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**PULMONARY FUNCTION AND RESPIRATORY MUSCLE ACTIVATION IN
INDIVIDUALS WITH CHRONIC SPINAL CORD INJURY**

By

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B.S., Instituto Jorge Coll, Mendoza, Argentina, 1999

M.S., University of Louisville, Louisville, USA, 2005

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Submitted to the Faculty of the
School of Medicine, University of Louisville
in Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy

Department of Physiology
University of Louisville
Louisville, Kentucky

August 2011

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A Dissertation Approved on July 15, 2011

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DEDICATION

I would like to dedicate this dissertation to my best friend and the love of my life
Andrés and to my precious children Benjamín and Jeremías.

They are the center of my universe.

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I would like to express my greatest gratitude to my mentor Dr. Susan Harkema, who gave me a lifetime opportunity in allowing me to work with her. Dr. Harkema not only gave me guidance and the tools to conduct my investigation; but also she gave me independence to explore and explode my own potential. I have learned in the past five years more than many would in their lifetime. She allowed me to truly understand that the main reason behind sciences is to improve people's life; translating lab findings to the clinic, giving hope and alleviating the pain of many who are suffering.

My special thanks are also to all the research participants and their families, for their time, unconditional commitment and for allowing me the honor of being part of their lives.

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ABSTRACT
PULMONARY FUNCTION AND RESPIRATORY MUSCLE ACTIVATION IN
INDIVIDUALS WITH CHRONIC SPINAL CORD INJURY

Daniela Terson de Paleville

July 15, 2011

Pulmonary complications associated with persistent respiratory muscle weakness and paralyses are critical problems faced by patients with chronic spinal cord injury (SCI). The aim of this dissertation is to investigate the role of neurological injury and neural plasticity on pulmonary function after SCI in humans. We tested the following hypotheses: 1) post-SCI respiratory insufficiency is related to the severity of the spinal lesion; 2) respiratory insufficiency is related to abnormal neuromuscular activity of respiratory muscles and that the electromyographic (EMG) amplitude of respiratory muscles is correlated to respiratory muscle strength and 3) locomotor training, an activity-based therapy, improves pulmonary function in individuals with cervical and upper thoracic SCI. We recorded standard sitting spirometry in SCI (n=35) and non-injured individuals (n=15). We also recorded EMG patterns from inspiratory, expiratory and accessory muscles during maximal respiratory pressures maneuvers. Motor incomplete SCI subjects have significantly higher pulmonary function than motor complete ($p = 0.006$). The respiratory muscle activation patterns were variable in SCI during all maneuvers.

After SCI, neural plasticity results in a compensatory recruitment of non-respiratory muscles for pulmonary function. Higher maximal inspiratory pressure (MIP) is related to higher EMG activity of the upper trapezius in the cervical incomplete group, suggesting that this muscle is being successfully recruited to create higher inspiratory pressure. EMG activity of the latissimus dorsi is negatively correlated with maximal expiratory pressure (MEP) in both cervical motor complete and incomplete subjects, suggesting that this muscle is being recruited as a compensatory strategy for forced expiration in individuals with cervical SCI. Locomotor training is a beneficial intervention for improving pulmonary function in SCI individuals. These findings can improve our understanding of the mechanisms that underlie respiratory insufficiency and may be useful to develop objective, quantitative evaluation of the neural control of respiration after neurologic injury.

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CHAPTER I

INTRODUCTION

A. Human Spinal Cord Anatomy and Physiology

The spinal cord processes motor and sensory information and mediates signals transmitted between brain and body ¹. In the adult human, the spinal cord extends from the foramen magnum of the skull to the first lumbar vertebrae. The cauda equine (horse tail in Latin) is formed by spinal nerves distal to L1-L2 extending from the bottom of the cord to the sacrum. The spinal cord is protected and supported by meningeal membranes, ligaments and vertebrae (7 cervical, 12 thoracic, 5 lumbar and 5 sacral). At the cellular level, the spinal cord contains longitudinally oriented spinal tracts (white matter) surrounding central areas (gray matter) where most spinal neuronal cell bodies are located ¹⁻⁴.

The gray matter is a brownish-gray nerve tissue found on the core of the spinal cord ³⁻⁸. As shown in figure 1.1, in cross section, the gray matter has a butterfly shape and it is composed of cell bodies of efferent neurons, dendritic processes and arborizations, neuroglial cells, projection neurons and interneurons, vascular support and high density of capillaries. The spinal cord is divided into ten regions, termed spinal lamina, denoted with roman numerals I-X. It is also subdivided into three horns: anterior or ventral horn (motor area), posterior or dorsal horn (sensory area); intermediate gray (associate neurons), and lateral horns (ie, part of the intermediate zone present in the thoracic and

lumbar segment where preganglionic sympathetic neurons are located). The metameric organization of the vertebrate body is clearly revealed by the specific innervation of successive portions of skin by a particular level of the spinal cord. The area of the skin innervated by a single spinal nerve is a dermatome which transmits sensory information to the spinal cord via dorsal roots. Similarly, neurons of each ventral root innervate a group of muscles or myotomes.

The white matter is the tissue of the central nervous system consisting mainly of myelinated nerve fibers, but with some unmyelinated nerve fibers, embedded in a spongy network of neuroglia and with low density of capillaries ^{3;4;6-8}. The white matter is divided into the dorsal, lateral, and ventral funiculi, each one containing multiple ascending and descending tracts and also fibers passing in both directions (i.e. dorsolateral fasciculus -of Lissauer-, fasciculus proprius and medial longitudinal fasciculus). Each column subdivides into tracts that are closely associated in function.

Figure 1.1: Diagram of the spinal cord showing ascending (blue) and descending (red) tracts. Taken from *"Human anatomy", Marieb, E.N. and Mallatt, J. Third edition, 2000.*

1. Ascending Tracts

Bundles of axons in the spinal cord are organized into the ascending and descending tracts^{2-4;7;9}. The ascending tracts within the spinal cord transmit sensory information from the periphery to the spinal cord. Ascending tracts include: spinocerebellar tracts, the dorsal column medial lemniscal system (fasciculus gracilis, fasciculus cuneatus), spinothalamic and spinoreticular tracts^{4;6-8}. Spinocerebellar tracts are composed of axons that originate in the spinal cord and terminate ipsilaterally in the cerebellum. Primary somatosensory axons transmitting information from muscles, joints, tendons, and cutaneous receptors enter the spinal cord via dorsal roots and synapse on secondary spinal sensory neurons. Sensory information from receptors representing spinal segments T6 and below is transmitted via the ventral and dorsal spinocerebellar tracts. Sensory information from receptors representing spinal segments T6 and above is transmitted via the rostral spinocerebellar and the cuneocerebellar tracts.

In the dorsal column medial lemniscal system, the primary sensory axons transmit information for conscious perception from receptors in superficial and deep tissue⁶. As shown in figure 1.2; these axons enter to the central nervous system via dorsal roots in the spinal cord or the brain stem and synapse on secondary sensory neurons which axons decussate, ascend as lemniscal pathways and end in the contralateral thalamus^{4;6;8}. These thalamic nuclei then project to sensory cortex and conscious perception of the transmitted information occurs. Sensation of vibration and joint position are transmitted via myelinated axons to neurons in the nuclei gracilis, nuclei cuneatus, and the medulla. Nuclei gracilis and cuneatus form the medial lemniscus pathway, which decussates and terminates in the ventral posterolateral nucleus of the thalamus^{4;6-8}.

The spinothalamic tracts are composed of axons that originate in the spinal cord, and terminate in the thalamus, and transmit information from skin and internal organs^{10;11}. Information about temperature, pain and light touch are transmitted via small myelinated and unmyelinated axons in the spinothalamic tract (Figure 1.2)^{4;6}. The lateral spinothalamic tracts transmit information related to light touch and temperature, and the ventral spinothalamic tracts transmit information related to touch and pressure (figure 1.3). The spinothalamic tract terminates in the tertiary sensory neurons in the ventral postero-lateral nucleus of the thalamus. These neurons synapse with primary sensory cortex neurons. Neurons of the spinoreticular tract originate primarily in the spinal cord and terminate in many sites throughout the brain stem reticular formation. Neurons from the reticular formation project to the hypothalamus, the thalamus, the limbic forebrain and neocortex transmitting information about slow excruciating pain.

Figure 1.2: Diagram of ascending spinothalamic tract (light blue) and the medial lemniscal system (blue) transmitting sensory information from proprioceptors and touch receptors. Taken from *"Human anatomy", Marieb, E.N. and Mallatt, J. Third edition, 2000.*

Figure 1.3: Diagram of ascending lateral spinothalamic tract (blue) transmitting sensory information from pain and temperature receptors. *Taken from "Human anatomy", Marieb, E.N. and Mallatt, J. Third edition, 2000.*

2. Descending Tracts

The ability to organize complex motor tasks and to execute fine movements with precision depends on control signals transmitted from motor areas in the cerebral cortex through the descending tracts. Descending tracts within the spinal cord transmit information from the brain downwards to initiate movement and influence body functions. Descending tracts include the pyramidal or corticospinal tracts, and the extrapyramidal tracts^{4;6-8}. Axon terminals from corticospinal tracts transmit motor information from the cerebral cortex and supplemental premotor cortex to the spinal motor neurons. Eighty percent of these fibers decussate at the junction of the medulla oblongata and the spinal cord (i.e. pyramidal decussation) and descend in the spinal cord where they form the lateral corticospinal tract^{12;13} (Figure 1.4). Axons that do not decussate, descend in anterior corticospinal tract and then decussate at the appropriate level to terminate directly or indirectly on motoneurons contralateral to cells of origin^{6; 12}.

Figure 1.4: Diagram of descending corticospinal tracts. Anterior corticospinal tract do not decussate at the decussation pyramid (red). Lateral corticospinal tract decussate at the pyramidal decussation (fuchsia) Taken from *"Human anatomy"*, Marieb, E.N. and Mallatt, J. Third edition, 2000.

