.

Liu, X., Wang, X., & Lu, J. (2015). Tenuifoliside A promotes neurite outgrowth in PC12 cells via the PI3K/AKT and MEK/ERK/CREB signaling pathways. *Mol Med Rep, 12*, 7637-7642.

Liu, Y., & Storm, D. R. (1989). Dephsophorylation of neuromodulation by calcineurin. *J Bio Chem, 264*, 12800-12804.

Lowitt, S., Malone, J. I., Salem, A. F., Korthals, J. K., & Benford, S. (1995). Acetyl-l-carnitine corrects the altered peripheral nerve functionof experimental diabetes. *Metabolism, 44*, 677-680.

Ly-Pen, D., Andreu, J. L., de Blas, G., Sanchez-Olaso, A., & Millan, I. (2005). Surgical decompression versus local steroid injection in carpal tunnel syndrome: a one-year prospective randomized, open controlled clinical trial. *Arthritis Rheum, 52*, 612-619.

Lyons, W. E., George, E. B., Dawson, T. M., Steiner, J. P., & Snyder, S. H. (1994). Immunosuppressant FK506 promotes neurite outgrowth in cultures of PC12 cells and sensory ganglia. *Proc Natl Acad Sci U S A, 91*, 3191-3195.

Lyons, W. E., Steiner, J. P., Dawson, T. M., & Snyder, S. H. (1992). Increased expression of FK-506 binding protein during peripheral nerve regeneration. *Soc Neurosci Abstr, 18*, 604.

Mackinnon, S. E. (2002). Pathophysiology of nerve compression. *Hand Clin, 18*, 231-241.

Mackinnon, S. E., Dellon, A. L., Hudson, A. R., & Hunter, D. A. (1985). A primate model for chronic nerve compression. *J Reconstr Microsurg, 1*, 185-194.

Mackinnon, S. E., Dellon, A. L., Hudson, A. R., & Hunter, D. A. (1986). Chronic human nerve compression—a histological assessment. *Neuropathol Appl Neurobiol, 12*, 547-565.

Mackinnon, S. E., & Dellon, L. (1988). *Surgery of the Peripheral Nerve* (S. E. Mackinnon Ed.). New York: Thieme.

Mackinnon, S. E., McCabe, S., Murray, J. F., Szalai, J. P., Kelly, L., Novak, C., . . . Burke, G. M. (1991). Internal neurolysis fails to improve the results of primary carpal tunnel decompression. *J Hand Surg Am, 16*, 211-218.

Maestri, A., De Pasquale Ceratti, A., Cundari, S., Zanna, C., Cortesi, E., & Crino, L. (2005). A pilot study on the effect of acetyl-L-carnitine in paclitaxel- and cisplatin-induced peripheral neuropathy. *Tumori, 91*, 135-138.

Malone, J. I., Lowitt, S., Salem, A. F., Miranda, C., Korthals, J. K., & Carver, J. (1996). The effects of acetyl-l-carnitine and sorbinil on peripheral nerve structure, chemistry, and funciton in experimental diabetes. *Metabolism, 45*, 902-907.

Manente, G., Torrieri, F., Di Blasio, F., Staniscia, T., Romano, F., & Uncini, A. (2001). An innovative hand brace for carpal tunnel syndrome: a randomized controlled trial. . *Muscle Nerve, 24*, 1020-1025.

Marie, P., & Foix, C. (1913). Atrophie isolée de l'éminence thenar d'origine névritique: role du ligament annulaire antérieur du carpe dans la pathogénie de la lésion. *Rev Neurol, 26*, 647-649.

Mazzoni, I. E., Said, F. A., Aloyz, R., Miller, F. D., & Kaplan, D. (1999). Ras regulates sympathetic neuron survival by suppressing the p53-mediated cell death pathway. *J Neurosci, 19*, 9716-9727.

McComas, A. J., Fawcett, P. R., Campbell, M. J., & Sica, R. E. (1971). Electrophysiological estimation of the number of motor units within the human hands. *J Neurol Neurosurg Psychiatry, 34*, 121-131.

Mencora, R. M. G., Fussell, T. S., & Elfar, J. C. (2013). Peripheral nerve trauma: Mechanisms of injury and recovery. *Hand Clin, 29*, 317-330.

Mishra, S., Prabhakar, S., Lal, V., Modi, M., Das, C. P., & Khurana, D. (2006). Efficacy of splinting and oral steroids in the treatment of carpal tunnel syndrome: a prospective randomized clinical and electrophysiological study. *Neurol India, 54*, 286-290.

Mondelli, M., Giannini, F., & Giacchi, M. (2002). Carpal tunnel syndrome incidence in a general population. *Neurology, 58*, 289-294.

Mondelli, M., Padua, L., & Reale, F. (2004). Carpal tunnel syndrome in elderly patients: results of surgical decompression. *J Peripher Nerv Syst, 9*, 168-176.

Mondelli, M., Reale, F., Padua, R., Aprile, I., & Padua, L. (2002). Clinical and neurophysiological outcome of surgery in extreme carpal tunnel syndrome. *Clin Neurophysiol, 112*, 1237-1242.

Monje, P. V., Athauda, G., & Wood, M. W. (2013). Protein kinase A mediated gating of neuregulin-dependent ErbB2-ErbB3 activation underlies the synergistic action of cAMP on schwann cell proliferation. *J Bio Chem, 283*, 34087-34100.

Mozaffar, T., Strandberg, E., Abe, K., Hilgenberg, L. G., Smith, M. A., & Gupta, R. (2009). Neuromuscular junction integrity after chronic nerve compression injury. *J Orthop Res, 27*, 114-119.

Navarro, X., Udina, E., Ceballos, D., & Gold, B. G. (2001). Effects of FK-506 on nerve regeneration and reinnervation after graft or tube repair of long nerve grafts. *Muscle Nerve, 24*, 905-915.

Neumann, S., Bradke, F., Tessier-Lavigne, M., & Basbaum, A. I. (2002). Regeneration of sensory axons within the injured spinal cord induced by intraganglionic cAMP elevation. *Neuron, 34*, 885-893.

Nordstrom, D. L., DeStefano, F., Vierkant, R. A., & Layde, P. M. (1998). Incidence of diagnosed carpal tunnel syndrome in a general population. *Epidemiology, 9*, 342-345.

O'Brien, J. P., Mackinnon, S. E., MacLean, A. R., Hudson, A. R., Dellon, A. L., & Hunter, D. A. (1987). A model of chronic nerve compression in the rat. *Ann Plast Surg, 19*, 430-435.

O'Connor, D., Marshall, S. C., Massy-Westropp, N., & Pitt, V. (2003). Non-surgical treatment (other than steroid injection) for carpal tunnel syndrome. *Cochrane Database Syst Rev, I*.

O'Gradaigh, D., & Merry, P. (2000). Corticosteroid injection for the treatment of carpal tunnel syndrome. *Ann Rheum Dis, 59*, 918-919.

Okada, K., Tanaka, H., Temporin, K., Okamoto, M., Kuroda, Y., Moritomo, H., . . . Yoshikawa, H. (2010). Methylcobalamin increases Erk1/2 and Akt activities through the methylation cycle and promotes nerve regeneration in a rat sciatic nerve injury model. *Exp Neurol, 222*, 191-203.

Osio, M., Muscia, F., Zampini, L., Nascimbene, C., Mailland, E., Cargnel, A., & Mariani, C. (2006). Acetyl-l-carnitine in the treatmentof painful antiretroviral toxic neuropathyin human immunodevifiency virus patients: An open label study. *J Peripher Nerv Syst, 11*, 72-76.

Ozdogan, H., & Yazici, H. (1984). The efficacy of local steroid injections in idiopathic carpal tunnel syndrome: a double-blind study. *Br J Rheumatol, 23*, 272-275.

Padua, L., LoMonaco, M., Gregori, B., Valente, E. M., Padua, R., & Tonali, P. (1997). Neurophysiologic classification and sensitivity in 500 carpal tunnel syndrome hands. *Acta Neurol Scand, 96*, 211-217.

Page, M. J., Massy-Westropp, N., O'Connor, D., & Pitt, V. (2012). Splinting for carpal tunnel syndrome. *Cochrane Database Syst Rev*.

Paget, J. (1854). *Lectures on surgical pathology.* Philidelphia: Lindsay and Blakinston.

Pal, B., Mangion, P., & Hossian, M. M. (1988). Should diuretics be prescribed for idiopathic carpal tunnel syndrome? Results of a controlled trial. *Clin Rehab, 2*, 299-301.