Other descending tracts include rubrospinal, vestibulospinal, reticulospinal and tectospinal tracts^{4;6-8}. The motor cortex sends inputs to the red nuclei and fibers descend forming the rubrospinal tract. As shown in figure 1.5, axons from this tract decussate and descend in the lateral brain stem and the lateral funiculus in the spinal cord. These axons then synapse directly or indirectly on alpha and gamma motoneurons associated with flexor activity of the extremities⁶. The vestibulospinal tract arises from the lateral vestibular nucleus and terminates on ipsilateral alpha and gamma motoneurons associated with extensor activity of the muscles. The vestibulospinal tract is involved in postural adjustments and antigravity muscle activation. The reticulospinal tract arises from the pons and axons descend ipsilaterally. This tract is involved in extensor activity and axial musculature, and reinforces the action of the vestibulospinal tract. The tectospinal tract arises from neurons in the midbrain, decussates in the dorsal tegmental decussation, descends contralaterally and terminates on motoneurons associated with head and neck movements in the cervical spinal cord^{4;6-8}.

Figure 1.5: Diagram of the rubrospinal tract. Axons from this tract decussate and descend in the lateral brain stem and the lateral funiculus of the spinal cord and terminate directly or indirectly on alpha and gamma motoneurons associated with flexor activity of the extremities. Taken from *"Human anatomy"*, Marieb, E.N. and Mallatt, J. Third edition, 2000.

3. Interneuronal System

The interneuronal system modulates the interaction between afferent input (ascending pathway) and efferent output (descending pathway)¹⁴. Last order interneurons are those that have excitatory or inhibitory synapse directly with motoneurons producing the motor output^{15;16}. Most of the literature about the interneuronal system are from feline studies¹⁷⁻²²; however most features of the interneuronal system are similar in cats and humans¹⁹. In mammals, inhibitory interneurons include: Ia inhibitory interneurons (reciprocal inhibition), nonreciprocal inhibitory interneurons, Renshaw cell (recurrent inhibition) and group II interneurons^{15;17;19}. Excitatory interneurons include: group I excitatory interneurons, mid-lumbar group II interneurons, excitatory interneurons mediating cutaneous inputs, and excitatory commissural interneurons^{15;17}.

Inhibitory interneurons are involved in many pathways. Reciprocal inhibition pathway include interneurons that induce inhibitory postsynaptic potentials in the motoneurons that innervate antagonist muscles²³. During movements that involve flexion and extension on a joint, there is an inhibition of the antagonist muscle during the activation of the agonist muscle that is mediated by Ia inhibitory interneurons. Nonreciprocal inhibition occur when there is an inhibition of synergist motoneurons as the strength of group I stimulation is increased. Nonreciprocal inhibition is mediated by Ib fibers originated in Golgi tendon organ^{14;15}. Recurrent inhibition is mediated by an interneuron termed Renshaw cell. Renshaw cells receive input from branches of axons of alpha motoneurons, by group II to IV afferents and by descending pathways^{15;19}. These interneurons result in inhibitory postsynaptic potentials in alpha motoneurons, Ia inhibitory interneurons, and gamma motoneurons. Group II afferent fibers arise from

muscle spindles, and the major effects of this pathway are mediated by the group II interneurons²⁴. These interneurons receive input from ipsilateral and contralateral group II afferents, Ia and Ib afferents, cutaneous and joint afferents and descending tracts. Group II interneurons produce excitation of alpha motor neurons that innervate flexors and inhibition of neurons innervating extensors^{14;19;24}.

Group I interneurons can also be excitatory^{13; 25}. Gossard, et al.,²⁵ report that in lumbar motoneurons that were evaluated during fictive locomotion in spinalized cats, stimulation of the ankle and knee extensor group Ib afferents evoked excitation of extensor motoneurons and not inhibition²⁵. Mid-lumbar group II interneurons can be excitatory or inhibitory and receive monosynaptic input from rubrospinal, corticospinal, vestibulospinal or reticulospinal neurons^{15;26}. There are excitatory interneurons related to cutaneous sensory afferents^{26;27}. Finally, there is another group of excitatory interneurons that receive reticulospinal and vestibulospinal input (commissural interneurons)¹⁵.

4. Propriospinal System

The propriospinal system is composed of spinal interneurons with axons that transverse several spinal segments¹⁴. Groups of propriospinal neurons are located in the cervical and lumbar regions of the spinal cord and receive both excitatory and inhibitory input from peripheral sources and transmit output to motoneurons^{28;29}. These neurons can integrate a variety of input and receive monosynaptic excitation from peripheral and corticospinal inputs and disynaptic inhibition from the same tracts.

Cervical propriospinal neurons project to motoneurons that innervate upper limbs^{30;31}. Studies demonstrate that the role of the cervical propriospinal system is to

transmit corticospinal input and convergent input from rubrospinal, tectospinal and reticulospinal tracts to motoneurons in segments C6 to T1 that innervates forelimbs^{29;32}. Evidence suggests that humans also have the cervical propriospinal system³³. The lumbar propriospinal neurons project to motor neurons that innervate lower limbs^{14;29}. The lumbar propriospinal system receive strong input from group I and II afferents from many muscles³².

Propriospinal neurons are classified for their anatomical characteristics as short or long²⁹. Short propriospinal neurons are those which extensions vary from one to six spinal segments. They can project either within or between spinal segments; they can be ascending or descending and can run contralaterally or ipsilaterally²⁹. Long propriospinal neurons project over more than six spinal segments. If the cell bodies are located rostrally within the cervical enlargement and project caudally, they are termed long descending proprioneurons, if they are located caudally and project rostrally, they are termed long ascending proprioneurons.

Reciprocal connections between cervical and lumbar motor circuits also exist^{29;32}. Animals studies demonstrate that there is coupling of the cervical and lumbar circuits^{34;35}. Central pattern generators in the cervical and lumbar enlargements bring about locomotor activity in the forelimbs and hindlimbs, respectively in quadrupeds²⁹. In humans, this coupling is flexible and allows switching from locomotor interlimb coordination to more fine motor control tasks³⁶.

5. Spinal Nerves

Axons from spinal sensory neurons enter and axons from motor neurons exit the spinal cord via segmental nerves or roots ^{2;3;9}. As axons enter the peripheral nervous system they associate with Schwann cells and become myelinated ^{4;6-8}. Figure 1.6 shows the four main groups of spinal nerves which exit different levels of the spinal cord ⁹. There are thirty-one pairs in descending order of the vertebral column: cervical (C1-C8), thoracic (T1-T12), lumbar (L1-L5), sacral (S1-S5) and a coccygeal pair. The cervical spinal nerves 1 through 7 exit above the first through seventh vertebrae, and the eighth cervical nerve exit between the seventh cervical and the first thoracic vertebrae. The presence of eight cervical nerves and seven cervical vertebrae highlights the vertebral origin of the posterior portion of the mammalian skull, and of the occipital in particular with at least one pre-atlas segment fused to the braincase: its nerve is identified as C1. Below the cervical nerves, each numbered nerve exits below the homonymous vertebrae.

Spinal nerves have overlapping supply; this is a protective mechanism against injury to assure innervation to a given organ or muscle. Each spinal nerve belongs to a network of intersecting nerves (i.e. plexus), innervates a particular number of muscles and organs, and receives sensory information from specific dermatomes. Figure 1.7 shows the innervation map for dermatomes.

Spinal Nerves

Figure 1.6: Diagram of the spinal nerves. The cervical spinal nerves 1 through 7 exit above the first through seventh vertebrae, and the eight cervical nerve exit between the seventh cervical and the first thoracic vertebrae. Below this segment, each numbered nerve exits below the homonymous vertebrae.

Figure 1.7: Dermatomes for cervical C1-C8 (green), thoracic T1-T12 (purple), lumbar L1-L5 (blue) and sacral S1-S5 (light blue) spinal nerves. Taken from *"Human anatomy"*, Marieb, E.N. and Mallatt, J. Third edition, 2000.

Cervical spinal nerve segments innervate muscles of the neck, arms, hands and some muscles of the trunk. Thoracic spinal nerve segments innervate trunk muscles and some muscle of the arm. Lumbar spinal nerve segments innervate muscles of the lower back, legs, and feet. Sacral and coccygeal nerves innervate muscles of the legs, feet and genitalia. C1-C4 spinal nerve segments correspond to the cervical plexus. C5 corresponds to cervical and brachial plexuses and C6-C8 to brachial plexus. Tables 1.1 shows muscles being innervated by cervical spinal nerve segments; table 1.2 shows muscles being innervated by thoracic spinal nerve segments; table 1.3 show muscles being innervated by lumbar spinal nerve segments and table 1.4 shows muscles being innervated by sacral and coccygeal spinal nerve segments.

Spinal nerve segment	Plexus	Muscles innervated
C1	cervical	longus capitis, superior belly omohyoid, thyrohyid and geniohyoid
C2	cervical	longus capitis, superior belly omohyoid, sternohyid, sternothyroid and longus colli
C3	cervical	scalene medius and posterior, levator scapulae, rhomboids, trapezius, diaphragm
C4	cervical	scalene medius and posterior, levator scapulae, rhomboids, trapezius, diaphragm, deltoid, teres minor, supraspinatus and infraspinatus.
C5	cervical and brachial	levator scapulae, rhomboids, scalenes anterior and posterior, deltoid, teres major and minor, subscapularis, serratus anterior, pectoralis major and minor, supraspinatus, infraspinatus, longus colli, biceps brachii, brachioradialis and diaphragm.
C6	brachial	latissimus dorsi, scalenes anterior and posterior, pectoralis major and minor, serratus anterior, supraspinatus and infraspinatus, teres major and minor, subscapularis, biceps brachii, brachioradialis, triceps, pronator teres, longus colli, extensor carpi radialis and ulnaris, extensor digiti quanti, and flexor carpi radialis.
C7	brachial	latissimus dorsi, scalenes anterior and posterior, pectoralis major and minor, serratus anterior, supraspinatus and infraspinatus, teres major and minor, subscapularis, biceps brachii, brachioradialis, triceps, pronator teres, longus colli, extensor carpi radialis and ulnaris, extensor digiti quanti, flexor carpi radialis and palmaris longus.
C8	brachial	latissimus dorsi, scalenes anterior and posterior, pectoralis major and minor, serratus anterior, supraspinatus and infraspinatus, teres major and minor, subscapularis, biceps brachii, brachioradialis, triceps, pronator teres, longus colli, extensor carpi radialis and ulnaris, extensor digiti quanti, flexor carpi radialis, palmaris longus, flexor digitorum superficialis and profundus, flexor pollicis longus and brevis.