Palmer, D. H., & Hanrahan, L. P. (1995). Social and economic costs of carpal tunnel surgery. *Instr Course Lect, 44*, 167-172.

Papaioannou, T., Rushworth, G., Atar, D., & Dekel, S. (1992). Carpal canal stenosis in men with idiopathic carpal tunnel syndrome. *Clin Orthop Relat Res, 285*, 210-213.

Patapoutain, A., & Reichardt, L. F. (2001). Trk receptors: Mediators of neurotrophin action. *Curr Opin Neurobiol, 11*, 272-280.

Peltier, A., Smith, A. G., Russell, J. W., Sheikh, K., Bixby, B., Howard, J., . . . Singleton, J. R. (2009). Reliability of quantitative sudomotor axon reflex testing and quantitative sensory testing in neuropathy of impaired glucose regulation. *Muscle Nerve, 39*.

Phalen, G. S. (1966). The carpal tunnel syndrome. *J Bone Joint Surg Am, 48*, 211-228.

Phalen, G. S., Gardner, W. J., & Lalonde, A. A. (1950). Neuropathy of the median nerve due to compression beneath the transverse carpal ligament. *J Bone Joint Surg Am, 48*, 109-112.

Pham, K., & Gupta, R. (2009). Understanding the mechanisms of entrapment neuropathies. Review article. *Neurosurg Focus, 26*, E7.

Phan, D. Q., & Schuind, F. (2012). Tolerance and effects of FK506 (tacrolimus) on nerve regeneration: a pilot study. *J Hand Surg Eur Vol, 37*, 537-543.

Phillips, T. J., Cherry, C. L., Cox, S., Marshall, S. J., & Rice, A. S. (2010). Pharmacological treatment of painful HIV-associated sensory neuropathy: a systematic review and meta-analysis of randomised controlled trials. *PLoS One, 5*, e14433.

Pisano, C., Pratesi, G., Laccabue, D., Zunino, F., Lo Giudice, P., Bellucci, A., . . . Cavaletti, G. (2003). Pacitaxel and cisplatin-induced neurotoxicity: A protective role of acetyl-l-carnitine. *Clin Cancer Res, 9*, 5756-5767.

Pop-Busui, R., Marinescu, V., Van Huysen, C., Li, F., Sullivan, K., Greene, D. A., . . . Stevens, M. J. (2002). Dissection of metabolic, vascular, and nerve conduction interrelationships in experimental diabetic neuropathy by cyclooxygenase inhibition and acetyl-l-carnitne administration. *Diabetes, 51*.

Premoselli, S., Sioli, P., Grossi, A., & Cerri, C. (2006). Neutral wrist splinting in carpal tunnel syndrome: a 3 and 6 month clinical and neurophysiologic follow-up evaluation of night only splint therapy. *Europa Medicophysia, 42*, 121-126.

Price, R. D., Yamaji, T., & Matsuoka, N. (2003). FK-506 potentiates NGF-induced neurite outgrowth via the Ras/Raf/MAP kinase pathway. *Br J Pharmacol, 140*, 825-830.

Putnam, J. J. (1880). A series of cases of paresthesias, mainly of the hand, or periodic recurrence and possibly of vaso-motor origin. *Arch Med, 4*, 147-162.

Read, D. E., & Gorman, A. M. (2009). Involvement of Akt in neurite outgrowth. *Cell Mol Life Sci, 66*, 2975-2984.

Reichert, F., Saada, A., & Rotshenker, S. (1994). Peripheral nerve injury induces Schwann cells to express two macrophage phenotypes: phagocytosis and the galactose-specific lectin MAC-2. *J Neurosci, 14*, 3231-3245.

Rempel, D., Evanoff, B., Amadio, P. C., de Krom, M., Franklin, G., Franzblau, A., . . . Pransky, G. (1998). Consensus criteria for the classification of carpal tunnel syndrome in epidemiologic studies. *Am J Public Health, 88*, 1447-1451.

Ricci, A., Ramacci, M. T., Ghirardi, O., & Amenta, F. (1989). Age-related changes in the mossy fibre system of the hippocampus; effects of long-term acetyl-l-carnitine. *Arch Gerontol Geriatr, 8*, 63-71.

Riccio, A., Ahn, S., Davenport, C. M., Blendy, J. A., & Ginty, D. D. (1999). Mediation by CREB family transcription factor of NGF-dependent survival of sympathetic neurons. *Science, 286*.

Sargent, S. (1921). Lesions of the Brachial Plexus Associated with Rudimentary Ribs. *Brain, 44*, 95-124.

Scarpini, E., Sacilotto, G., Baron, P., Cusini, M., & Scarlato, G. (1997). Effect of acetyl-L-carnitine in the treatment of painful peripheral neuropathies in HIV+ patients. *J Peripher Nerv Syst, 2*, 250-252.

Scheenberger, S., Khalifian, S., & Brandacher, G. (2013). Immunosuppression and monitoring of rejection in hand transplantation. *Tech Hand Surg, 17*, 208-214.

Schumacher, M., Guennoun, R., Stein, D. G., & Nicola, A. F. (2007). Progesterone:therapeutic opportunities for neuroprotection and myelin repair. *Pharmacol Ther, 116*, 77-106.

Seddon, H. J. (1943). The three types of nerve injury. *Brain, 66*, 237-288.

Shores, J. T., Malek, V., Lee, W. P., & Brandacher, G. (2017). Outcomes after hand and upper extremity transplantation. *J Mater Sci Mater Med, 28*, 72.

Siao, P., & Cros, D. P. (2003). Quantitative sensory testing. *Phys Med Rehab Clin N Am, 14*, 261-286.

Sima, A. A., Ristic, H., Merry, A., Kamijo, M., Lattimer, S. A., Stevens, M. J., & Greene, D. A. (1996). Primary preventive and secondary interventionary effects of acetyl-L-carnitine on diabetic neuropathy in the bio-breeding Worcester rat. *J Clin Invest, 97*, 1900-1907.

Sima, A. A. A. F., Calvani, M., Mehra, N., & Amato, A. (2005). ALCAR improves pain, nerve regeneration and vibratory perception in patients with chronic diabetic neuropathy: an analysis of two randomized control trials. *Diabetes Care, 28*, 96-101.

Simon, J., Lutsky, K., Maltenfort, M., & Beredjiklian, P. K. (2017). The Accuracy of the Scratch Collapse Test Performed by Blinded Examiners on Patients With Suspected Carpal Tunnel Syndrome Assessed by Electrodiagnostic Studies. *J Hand Surg Am, 42*, 386 e381-386 e385.

Singh, M., Sisk, G., Carty, M., Sampson, C., Blazar, P., Dyer, G., . . . Talbot, S. G. (2016). Functional Outcomes after Bilateral Hand Transplantation: A 3.5-Year Comprehensive Follow-Up. *Plast Reconstr Surg, 137*, 185-189.

Skaper, S. (2008). The Biology of Neurotrophins, Signalling Pathyways and their receptorss.pdf. *CNS & Neurological Disorders - Drug Targets, 7*, 46-62.

Snyder, A. K., Fox, I. K., Nichols, C. M., Rickman, S. R., Hunter, D. A., Tung, T. H., & Mackinnon, S. E. (2006). Neuroregenerative effects of preinjury FK-506 administration. *Plast Reconstr Surg, 118*, 360-367.

Soneru, I., Khan, T., Orfalian, Z., & Abraira, C. (1997). Acetyl-L-carnitine effects on nerve conduction and glycemic regulation in experimental diabetes. *Endocr Res, 23*, 27-36.

Song, C. H., Gong, H. S., Bae, K. J., Kim, J. H., Nam, K. P., & Baek, G. H. (2014). Evaluation of female hormone-related symptoms in women undergoing carpal tunnel release. *J Hand Surg Eur Vol, 39*, 155-160.

Starzl, T. E., Todo, S., Fung, J., Demetris, A. J., Venkataramman, R., & Jain, A. (1989). FK506 for liver, kidney, and pancreas transplantation. *Lancet, 2*, 1000-1004.

Steinberg, D. R. (2002). Surgical release of the carpal tunnel. *Hand Clin, 18*, 291-298.

Stevens, J. C., Sun, S., Beard, C. M., O'Fallon, W. M., & Kurland, L. T. (1988). Carpal tunnel syndrome in Rochester, Minnesota, 1961 to 1980. *Neurology, 38*, 134-138.