- **Table 1.1:** Cervical spinal nerve segments. C1-C5 form the cervical plexus; C5 also form brachial plexus; and C6-C8 form the brachial plexus. Each segment innervates a large number of muscles.

Spinal nerve segment	Plexus	Muscles innervated
T1	brachial	intercostal of the respective level, levator costarum, serratus posterior and superior, semispinalis thoracis, pectoralis major and minor transversus thoracis, flexor digitorum superficialis and profundus, interossei, lumbricales, flexor pollicis longus, pollicis brevis and abductor pollicis brevis
T2	brachial (variably)	intercostals of the respective levels, spinalis thoracic and intrinsic muscles of the back
T3	none	intercostals of the respective levels, spinalis thoracic and intrinsic muscles of the back
T4	none	intercostals of the respective levels, spinalis thoracic and intrinsic muscles of the back
T5	none	intercostals of the respective levels, spinalis thoracic and intrinsic muscles of the back
T6	none	intercostals of the respective levels, spinalis thoracic and intrinsic muscles of the back, rectus abdominis, internal and external obliques
T7	none	intercostals of the respective levels, spinalis thoracic and intrinsic muscles of the back, rectus abdominis, internal and external obliques
T8	none	intercostals of the respective levels, spinalis thoracic and intrinsic muscles of the back, rectus abdominis, internal and external obliques
T9	none	intercostals of the respective levels, spinalis thoracic and intrinsic muscles of the back, rectus abdominis, internal and external obliques
T10	none	intercostals of the respective levels, spinalis thoracic and intrinsic muscles of the back, rectus abdominis, internal and external obliques
T11	none	intercostals of the respective levels, spinalis thoracic and intrinsic muscles of the back, rectus abdominis, internal and external obliques
T12	lumbar	intercostals of the respective levels, spinalis thoracic and intrinsic muscles of the back, rectus abdominis, internal and external obliques

- **Table 1.2:** Thoracic spinal nerve segments. T1 (and variably T2) form part of the brachial plexus; T12 form part of the lumbar plexus. The rest of the thoracic segments do not form plexi. Each segment innervates a large number of muscles.

Spinal nerve segment	Plexus	Muscles innervated
L1	lumbar	external and internal obliques, rectus abdominis, transverse abdominis, cremaster, quadratus lumborum, iliopsoas and iliacus
L2	lumbar	external and internal obliques, rectus abdominis, transverse abdominis, cremaster, quadratus lumborum, iliopsoas and iliacus
L3	lumbar and sacral	internal obliques, rectus abdominis, transverse abdominis, cremaster, quadratus lumborum, iliopsoas, iliacus, psoas major and minor, sartorius, rectus femoris, vastus lateralis, vastus intermedius, vastos medialis, adductor longus and brevis, pectineus
L4	sacral	internal obliques, rectus abdominis, transverse abdominis, cremaster, quadratus lumborum, iliopsoas, iliacus, psoas major and minor, sartorius, rectus femoris, vastus lateralis, vastus intermedius, vastos medialis, adductor longus and brevis, pectineus and adductor magnus and gracilis
L5	sacral	internal obliques, rectus abdominis, transverse abdominis, cremaster, quadratus lumborum, iliopsoas, iliacus, psoas major and minor, sartorius, rectus femoris, vastus lateralis, vastus intermedius, vastos medialis, adductor longus and brevis, pectineus and tensor fasciae latae

- **Table 1.3:** Lumbar spinal nerve segments. L1-L3 form part of the lumbar plexus; L3-L5 form the sacral plexus. Each segment innervates a large number of muscles.

Spinal nerve segment	Plexus	Muscles innervated
S1	sacral	gluteus maximus, medius and minimus; superior and inferior gemelli, obturator internus, rectus femoris, vastus lateralis, vastus intermedius, vastus medialis biceps femoris, semitendinosus, semimembranosus, tibialis posterior, extensor hallucis longus, extensor digitorum longus, extensor digitorum brevis, peroneus tertius, abductor hallucis, abductor digiti minimi, quadratus plantae, flexor digiti minimi brevis, dorsal interossei and plantar interossei
S2	sacral	gluteus maximus, medius and minimus; superior and inferior gemelli, obturator internus, rectus femoris, vastus lateralis, vastus intermedius, vastus medialis biceps femoris, semitendinosus, semimembranosus, tibialis posterior, extensor hallucis longus, extensor digitorum longus, extensor digitorum brevis, peroneus tertius, abductor hallucis, abductor digiti minimi, quadratus plantae, flexor digiti minimi brevis, dorsal interossei and plantar interossei
S3	sacral	levator ani, coccygeus, transversus perineae superficialis, bulbocavernosus, ischiocavernosus, transversus perineae profundus, sphincter urethrae and sphincter ani
S4	sacral and coccygeal	levator ani, coccygeus, transversus perineae superficialis, bulbocavernosus, ischiocavernosus, transversus perineae profundus, sphincter urethrae and sphincter ani
S5	coccygeal	levator ani, coccygeus, transversus perineae superficialis, bulbocavernosus, ischiocavernosus, transversus perineae profundus, sphincter urethrae and sphincter ani
Coccygeal	coccygeal	levator ani, coccygeus, transversus perineae superficialis, bulbocavernosus, ischiocavernosus, transversus perineae profundus, sphincter urethrae and sphincter ani

- **Table 1.4:** Sacral and coccygeal spinal nerve segments. S1-S4 form part of the sacral plexus; S4-coccygeal form the coccygeal plexus. Each segment innervates a large number of muscles.

B. Spinal Cord Injury in Humans

1. Incidence Functional and Economic Consequences

Spinal cord injury (SCI) affects millions of persons worldwide ³⁷; and results in devastating economic, functional and psychological consequences³⁸. Statistics from a large study conducted by the Christopher and Dana Reeve Foundation reveal that spinal cord injury affects approximately 1,275,000 persons in the United States ³⁹. According to the National Spinal Cord Injury Statistics Center, traumatic SCI affects approximately 262,000 persons in the United States ³⁸. Each year there are around 12,000 new cases of SCI in the United States, the average age is 28.7 years old and around 81 % occur in males. Further, 34% are cervical incomplete, 23% are thoracic complete; 18% are cervical complete; 18% thoracic incomplete and 7% are lumbo-sacral. The majority of the SCI are caused by motor vehicle accidents (41%) followed by falls (27%), gunshots (15%) and sports accidents (8%). The high incidence of SCI cases and the secondary complications result in enormous financial costs.

The secondary medical conditions increase the morbidity and mortality in people with chronic spinal cord injuries. These conditions include renal and urinary tract infections ⁴⁰, cardiovascular disease ⁴¹, pressure ulcers ^{42;43}, and pulmonary dysfunction ^{37;44-46}. Renal and urinary tract infections were for decades, the leading cause of death among SCI. However, advances in the urologic and medical management of these patients have significantly decreased the morbidity and mortality⁴⁰. Cardiovascular disease is the second leading cause of death among SCI ³⁸. Cardiac dysfunction in the chronic SCI includes autonomic dysreflexia and coronary heart disease⁴⁷. In both, chronic and acute phases cardiac dysrhythmias occur frequently. These cardiac dysrhythmias

include: bradyarrhythmias, such as bradycardia or tachyarrhythmias, such as paroxysmic supraventricular tachycardia, sinus tachycardia, atria flutter, atria fibrillation and cardiac deconditioning occur in both acute and chronic phases.

Pulmonary dysfunction is the leading cause of death in both acute and chronic SCI³⁸. These pulmonary complications include atelectasis, pneumonia and ventilator failures, and it is related to level and severity of the spinal lesion. Other frequent secondary complications after SCI include depression⁴⁸ and spasticity. A large multicenter study demonstrates that 24% of the SCI individuals suffered from major depression and many others suffer from mild to moderate depression, anxiety and other mood disorders⁴⁸. Spinal cord injured individuals may also suffer from spasticity which results from disordered intermittent or sustained involuntary activation of skeletal muscles⁴⁹. The incidence of spasticity in the extremities is higher among persons with cervical and upper thoracic lesions than in lower thoracic and lumbo-sacral levels of injury⁵⁰.

The functional consequences of SCI are variable and depend on the severity of the lesion⁵¹. SCI may cause death of neurons, detachment and/or demyelination of afferent and efferent axons with the consequent loss of sensory and motor function. These losses can negatively affect independence, mobility, locomotion, self-care, bowel and/or bladder function, communication and social interaction. The national SCI statistic center reports that sixty-six percent of individuals who suffer from SCI are unable to walk 150 feet³⁸. They also receive low scores on the Functional Independence Measure (FIM) scale and require some assistance for bowel and/or bladder function. Motor incomplete SCI are more functionally independent than motor complete SCI subjects, similarly, younger SCI are more independent in self-care than those who are older.

The average cost for individuals with high cervical SCI (cervical segments 1-4) is approximately US\$ 900,000 in the first year after injury; US\$ 500,000 for a lower cervical SCI (C5-C8) and US\$ 300,000 for lower injuries ^{37;38}. Additionally, the average annual expenses are US\$ 150,000; US\$ 60,000 and US\$ 30,000 each subsequent year, respectively. Considering other factors like loss of wage and productivity and the average age (40-years old) of these individuals at the time of injury the lifetime costs of SCI are astounding ⁵².