Sulaiman, O. A., Voda, J., Gold, B. G., & Gordon, T. (2002). FK506 increases peripheral nerve regeneration after chronic axotomy but not after chronic schwann cell denervation. *Exp Neurol, 175*, 127-137.

Sun, H. H., Saheb-Al-Zamani, M., Yan, Y., Hunter, D. A., Mackinnon, S. E., & Johnson, P. J. (2012). Geldanamycin accelerated peripheral nerve regeneration in comparison to FK-506 in vivo. *Neuroscience, 223*, 114-123.

Sun, Y., Shu, Y., Liu, B., Liu, P., Wu, C., Zheng, R., . . . Yao, Y. (2016). A prospective study to evaluate the efficacy and safety of oral acetyl-L-carnitine for the treatment of chemotherapy-induced peripheral neuropathy. *Exp Ther Med, 12*, 4017-4024.

Sunderland, S. (1951). A classification of peripheral nerve injuries producing loss of function. *Brain, 74*, 491-516.

Taglialatela, G., Angelucci, L., Ramacci, M. T., Werrbach-Perez, K., Jackson, G. R., & Perez-Polo, J. R. (1992). Stimulation of nerve growth factor receptors in PC12 by acetyl-l-carnitine. *Biochem Pharmacol, 44*, 577-585.

Tajdaran, K., Shoichet, M. S., Gordon, T., & Borschel, G. H. (2015). A novel polymeric drug delivery system for localized and sustained release of tacrolimus (FK506). *Biotechnol Bioeng, 112*, 1948-1953.

Taleisnik, J. (1973). The palmar cutaneous branch of the median nerve and the approach to carpal tunnel. *J Bone Joint Surg, 55*, 1212-1217.

Tapadia, M., Mozaffar, T., & Gupta, R. (2010). Compressive neuropathies of the upper extremity: an update on pathophysiology, classification, and electrodiagnostic findings. *J Hand Surg Am, 35*, 668-677.

Thomsen, N. O. B., Cederlund, R. I., Andersson, G. S., Rosen, I., Bjork, J., & Dahlin, L. B. (2014). Carpal tunnel release in patients with diabetes: A 5-year follow-up with matched controls. *J Hand Surg Am, 39*, 713-720.

Topilko, P., Schneider-Maunoury, S., Levi, G., Baron-Van Evercooren, A., Chennoufi, A. B., Seitandou, T., . . . Chamay, P. (1994). Krox-20 controls myelination in the peripheral nervous system. *Nature, 371*, 796-799.

Tsuda, Y., Kanje, M., & Dahlin, L. B. (2011). Axonal outgrowth is associated with increased ERK 1/2 activation but decreased caspase 3 linked cell death in Schwann cells after immediate nerve repair in rats. *BMC Neurosci, 12*, 12.

Udina, E., Ceballos, D., Verdú, E., Gold, B. G., & Navarro, X. (2002). Bimodal dosedependence of FK506 on the rate of axonal regeneration in mouse peripheral nerve. *Muscle Nerve, 26*, 348-355.

Upton, A. R. M., & McComas, A. J. (1973). The double crush in nerve-entrapment syndromes. . *Lancet, 2*, 359-362.

Valcour, V., Yeh, T. M., Bartt, R., Clifford, D., Gerschenson, M., Evans, S. R., . . . team, A. C. T. G. p. (2009). Acetyl-l-carnitine and nucleoside reverse transcriptase inhibitor-associated neuropathy in HIV infection. *HIV Med, 10*, 103-110.

Vanhaesebroeck, B., & Alessi, D. R. (2000). The PI3K-PDK1 connection: more than just a road to PKB. *Biochem J, 346*, 561-576.

Vasiliadis, H. S., Georgoulas, P., Shrier, I., Salanti, G., & Scholten, R. J. P. M. (2014). Endoscopic release for carpal tunnel syndrome. *Cochrane Database Syst Rev*.

Walker, W. C., Metzler, M., Cifu, D. X., & Swartz, Z. (2000). Neutral wrist splinting in carpal tunnel syndrome: A comparison of night-only versus full-time wear instructions. *Arch Phys Med Rehabil, 81*, 424-429.

Waller, A. (1850). Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced theraby in the structure of their primitive fibres. *Phil Trans R Soc Lond, 140*, 423-429.