2. Classification of Human Spinal Cord Injury Level and Severity

A variety of neurological assessments have been developed in order to document sensory and motor function following SCI ⁵³. Spinal cord injuries are classified as cervical (C1-C8, i.e., when the lesion occurs between cervical segments 1 through 8), thoracic (T1-T12), lumbar (L1-L5) or sacral (S1-S5). By testing and mapping for deficits in skin sensation and motor function, it is possible to estimate at which level of the spinal cord the injury has occurred ¹. These clinical assessments include: the Frankel scale ^{54;55}, ⁵⁶, the modified Frankel scale ^{57 58}, the Lucas and Ducker's Neurotrauma motor index ⁵⁹, and the Yale scales ⁶⁰, the Botsford scale ⁶¹, the National Acute Spinal Cord Injury Studies (NASCIS) ⁶², the American Spinal Injury Association (ASIA) scale ^{1;57;58} and the International Standards for the Neurological Classification of Spinal Cord Injury (ISNCSCI) ⁶³

The first scale to estimate neurological deficits was the Frankel scale. This scale was originally created in a five grade scale from A to E. Grade A patients had complete motor and sensory impairment; grade B had only sensory function preserved below the

level of the injury; grade C had motor and sensory function below the level of the injury, but the motor function was not useful. Grade D patients had motor and sensory function below the level of the injury with useful motor function; grade E patients had recovery with no motor or sensory disturbances^{54;55}. This scale was very useful but the distinction between grades C and D presented the main limitation. In order to overcome this limitation, modifications to the Frankel scale were developed.

Other neurological assessment scales were developed over years. The Yale scale has two subscales: the sensory severity scale and the motor severity scale⁶⁴. The sensory severity scale assesses the preserved sensory function by checking selected dermatomes in a numeric scale from 0 to 7 points. The motor severity scale assesses the preserved motor function by checking selected myotomes in a numeric scale ranging from 0 to 5 points. This scale does not assess bladder or bowel function and has the limitation that it is difficult to apply in the acute stages⁵³. The Sunnybrook scale is a ten-point numerical neurological assessment scale with higher sensitivity in the evaluation of sensory function preservation. The Botsford scale includes the evaluation of rectal tone and bladder function and it is more sensitive than the previous ones in tracking changes in function after SCI⁶¹. All these scales present improvements from the original Frankel scale, but still they have the limitation that they do not include motor evaluation of the thoracic segments.

In 1979, Lucas and Ducker developed a scale in which motor function of thoracic segments were evaluated by including voluntary breathing tasks (tidal volume, vital capacity and forced expiratory volume in 1 second) in the assessment⁵⁹. This scale was later modified by the American Spinal Cord Injury Association (ASIA) into a motor

index score ^{50;65}. The American Spinal Cord Injury Association created standards for the classification of SCI individuals. This ASIA scale has had many revisions over the years ^{50;55-58;65}, and has recently changed its nomenclature to the International Standards for the Neurological Classification of Spinal Cord Injury (ISNCSCI) ⁶³.

In the ISNCSCI, dermatomes and myotomes are evaluated to estimate the preserved sensory and motor function below the level of the injury ¹. According to this scale, individuals can be classified as cervical (C1-C8), thoracic (T1-T12), lumbar (L1-L5) or sacral (S1-S5). Spinal cord injuries are also classified as A, B, C, D or E depending on the degree of sensory and motor function preserved in the key muscles evaluated. The sensory examination includes the evaluation of 28 key dermatomes, in which 2 elements are being evaluated: pin prick and light touch in both sides of the body; each of which are being evaluated with a 3-point score scale: 0= absent; 1=impaired sensation; and 2= normal. The motor examination includes the evaluation of 10 paired myotomes. Each myotome is evaluated in the rostral-caudal sequence. The strength of each muscle is being evaluated in a 6-point score scale: 0= total paralysis; 1= palpable contraction; 2= active movement, full range of motion (ROM) with gravity eliminated; 3= active movement, full ROM against gravity; 4= active movement, full ROM against moderate resistance; 5= normal full ROM against full resistance. For those myotomes that are not testable by manual exam (C1-C4; T2-T12 and S2-S5), the motor level is considered to be the same as the sensory level (Figures 1.8 and 1.9).

There are several steps in the classification of the SCI using the International Standards for the Neurological Classification of Spinal Cord Injury. First, dermatomes are evaluated and sensory levels for right and left side are determined. Second, myotomes

are evaluated and motor levels for right and left side are determined. Then, the single neurological level (i.e. the lowest segment where motor and sensory function is normal on both sides) is determined. Finally, the examiner determines whether the injury is complete or incomplete and determines American Spinal Cord Injury Impairment Scale (AIS) Grade.

Spinal cord injury “A” refers to motor and sensory complete injuries in which no sensory or motor function is preserved in the sacral segments S4-S5. Spinal cord injury B refers to motor complete sensory incomplete injuries in which sensory but not motor function is preserved below the neurological level including the sacral segments S4-S5. Spinal cord injury C is for motor and sensory incomplete injuries in which motor function is preserved below the neurological level, and more than half of the key muscles below the neurological level have a muscle grade less than 3. Motor and sensory incomplete “D” is for those injuries in which motor function is preserved below the neurological level, and at least half of the key muscles below the neurological level have a muscle grade greater than or equal to 3. Finally, spinal cord injury “E” is for those injuries in which motor and sensory function are normal (Figures 1.8 and 1.9).

The international Standards for the Neurological Classification of Spinal Cord Injury scale is the most widely implemented scale in both acute and chronic SCI evaluations. A key limitation is that the motor evaluation does not evaluate thoracic segment of the spinal cord (figure 1.9). For this reason, it is difficult to predict if an individual with SCI is at risk of developing respiratory dysfunction, since the majority of the respiratory muscles are innervated by thoracic spinal nerves.

Patient Name _____

Examiner Name _____ Date/Time of Exam _____

ASIA **STANDARD NEUROLOGICAL CLASSIFICATION** **ISCOS**
AMERICAN SPINAL INJURY ASSOCIATION OF SPINAL CORD INJURY

MOTOR
KEY MUSCLES (scoring on numeric scale)

	R	L
C5		
C6		
C7		
C8		
T1		

UPPER LIMB TOTAL (MAXIMUM) (25) (25) (50)

Comments: _____

KEY MUSCLES (scoring on numeric scale)

	R	L
L2		
L3		
L4		
L5		
S1		

LOWER LIMB TOTAL (MAXIMUM) (25) (25) (50)

Voluntary anal contraction (Yes/No) ☐

SENSORY
KEY SENSORY POINTS

0 = absent
1 = impaired
2 = normal
NT = not testable

Any anal sensation (Yes/No) ☐

PIN PRICK SCORE (max: 112)
LIGHT TOUCH SCORE (max: 112)

NEUROLOGICAL LEVEL
The most caudal segment with normal function

COMPLETE OR INCOMPLETE?
Incomplete - Any sensory or motor function is saved

ASSA IMPAIRMENT SCALE

ZONE OF PARTIAL PRESERVATION
Caudal extent of partially innervated segments

SENSORY MOTOR

Key Sensory Points

- **Figure 1.8:** American Spinal Injury Association Impairment Scale. (AIS). Based on the evaluation of dermatomes and myotomes, the examiner determines whether the injury is complete or incomplete and determines AIS Grade.

MUSCLE GRADING

- 0 total paralysis
- 1 palpable or visible contraction
- 2 active movement, full range of motion, gravity eliminated
- 3 active movement, full range of motion, against gravity
- 4 active movement, full range of motion, against gravity and provides some resistance
- 5 active movement, full range of motion, against gravity and provides normal resistance
- 5* muscle able to exert, in examiner's judgement, sufficient resistance to be considered normal if identifiable inhibiting factors were not present

NT not testable. Patient unable to reliably exert effort or muscle unavailable for testing due to factors such as immobilization, pain on effort or contracture.

ASIA IMPAIRMENT SCALE

- ☐ **A = Complete:** No motor or sensory function is preserved in the sacral segments S4-S5.
- ☐ **B = Incomplete:** Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-S5.
- ☐ **C = Incomplete:** Motor function is preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3.
- ☐ **D = Incomplete:** Motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a muscle grade of 3 or more.
- ☐ **E = Normal:** Motor and sensory function are normal.

CLINICAL SYNDROMES (OPTIONAL)

- ☐ Central Cord
- ☐ Brown-Sequard
- ☐ Anterior Cord
- ☐ Conus Medullaris
- ☐ Cauda Equina

STEPS IN CLASSIFICATION

The following order is recommended in determining the classification of individuals with SCI.

- Determine sensory levels for right and left sides.
- Determine motor levels for right and left sides.
Note: in regions where there is no myotome to test, the motor level is presumed to be the same as the sensory level.
- Determine the single neurological level.
This is the lowest segment where motor and sensory function is normal on both sides, and is the most cephalad of the sensory and motor levels determined in steps 1 and 2.
- Determine whether the injury is Complete or Incomplete (sacral sparing).
If voluntary anal contraction = No AND all S4-5 sensory scores = 0 AND any anal sensation = No, then injury is COMPLETE. Otherwise injury is incomplete.

5. Determine ASIA Impairment Scale (AIS) Grade:

Is injury Complete?

NO

If YES, AIS=A Record ZPP
(For ZPP record lowest dermatome or myotome on each side with some (non-zero score) preservation)

Is injury motor incomplete?

YES

If NO, AIS=B
(Yes=voluntary anal contraction OR motor function more than three levels below the motor level on a given side.)

Are at least half of the key muscles below the (single) neurological level graded 3 or better?

NO

AIS=C

YES

AIS=D

If sensation and motor function is normal in all segments, AIS=E
Note: AIS E is used in follow up testing when an individual with a documented SCI has recovered normal function. If at initial testing no deficits are found, the individual is neurologically intact; the ASIA Impairment Scale does not apply.

- **Figure 1.9:** American Spinal Injury Association Impairment Scale. (AIS) grade sheet. The examiner evaluates sensory and motor preservation. Muscle grading ranges from 0 (total paralysis) to 5 (muscle able to exert against resistance in full range of motion). According to this, a SCI individual can be classified as A,B, C, D or E depending on the motor and sensory function preserved.

C. Respiratory Function after Human Spinal Cord Injury

Individuals with spinal cord injury frequently suffer from pulmonary dysfunction in both their acute and chronic phases. In the acute phase, there is an incidence of respiratory complications of 36% to 83% of all the non-traumatic and traumatic SCI cases. Eighty percent of deaths in patients hospitalized with cervical SCI are secondary to pulmonary dysfunction ^{66;67}. Chronic spinal cord injured individuals also suffer respiratory complications ^{68;69}. These respiratory complications include: pneumonia, atelectasis, and ventilator-dependent respiratory failure ^{70;71}. A database of self-reported medical problems among U.S. military veterans with different levels of SCI revealed that 12% of participants reported respiratory problems as their principal secondary medical problem after SCI ⁷².

Another clinically relevant aspect of impaired respiration in individuals with SCI is the inability to cough adequately probably caused by weakness of the abdominal muscles. Neurophysiologically, the cough has been described as a phenomenon initiated from structures innervated by the vagus cranial nerve. Afferent fibers from rapid-adapting receptors run within the vagus nerve. When being stimulated, these receptors are responsible for initiating the cough reflex⁷³. Vagal fibers bypass the spinal cord; therefore, these fibers should not be affected by SCI, even in complete cervical lesions. In fact, cough reflex is preserved in cervical and upper thoracic SCI. However, these individuals suffer from ineffective coughing ⁷³ resulting in accumulation of secretions that can cause airway obstruction and provide growth media for the development of pneumonia ⁷¹.

D. Control of Respiration

The primary role of the respiratory system is to bring in air rich with oxygen and to exhale air with high content of carbon dioxide from the alveolar gas exchange to keep arterial blood gases within the acceptable values for living ⁷⁴. Oxygen from the inhaled air travels down airways to alveoli in lungs. Then it diffuses and travels in the arterial blood bound to hemoglobin to reach tissues (i.e. systemic circulation). Oxygen dissociates from hemoglobin and is used by tissues to keep up with specific metabolic demands. As a result, a higher carbon dioxide concentration travels in the venous blood. Carbon dioxide is transported in blood in three forms: 10 % travels dissolved in plasma, 20% travels bound to hemoglobin (called carbamino-hemoglobin), and about 70% is transported as bicarbonate. Once in lungs, carbon dioxide diffuses to alveoli and then is exhaled during expiration. All of these biochemical reactions are under sophisticated neural controls.

Respiration is principally controlled by the respiratory centers in the midbrain (Figure 1.10). The upper motoneurons of the system originate in cell bodies in the pons and the medulla. The apneustic center is located in the lower pons and promotes inspiration by signaling medullary inspiratory neurons in the dorsal respiratory group to fire. The pontine respiratory center (or pneumataxic center) is a group of neurons which opposes the apneustic center by inhibiting inspiration in cycles. The dorsal respiratory group and ventral respiratory group in the pons are involved in inspiration, but their mechanisms are different ⁷⁵.

Inspiration occurs when neurons from the dorsal respiratory group in the medulla oblongata fire causing the inspiratory muscles to contract. Axons from the dorsal

respiratory group synapse on motoneurons in the spinal cord at levels C3-C5 with lower motor neurons forming the phrenic nerve⁷⁶. When the dorsal respiratory group fires it signals the phrenic nerve to contract the diaphragm and intercostal nerves to contract external intercostals and the intercondral portion of the internal intercostals (Figure 1.10). Scalenes and sternocleidomastoid serve a secondary role during inspiration. When these two inspiratory muscle groups contract, the thoracic cavity expands and due to its negative pressure air travels into the lungs. When the pulmonary stretch receptors are stimulated, afferent axons send inhibitory signals to the dorsal respiratory group, and expiration occurs. This expiration is due to the elastic recoil of the lungs and, if necessary by the active involvement of the intracostal portion of the internal intercostal and abdominal musculature. The dorsal respiratory group is part of the solitary tract and also convey sensory information from mechanoreceptors and chemoreceptors to increase or decrease respiratory rhythm. The ventral respiratory group contains both inspiratory and expiratory neurons; however it is only active during high respiratory demand.

The respiratory centers receive a variety of neural and humoral inputs from different peripheral receptors. As shown in figure 1.11, these peripheral receptors include chemoreceptors in the carotid bodies, arterial chemoreceptors, mechanoreceptors and peripheral receptors for neural inputs to the inspiratory center⁷⁷. Carotid and aortic bodies are stimulated by low partial pressure of oxygen or low pH. The carotid bodies are located at the bifurcation of the common carotid artery. Afferent inputs from the carotid bodies reach the respiratory centers in the medulla oblongata via the carotid sinus and glossopharyngeal nerves, and ventilation is increased to counteract it. In addition, aortic

chemoreceptors are sensitive to increases in carbon dioxide concentrations, small increases in carbon dioxide concentrations increase breathing rate exponentially ⁷⁸.

Mechanoreceptors include muscle spindles, Golgi tendon organ and joint receptors; these receptors send afferent input to the sensory cortex which in turn transmits information to the respiratory centers to increase ventilation. Additionally, another group of stretch receptors in the smooth muscle of airways have an inhibitory action for inspiration via vagus nerves ⁷⁹. The Hering-Breuer inflation reflex describes how these mechanoreceptors inhibit inspiration to prevent over-inflation of the lungs and start expiration. Additionally, neural input from muscles to the respiratory centers provides information for the control of ventilation. Contracting muscles accumulate lactate which stimulates muscle receptors; these signals are transmitted by group III and IV afferent nerve fibers to the brain to increase breathing rate. This later mechanism composes the redundant mechanism for ventilatory control in humans ⁸⁰.

Figure 1.10: Neurons from the respiratory groups in the medulla oblongata fire causing the inspiratory muscles to contract. When the diaphragm and the external intercostals contract the thoracic cavity expands and due to its negative pressure air travels in to the lungs. When the intrathoracic pressure is higher than the atmospheric pressure, the inspiratory neurons stop firing and expiration occurs due to the elastic recoil of the lungs. *Taken from Pearson Education, Inc publishing as Benjamin Cumming 2004.*

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Figure 1.11: Central and peripheral chemoreceptors, irritant receptors, stretch receptors in the lung and muscles, joint receptors and other receptors send information to the respiratory centers in the pons and the medulla to increase ventilation as needed. *Taken from Pearson Education, Inc publishing as Benjamin Cumming 2004.*

E. Respiratory muscles

Respiratory muscle control depends on the coordinated involuntary and voluntary output of electrical impulses that originate in regulatory neurons within the central nervous system⁸¹. These impulses are transmitted via motor nerves to the neuromuscular junctions of the muscles and propagate along the muscle fiber membranes activating the actin and myosin contractile protein structures to produce the contraction. This activity can then be recorded by surface electromyography (EMG)⁸².

Respiratory muscles are skeletal muscles whose primary role is to displace the chest wall rhythmically to inspire air rich with oxygen and to expire air with high content of carbon dioxide from the alveolar gas exchange in order to keep arterial blood gases within the acceptable values for living⁷⁴. These muscles work synergistically and their functionality is related to mechanics of the chest wall. In humans, the trunk consists of the rib cage and the abdomen, separated by the diaphragm. The rib cage consists of a complex bony structure, cartilage and several skeletal muscles. Components of the rib cage include the thoracic vertebrae, ribs, costal cartilages, sternum and articulations and ligaments that allow a variety of movements. There are 12 pairs of ribs; the first 7 pairs (vertebrosternal ribs) attach directly to the sternum, the following 3 pairs (vertebrochondral ribs) attach indirectly to sternum and the lowest 2 pairs are the floating ribs (vertebral ribs) and their ends are free. The dorsal ends of the ribs are attached to the vertebra by ligaments and the ventral ends of the non-floating ribs are joined to the sternum directly or indirectly by cartilaginous articulations. The costo-sternal attachments are shorter and more restrictive in the upper than in the lower ribs, allowing the latter ones to have more freedom of movement.

Breathing is often divided into two types: quiet breathing (at rest) and forced breathing^{83;84}. The diaphragm and the external intercostals are the prime movers for quiet inspiration. As ventilatory demands increase, for example during exercise, other accessory muscles for inspiration are recruited^{2;74;75;85}. The accessory muscles for inspiration include: sternocleidomastoids, scalenes, and upper trapezii. Expiration during quiet breathing is passive and due to the elastic recoil of the lungs. Nevertheless, during forced expiration, muscles that depress the ribs and reduce the thoracic cavity (i.e. expiratory muscles) are recruited. Expiratory muscles include: internal intercostals, rectus abdominis, external and internal obliques. Additionally, other muscles, such as pectoralis major^{44;86-88} and latissimus dorsi⁴⁴ may be recruited for respiratory tasks.

Muscle	Quiet Inspiration	Forced Inspiration	Passive Expiration	Forced Expiration	Cough	Accessory during Forced Expiration in SCI	Innervation	Action
Diaphragm	√	√			√		phrenic nerve (C3-C5)	Descends enlarging thoracic cavity
External Intercostals	√	√			√		Intercostal nerve (T2-T6)	Elevation of the ribs
Scalenes		√			√		Spinal nerves C2-C7	Elevation of the 1st rib
Sternocleidomastoid		√			√		Cranial nerve XI (accessory nerve)	Elevation of the upper ribs and sternum
Upper Trapezius		√			√		Cranial nerve XI (accessory nerve)	Elevation of the ribs
Internal Intercostals	√ *	√		√	√		Spinal nerves T1-T6	Reduce thoracic cavity
Rectus Abdominus				√	√		Spinal nerves T5-L1	Reduce thoracic cavity
External Obliques				√	√		Lower 6 intercostal nerves and subcostal nerve	Reduce thoracic cavity
Internal Obliques				√	√		Lower 6 intercostal nerves and subcostal nerve	Reduce thoracic cavity
Pectoralis Major (clavicular portion)						√	Spinal nerves C5-C7	Reduce thoracic cavity
Latissimus Dorsi						√	Spinal nerves C6-C8	Reduce thoracic cavity

Table 1.5: Respiratory muscles, actions and innervations. Inspiratory muscles include diaphragm and external intercostals, and parasternal part of the internal intercostals (*). Accessory muscles for inspiration include scalenes, sternocleidomastoid and upper trapezius. Expiratory muscles include internal intercostals, rectus abdominus and obliques. Some studies also suggest that pectoralis and latissimus dorsi are accessory muscles for expiration in individuals with SCI.