Wang, M. S., Zeleny-Pooley, M., & Gold, B. G. (1997). Comparative dose dependence study of FK506 and cyclosporin A on the rate of axonal regeneration in the rat sciatic nerve. *J Pharmacol Exp Ther, 292*, 1084-1093.

Wang, S. M., Han, C., Lee, S. J., Patkar, A. A., Masand, P. S., & Pae, C. U. (2014). A review of current evidence for acetyl-l-carnitine in the treatment of depression. *J Psychiatr Res, 53*, 30-37.

Werner, R. A., & Andary, M. (2002). Carpal tunnel syndrome: pathophysiology and clinical neurophysiology. *Clin Neurophysiol, 113*, 1373-1381.

Wilson, A. D., Hart, A., Brannstrom, T., Wiberg, M., & Terenghi, G. (2003). Primary sensory neuronal rescue with systemic acetyl-L-carnitine following peripheral axotomy. A dose response analysis. *Br J Plas Surg, 56*, 732-739.

Wilson, A. D., Hart, A., Wiberg, M., & Terenghi, G. (2010). Acetyl-l-carnitine increases nerve regeneration and target organ reinnervation - a morphological study. *J Plast Reconstr Aesthet Surg, 63*, 1186-1195.

Wolf, S., Barton, D., Kottschade, L., Grothey, A., & Loprinzi, C. (2008). Chemotherapy-induced peripheral neuropathy: Prevention and treatment strategies. *Eur J Cancer, 44*, 1507-1515.

Wong, S. M., Hui, A. C. F., & Tang, A. (2002). Local vs systemic corticosteroids in the treatment of carpal tunnel syndrome. *Neurology, 56*, 1565-1567.

Yamaji, T., Yamazaki, S., Li, J., Price, R. D., Matsuoka, N., & Mutoh, S. (2008). FK1706, a novel non-immunosuppressant neurophilin ligand, ameliorates motor dysfunction following spinal cord injury through its neuroregenerative action. *Eur J Pharmacol, 591*, 147-152.

Yan, Y., Wood, M. D., Moore, A. M., Snyder-Warwick, A. K., Hunter, D. A., Newton, P., . . . Mackinnon, S. E. (2016). Robust Axonal Regeneration in a Mouse Vascularized Composite Allotransplant Model Undergoing Delayed Tissue Rejection. *Hand (N Y), 11*, 456-463.

Yang, R. K., Lowe, J. B., 3rd, Sobol, J. B., Sen, S. K., Hunter, D. A., & Mackinnon, S. E. (2003). Dose-dependent effects of FK506 on neuroregeneration in a rat model. *Plast Reconstr Surg, 112*, 1832-1840.

Yeh, C., Bowers, D., & Hadlock, T. A. (2007). Effects of FK506 on functional recovery after facial nerve injury. *Arch Facial Plast Surg, 9*, 333-339.

Yildiz, N., Atalay, N. S., Gungen, G. O., Sanal, E., Akkaya, N., & Topuz, O. (2011). Comparison of ultrasound and ketoprofen phonophoresis in the treatment of carpal tunnel syndrome. *J Back Musculoskelet Rehabil, 24*, 39-47.

Youle, M. (2007). Acetyl-l-carnitine in HIV-associated anitretroviral toxic neuropathy. *CNS Drugs, 21*, 25-30.

Youle, M., & Osio, M. (2007). A double-blind, placebo-controlled, multicentre study of acetyl-L-carnitine in the symptomatic treatment of antiretroviral toxic neuropathy in patients with HIV-1 infection. *HIV Medicine, 8*.

Zhao, J., Zheng, X., Fu, C., Qu, W., Wei, G., & Zhang, W. (2014). FK506 loaded chitosan conduit promotes the regeneration of injured sciatic nerves in the rat through the upregulation of brain-derived neurotrophic factor and TrkB. *J Neurol Sci, 344*, 20-26.

Zhou, X., Liu, F., & Zhai, S. (2007). Effect of L-carnitine and/or acetyl-L-carnitine in nutrition treatment of male infertility: a systematic review. *Asia Pac J Clin Nutr, 16 Supp1*, 383-390.

Zochodne, D. W. (2008). *Neurobiology of Peripheral Nerve Regeneration.* Edinburgh: Cambridge University Press.