1. Inspiratory Muscles

a. The diaphragm

The diaphragm is the major muscle contributing to inspiration. The vertebral (crural) portion of the diaphragm inserts on the anterolateral aspect of the first three lumbar vertebrae and on the aponeurotic arcuate ligaments; fibers of the costal portion insert on the xyphoid process of the sternum and the upper margins of the lower six ribs⁷⁴. The diaphragm is shaped like a dome, with a centrally located tendon, and a zone of apposition, directly apposed to the inner aspect of the lower ribs. The apposition zone constitutes 30 percent of the total surface area of the rib cage. During inspiration, as muscle fibers shorten, the apposed area decreases and the dome descends axially, increasing the thoracic cavity and displacing the abdominal contents caudally. The diaphragm is innervated by the phrenic nerves which arise from cervical nerve roots C3 to C5.

b. External Intercostals

Intercostals are two very thin muscle layers separated by an irregular aponeurotic membrane^{2;83;84;89}. The external intercostals are superficial to the internal intercostals. These muscles are innervated by the corresponding thoracic spinal nerves. The external intercostals and the parasternal part of internal intercostals have inspiratory actions and the interosseous part of the internal intercostal has expiratory action^{2;74;83;84;85}. The external intercostals have a synergistic action with diaphragm during inspiration. Each external intercostal attaches from the inferior border of one rib to the superior border of the rib directly inferior^{2;74;83;84}. Fibers of the external intercostal slope obliquely caudal

and ventrally from the rib above to the one below (front-pocket)⁸⁹. The major respiratory action of the external intercostal is to elevate ribs (from the 2nd to the 12th ribs) at the sternocostal and costospinal joints⁸⁴. De Troyer et al.⁷⁴, report that both internal and external intercostals have a net lowering action on ribs, and that the same directional rib motion was obtained when stimulating either intercostal group.

c. Internal intercostals (parasternal part)

The internal intercostals are located in the intercostal spaces of ribs one through twelve^{2;74}. These muscles are innervated by the corresponding thoracic spinal nerves. Fibers of the internal intercostals have the opposite direction than the external intercostals (back-pocket)⁸⁹. Each internal intercostal attaches from the superior border of one rib and its costal cartilage to the inferior border of the rib and its costal cartilage that is directly superior⁸⁴. The major respiratory action of the parasternal part of internal intercostals is to assist external intercostals during inspiration increasing thoracic cavity size.

2. Expiratory muscles

a. Internal intercostals

The internal intercostals are located in the intercostal spaces of ribs one through twelve^{2;74}. These muscles are innervated by the corresponding thoracic spinal nerves. Fibers of the internal intercostals have the opposite direction than the external intercostals (back-pocket)⁸⁹. Each internal intercostal attaches from the superior border of one rib and its costal cartilage to the inferior border of the rib and its costal cartilage that is

directly superior ⁸⁴. The major respiratory action of the internal intercostals is to lower ribs (from the 2nd to the 12th ribs) at the sternocostal and costospinal joints ⁸⁴.

b. Rectus abdominis

Rectus abdominis is an abdominal muscle of forced expiration. This muscle originates from the ventral aspect of the fifth, sixth and seventh costal cartilages and the sternum. It is innervated by the lower thoracic nerves T5-T12 ^{2;83}.

c. External and internal obliques

Internal and external obliques are muscles of forced expiration. External oblique is the most superficial, it originates in the lower eight ribs and intercostals and its fibers run in an oblique line inferiorly and posteriorly to the iliac crest and the inguinal ligament. It is innervated by the lower six intercostal nerves. Underneath the external oblique lies the internal oblique. Its fibers run perpendicular to the external oblique. It is innervated by the lower intercostal nerves, as well as the iliohypogastric and the ilioinguinal nerve.

3. Accessory Muscles

When the ventilatory demands are higher than normal, the so called accessory muscles for inspiration are recruited. Accessory muscles of inspiration include the sternocleidomastoid and scalenes ⁷⁵. Muscles being innervated by the cranial XI or spinal accessory nerve receive the name of accessory muscles of inspiration ⁹⁰. The XI nerve has two parts: the cranial and the spinal parts. The spinal part innervates the

sternocleidomastoid and the upper trapezius. Scalenes are considered accessory muscles for inspiration, even though they are innervated by cervical spinal nerves ⁷⁴. Other muscles have been reported to contribute to breathing, but their actions are still unclear. These muscles include: upper trapezius ⁹¹, pectoralis ^{86;92}, latissimus dorsi ^{44;45} and serratus anterior ⁹³.

a. Sternocleidomastoid

Origins of the sternocleidomastoids are bilaterally at the sternum and the clavicle, and the insertion is in the mastoid process ^{6;83;84}. Sternocleidomastoid is innervated by the cranial nerve XI (accessory nerve). When this muscle is recruited for inspiration there is a cranial displacement of the sternum and an expansion of the upper rib cage. This muscle is activated at high lung volumes or in situations of very high ventilatory demands⁸⁵ and in individuals with high cervical complete spinal cord injuries⁷⁴.

b. Scalenes

Deeper to the sternocleidomastoid muscle are positioned the three scalene muscles: the anterior, the middle and the posterior scalene ^{83;84;94}. The anterior scalene originates in the transverse process of C3 through C6 and inserts into the superior surface of the first rib. The middle scalene originates in the transverse process of C2 through C7 and also inserts into the superior surface of the first rib. The posterior scalene is the deepest one and originates in the transverse process of C5 through C7 and inserts into the second rib. Because they all perform the same action and are located in close proximity to

each other, it is unnecessary to distinguish them apart ⁸³. Scalenes are innervated by cervical spinal segments C2-C7 ⁷⁴.

The major respiratory action of the scalenes is to elevate ribs at the sternocostal and costospinal joints ⁸⁴. Scalenes are usually inactive in quiet breathing but they are recruited when ventilation increases, lifting and expanding the rib cage as they contract ⁸⁵. Nevertheless, some investigators have suggested that scalenes are also recruited during quiet breathing ^{74;95}.

c. Upper trapezius

Upper trapezius originates from the external occipital protuberance and the nuchal ligament of the first cervical vertebra. It inserts on the lateral end of the clavicle and acromion process. Upper trapezius is a prime mover in scapular elevation ^{83;84}. This muscle is active in inspiration in individuals with cervical and upper thoracic SCI and in healthy individuals during maximal forced inspiratory tasks ⁹¹. The upper portion of the trapezius is also innervated by the cranial nerve XI (accessory nerve).

d. Pectoralis major

The clavicular portion of pectoralis major attaches to the medial third of the clavicle and inserts on the lateral lip of bicipital groove of the humerus. The pectoralis major receives dual motor innervation by the medial pectoral nerve and the lateral pectoral nerve. One route of innervation of the pectoralis major originates in the C7, C8 and T1 nerve roots, which merge to form the lower trunk of the brachial plexus.

De Troyer et al., ^{45;86;87;96} report a series of investigations in SCI individuals concluding that the clavicular portion of the pectoralis major is active in expiration in individuals with cervical and upper thoracic SCI. Individuals with cervical and upper thoracic SCI contract the clavicular portion of their pectoralis during cough as well as during slow expiration ⁴⁵.

e. Latissimus dorsi

Latissimus dorsi is the largest, flat, dorso-lateral muscle on the trunk, partly covered by the trapezius on its median dorsal region. It arises by tendinous fibers from the spinous processes of the lower six thoracic vertebræ and from the posterior layer of the lumbodorsal fascia by which it is attached to the lumbar and sacral vertebræ, to the supraspinal ligament, and to the posterior part of the crest of the ilium. It also arises by muscular fibers from the external lip of the crest of the ilium lateral to the margin of the sacrospinalis, and from the three or four lower ribs by fleshy digitations. It is innervated by the thoracodorsal nerve (C6-C8 segments). Latissimus dorsi also has been reported as active during expiration in SCI individuals ^{44;45}.

F. Effect of Level of Injury on Respiratory Function

Symptoms of respiratory insufficiency are highly correlated with the level and severity of the spinal lesion ^{97;98}. Injury of the cervical or upper thoracic spinal cord affects spinal nerves that innervate respiratory muscles. The more rostral the level of injury, the more significant will be the respiratory impairment ⁴⁶. Spirometrical values of forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) are

increased with descending level of SCI and are not altered in lower thoracic injuries ^{98;99}. Severity of the spinal cord lesion is related to decreased functional residual capacity, total lung capacity, expiratory reserve volumes and to increased residual volumes ¹⁰⁰. Individuals with complete or incomplete SCI often suffer from these respiratory insufficiencies due to paralysis ^{69;101}, muscle weakness and/or spastic contractions of muscles involved in respiration ^{102;103}.

1. Respiratory Muscle Paralysis

Injuries to the cervical segments of the spinal cord often result in paralysis of the respiratory muscles ⁴⁶. Complete cervical injuries above the level of phrenic motoneurons may cause paralysis of muscles of both inspiration and expiration, leaving the individual mechanical ventilator-dependent. High cervical incomplete (C2-C4) or cervical lesions below C5 (C5-C8) are likely to produce paralysis, weakness, or spasticity in the muscles used to perform respiration. In these individuals neural control of the diaphragm is preserved and inspiration occurs independently ⁸⁵. Independent breathing may occur even with a partially paralyzed diaphragm ¹⁰⁴. Canine studies demonstrate that following unilateral phrenic nerve transection, non paralyzed contralateral hemi-diaphragm and the intercostal muscles can compensate that partially paralyzed diaphragm ¹⁰⁴.

Lesions in the thoracic segments of the spinal cord are also related to respiratory complications. In 2005, Cotton et al., ⁷¹ report the respiratory complications and mortality risk associated with thoracic spinal cord injury. They found that 51% of high thoracic (T1-T6) and 28% of T7-T12 SCI individuals suffer serious respiratory complications such as pneumonia and recurrent respiratory infections. Individual with either cervical or

thoracic SCI are at risk of developing respiratory insufficiency due to total or partial paralysis of the muscles involved in breathing.

2. Respiratory Muscle Weakness

In addition to paralysis, muscle weakness is another feature frequently observed in SCI individuals ^{105;106}. Muscle weakness is another frequent sequelae suffered by individuals with SCI. Weakness after SCI occurs in limbs¹⁰⁶ and abdominal muscles ¹⁰⁵. Although muscle weakness of the respiratory muscles has not been intensely studied, others suggest that it is related to the paradoxal breathing in individuals with SCI ⁹⁶. In contrast to uninjured individuals, the upper anterior rib cage moves inward during inspiration in persons with cervical or high thoracic SCI ¹⁰⁷. This inappropriate rib-cage movement is the result of a lack of spinal motor activation of the external intercostals combined with the excessive compliance of the abdominal wall due to weak muscle contraction ⁹⁶. This abnormal breathing pattern is more frequent in cervical than thoracic SCI; however, it does not occur uniformly across all cervical SCI individuals ¹⁰⁸⁻¹¹¹. It depends on rib cage elasticity and on the electrical activity of the accessory muscles for inspiration. When electromyographic (EMG) recording from scalenes is silent (i.e. paralyzed scalene muscles), this paradoxal movement of the rib cage is consistently present ¹⁰⁸. Therefore, abnormalities in respiration mechanics are related to abnormal neuromuscular activity including paralysis, muscle weakness and inappropriate involuntary activation of muscles (i.e. spasticity).

3. Respiratory Muscle Spasticity

Spastic contractions of the abdominal muscles impose a substantial load on inspiratory muscles, causing an imbalance between the respiratory load and the capacity of the inspiratory muscles to carry that load. Laffont et al.,¹⁰² report an investigation in a C4 AIS A SCI individual in which EMG activity of the abdominal muscles was simultaneously recorded with pulmonary air flow (L/sec) esophageal pressure (cm H₂O), gastric, transdiaphragmatic pressure (cm H₂O) and spirometrical variables. They found that in spite of the diaphragmatic weakness, the patient did not suffer breathlessness episodes unless spastic contractions of the abdominal muscles occurred. Transient spastic contractions of the abdominal muscles occur with increases in gastric and esophageal pressures of more than 30 cm H₂O. This additional pressure must be overcome for inspiration to occur, imposing a considerable load on the inspiratory muscles resulting in dyspnea.

In 1997, Roth et al.,¹⁰³ report an investigation to determine whether ventilatory dysfunction is related to the level of injury and spasticity by testing pulmonary function and correlating spirometrical values with spasticity scores obtained using the Ashworth scale in humans after SCI. The Ashworth scale is a subjective method used to assess spasticity in which the examiner rates the amount of muscle tone (spasticity) felt as a limb is moved passively through its arc of motion¹¹²⁻¹¹⁴. They find that the level of the injury is correlated with respiratory variables, but no correlation occurs between pulmonary function and limb spasticity¹⁰³. A key limitation of this study is that Ashworth scale has not been designed to evaluate trunk spasticity.

G. Measurements of Pulmonary Function

1. Spirometry

Spirometry is a test that measures pulmonary function, specifically the dynamic measurement of the volume and flow of air that can be inhaled and exhaled to quantify the mechanical properties of the respiratory system¹¹⁵. It provides values for forced vital capacity (FVC) or unforced vital capacity (VC), force expiratory volume in one second (FEV₁), and the ratio between these two volumes (FVC/ FEV₁). To achieve optimal results, forced vital capacity maneuver must be performed with maximal effort from the participant doing forced breathing through a mouthpiece and with a nose clip. After a full inspiration the participant should exhale as fast and as far as possible until the lungs are empty, followed by a full forced inspiration. The initial inspiration should be done from the end of quiet expiration (functional residual capacity) to total lung capacity.

2. Maximal Inspiratory and Expiratory Pressure

Maximal respiratory maneuvers are used to evaluate the strength that respiratory muscles can exert against resistance¹¹⁶. Maximal inspiratory pressure is measured during maximal inspiratory effort from near residual volume. Maximal expiratory pressure is measured during maximal expiratory effort from near total lung capacity. The maximum pressure is taken as the highest value that can be sustained for a minimum of one second⁹¹. The assessment of maximal pressures requires a sharp, forceful effort maintained for a minimum of 2 s. The pressure meter incorporates a 1 mm leak to reduce buccal muscle contribution during the maximal expiratory pressure^{116;117}.

H. Electromyography

When an end-plate potential is generated at the neuromuscular junction, it results in a muscle fiber action potential that propagates to the end of muscle fibers. These currents can be measured with electrodes; a technique termed electromyography (EMG)^{14;118-120}. The muscle action potential generates extracellular currents that spread out from the membrane to the electrode. These currents travel from the electrode to an amplifier, a process called signal transduction. These electrical signals can be measured in microvolts (uV). When a muscle contracts a value ranging from 10 to 500 uV will be apparent^{14;119;121}, depending on the number of motor units recruited and the intensity of the stimuli. The amplitude can be estimated using root mean squared (RMS) or rectified EMG values¹¹⁹⁻¹²¹.

EMG is an essential technique for evaluating mechanisms involving neuromuscular physiology^{120;122}. It is a widespread technique used in both clinical and research settings that allows the characterization of neurological disorders and provides valuable information about the severity of the lesion¹¹⁸. EMG studies can help to identify underlying neurological disorders, and determine causes and the extent of damage¹⁴. EMG provides information about the intensity and timing of muscle activation¹⁴. EMG can be used to distinguish between a paralyzed, a weak or a spastic muscle. It also helps the clinician or researcher to accurately diagnose neurological disorder¹¹⁸. EMG is also an effective tool to track recovery¹²³, inappropriate muscle activation¹²⁴, effect of therapy and plasticity after injury¹²⁵⁻¹²⁷.

1. Surface EMG

There are two types of EMG electrodes: indwelling and surface electrodes. Indwelling electrodes are inserted through the skin directly into the muscle^{14;82;120}. Surface electrodes are placed on top of the skin directly over the muscle. Both of these electrodes are used for signal transduction. Surface EMG electrodes have the advantage that are non-invasive and have a larger recording area, providing information about electrical activation of the whole muscle¹²⁰. Surface EMG electrodes are usually bipolar. Bipolar electrodes measure the voltage differences between two electrodes placed over a muscle in close proximity to each other^{82;120;119;121}.

2. Electromyography of Respiratory Muscles

A number of investigations have suggested that there is a correlation between pulmonary function and the neural activation of muscles involved in respiration^{44;128-130}. Peak expiratory flow rate is correlated with the EMG activity of pectoralis major and latissimus dorsi^{44;45}. EMG of intercostal muscles show significant increases with incremental respiratory loads in inspiratory muscle endurance tests in healthy female individuals, and sternocleidomastoid muscles do not show significant differences¹²⁸. This is similar to the findings of Yokoba et al.,¹²⁹ on healthy individuals, in which sternocleidomastoid muscle start to contract at around 34% of the maximal inspiratory pressure. In addition, they found that scalenes and transversus abdominis EMG activity showed a significant linear correlation (R^2 : 0.98) during gradual production of expiratory and inspiratory mouth pressure to maximal. Similarly, EMG activity of the sternocleidomastoid showed a strong linear correlation with maximal inspiratory pressure

(R^2 : 0.97) and trapezius a non-linear correlation (R^2 : 0.50). Further, trapezius is recruited at 90% of the maximal inspiratory mouth pressure. EMG activity of parasternal intercostals, triangularis sterni ¹³¹ and scalene muscles in anesthetized dogs increases as lung volume increases during inspiration. Further, lower and upper abdominal muscles, external obliques and transverses abdominis are electrically active when expiration occurs ⁷⁴.

I. Therapies to Restore Respiratory Function after Spinal Cord Injury

Different therapies have been implemented to improve respiratory function in individuals with SCI. Abdominal strapping consist of a non-elastic binder that is applied around the abdomen in order to prevent the paradoxical expansion of the abdomen that is common in cervical SCI ¹³². This technique has been shown to improve forced expiration and coughing in patients with paralyzed or weak abdominal muscles. Conversely, several studies have demonstrated that abdominal strapping have minimal effect in improving forced expiration at rest ^{46;132;133} or during exercise ¹³⁴.

1. Exercise

Exercise has also been used as a successful stimulation tool to increase fitness and improve the ventilatory function in individuals with acute ¹³⁵ and chronic SCI ¹³⁶. Physical capacity is significantly decreased in people with cervical or thoracic SCI due paralysis of the muscles below the level of the injury, altered autonomic control and inactivity ¹³⁷. Exercise in individuals with chronic SCI elicits a metabolic response characterized by increased oxygen consumption, minute ventilation, and heart rate ^{138;139}. In addition to these general factors

involved in increasing respiratory activity, exercise effects is associated with the excitation of cerebral cortex, limbic and reticular activating systems, hypothalamus, and central chemoreceptors¹⁴⁰. Specifically, strength training of the pectoralis major in complete lower cervical SCI individuals significantly improves expiratory function by significantly increasing expiratory reserve volume and decreasing residual volume⁸⁶.

2. Functional Electrical and Magnetic Stimulation

Functional electrical stimulation is an experimental technique in which electrical stimulation is delivered to an intact lower motor neuron to activate partially or completely paralyzed muscles causing them to contract^{140;141}. Functional electrical stimulation applied to respiratory muscles is effective in activating expiratory muscles with the related improvements on respiratory function and cough in animals^{76;142} and humans^{101;143;144}. Functional electrical stimulation used to stimulate expiratory muscles applied to abdominal muscles and pectoralis produce a significant increase in maximal expiratory pressure, cough capacity^{101;143;144}, peak expiratory flow¹⁴⁵, forced expiratory volume in one second, forced vital capacity, and maximal inspiratory pressure¹⁴³. In addition, one acute C4 SCI individual was decannulated from tracheotomy and able to cough voluntarily after 3 weeks of functional electrical stimulation, when no other method of respiratory technique for improving coughing was effective¹⁴⁴.

Electrical stimulation also has been used to activate inspiratory muscles. Production of ventilation by phrenic nerve pacing has been successfully in the United States, Finland and Austria as an alternative to the mechanical ventilator^{76;146} for

individuals with paralyzed diaphragm. In addition, ventilation can occur and tidal volume can increase by electrically stimulating abdominal muscles superficially ^{147;148}.

Functional magnetic stimulation improves coughing in cervical SCI ^{148;149;149;150} and pulmonary function in animals ¹⁵¹, healthy individuals ^{150;152;153} and other SCI individuals ¹⁵⁰. Magnetic stimulation applies the law of Faraday that: *whenever a magnetic field changes, an electrical field is induced* ¹⁵². A magnetic field generated from the magnetic coil is able to pass through high-resistance structures, such as skin, fat, or bone, to stimulate underlying nerves, including the brain ¹⁵¹.

Lin et al., ¹⁴⁹⁻¹⁵² report a series of investigations about the effectiveness of functional magnetic stimulation as a method for restoring maximal expiratory pressure with the associated restoration of cough. Functional magnetic stimulation's magnetic coil was located along the lower thoracic spine (between spinous processes T6 –T12) to stimulate expiratory muscles. The authors find that this technique is also effective in increasing maximal expiratory pressure, as well as forced expiratory flow ¹⁵². In addition, after 4-week functional magnetic stimulation expiratory muscle training in SCI individuals, values of maximal expiratory pressure and forced expiratory flow at total lung capacity and at functional residual capacity significantly increased. The most relevant finding is that expiratory reserve volume increased significantly, indicating improvements in expiratory muscle function ¹⁵⁰.

3 Respiratory Muscle Training

Respiratory muscle training improves inspiratory and expiratory pressure, total lung capacity, peak oxygen consumption, and minute ventilation. It is believed to induce central plasticity ¹⁵⁴ in patients with restrictive thoracic disease ¹⁵⁵ and in acute and chronic SCI individuals ^{97;156}. Respiratory muscle training is performed in two modalities: inspiratory muscle strength training and expiratory muscle strength training. Inspiratory muscle training is widely recommended as an effective respiratory therapy for subjects with chronic obstructive pulmonary disease ¹⁵⁷. Expiratory muscle training results in significant improvements on expiratory muscle strength with the concomitant improvements in pulmonary function and coughing in patients with restrictive thoracic disease ¹⁵⁴ and SCI ⁹⁷.

Van Houtte et al., ⁹⁷ reviewed effectiveness of respiratory muscle training on respiratory muscle strength and endurance, as well as pulmonary function, quality of life, respiratory complications and exercise capacity in SCI individuals. No significant improvements occur in experimental groups in three studies when the effect of inspiratory muscle training was assessed. However, one of the studies ⁹⁷ suggests that expiratory muscles showed to be significantly improved after expiratory muscle training in SCI individuals. Training of the expiratory muscles may increase effectiveness of coughing, thus minimize the risk for respiratory complications ^{86;92}. Finding strategies for improving expiration seems to be essential in restoring pulmonary function in cervical and thoracic SCI individuals.

4. Locomotor Training

Locomotor training is an activity-based therapy that improves metabolic (i.e. oxygen consumption) and cardiorespiratory function (heart rate and pulmonary ventilation) ¹³⁹. Additionally, it has been reported that it improves well-being in persons with chronic SCI ¹⁵⁸ and significantly increases neuromuscular activity and motor activation below the level of the lesion ^{159;160}.

Locomotor training is a task-specific rehabilitation, in which patients are placed on a treadmill with partial body weight support supported by a harness, and therapists manually facilitate the participant to accomplish movements of gait ^{161;162}. Results from our laboratory indicate that spinal interneuronal networks are highly dependent on afferent feedback specific to the motor tasks providing intensive proprioceptive feedback to the spinal network to elicit appropriate output to lower limb muscles ^{159;160}. Further, locomotor training has been successful in improving walking over ground, head and trunk control, ¹⁶² and it may induces cortical reorganization of the representation of walking areas ¹⁶³.

5 Summary and Rationale

Pulmonary function is affected in individuals with cervical and upper thoracic SCI. Even though it is well established that the level of the injury is negatively correlated to pulmonary function, the effect of severity of the injury remains unclear. We propose that pulmonary function is related not only with level but, more prominently, by the severity of the spinal cord injury. Additionally, we suggest that lower pulmonary function values are associated to higher EMG activity of the respiratory muscles above the level of the injury (i.e. accessory muscles). Therefore, we suggest that neuromuscular activation plays a role in

pulmonary function in individuals with SCI. Finally, we propose that responses from afferent input involved in locomotion during locomotor training may also activate the respiratory motoneurons below the level of the spinal cord injury, and thus, improve respiratory function in cervical and upper thoracic SCI.

J. Hypotheses and Specific Aims

1. Overall Aim:

To investigate the role of neurological injury and neural plasticity on pulmonary function after spinal cord injury in humans.

a. Specific Aim 1: To evaluate the effect of injury severity on pulmonary function after spinal cord injury.

i. Hypothesis 1.1: Individuals with cervical motor complete SCI will have lower forced vital capacity, forced expiratory volume in 1 second and maximal respiratory pressures than individuals with cervical motor incomplete SCI.

ii. Hypothesis 1.2: Individuals with upper thoracic motor complete SCI will have lower forced vital capacity, forced expiratory volume in 1 second, maximal and maximal respiratory pressures than individuals with upper thoracic motor incomplete SCI.

1. Measures:

- Forced vital capacity, forced expiratory in one second, maximal inspiratory pressure and maximal expiratory pressure.

b. Specific Aim 2: To investigate the role of neuromuscular activation in pulmonary function in individuals with SCI.

- i. **Hypothesis 2.1:** EMG amplitude of the expiratory muscles (rectus abdominis and obliques) will be directly related to maximal expiratory pressure values in SCI individuals.
- ii. **Hypothesis 2.2:** EMG amplitude of the compensatory muscles for expiration (Latissimus dorsi and pectoralis) will be negatively correlated maximal expiratory pressure values in SCI individuals.
- iii. **Hypothesis 2.3:** EMG amplitude of the inspiratory muscle (external intercostals) will be directly related to maximal inspiratory pressure values in SCI individuals.
- iv. **Hypothesis 2.4:** EMG amplitude of the accessory muscles for inspiration (sternocleidomastoid, scalene and upper trapezius) will be negatively related maximal inspiratory pressure values in SCI individuals.

1. Measures:

- Maximal inspiratory pressure and maximal expiratory pressure
- Simultaneously we recorded integrated EMG of sternocleidomastoid, scalenes, upper trapezius, clavicular portion of pectoralis major, intercostals (6th intercostal space), rectus abdominus, external obliques and latissimus dorsi.

c. **Specific Aim 3:** To investigate effects of locomotor training on pulmonary function in individuals with SCI.

- i. **Hypothesis 3.1:** Individuals with SCI will have significantly higher values of forced vital capacity, forced expiratory volume in 1 second, maximal inspiratory and maximal expiratory pressure after 60 sessions of locomotor training.

1. Measures:

- Maximal inspiratory pressure and maximal expiratory pressure before and after 60 sessions of locomotor training.

CHAPTER II

RESEARCH DESIGN AND METHODS

A. Ethical Approval for Human Studies

This study was formally approved by the University of Louisville Institutional Review Board (University of Louisville IRB: 06.382, 09.0321 and 10.0411) in compliance with all the institutional and federal regulations concerning the ethical use of human volunteers for research studies. Prior to the study, potential subjects were contacted by our research team (University of Louisville IRB: 06. 647) and screened under our screening protocol (University of Louisville IRB: 07.0224).

B. Experimental Procedures

1. Facilities/Resources

The Neuroscience Collaborative Center at the Frazier Rehab Institute (1590 square feet) was the performance site of these research projects. This state-of-the-art rehabilitation facility is equipped with preVent™ pneumotach BreezeSuite (MedGraphics, St. Paul, MN), Cardio Respiratory Diagnostic Systems (MedGraphics, St. Paul, MN) and Respiratory Muscle Analyzer RMA100 (MicroDirect, Lewiston, ME). Eclipse Neurological Workstation (Axon Systems Inc., Hauppauge, NY) for EMG recording.

2. Participants:

Individuals with chronic cervical or thoracic SCI and individuals without SCI (non-injured) participated in this study. Non-injured subjects were included in this study to evaluate normal baseline variables and improve the protocol efficiency of procedures involving individuals with SCI. The majority of the SCI and all of the non-injured participants were involved in this study for 1-2 weeks. The SCI individuals who participated in locomotor training ($n=8$) were involved in the study for about 12-14 weeks which included: baseline screening, pre-training testing, training, post-training testing. Participants' characteristics are shown in table 2.1.

We assessed the pulmonary function in both non-injured and individuals with SCI by using spirometry and surface electromyography (EMG). These methods are routinely used in clinical and research practices.

Group	Neurological level	AIS grade	Gender	Age	Weight (Lb)	Height (in)
Cervical motor complete (n=12)	C	A-B	4F; 8M	37 ($\pm$ 13)	169 ($\pm$ 47)	70 ($\pm$ 3)
Cervical motor incomplete (n=11)	C	C-D	2F; 9M	30 ($\pm$ 8)	184 ($\pm$ 33)	71 ($\pm$ 3)
Thoracic motor complete (n=7)	T	A-B	1F; 6M	37 ($\pm$ 17)	175 ($\pm$ 53)	69 ($\pm$ 5)
Thoracic motor incomplete (n=5)	T	C-D	1F; 4M	40 ($\pm$ 13)	160 ($\pm$ 26)	70 ($\pm$ 4)
Non-injured (n=15)	N/A	N/A	7F; 8M	39 ($\pm$ 10)	165 ($\pm$ 34)	68 ($\pm$ 3)

Table 2.1: Clinical characteristics of all the participants: 12 cervical motor complete SCI, 11 cervical motor incomplete SCI, 7 thoracic motor complete SCI, 5 thoracic motor incomplete SCI and 15 non-injured volunteers.

Participant ID	Neurol. Level	ASIA Grade	Gender	Weight (lb)	Height (in)	Age	Time after injury
A33	C2	A	M	232	74	51	24
A42	C3	A	M	240	71	71	16
C14	C3	C	M	130	76	41	12
C20	C3	C	M	157	72	63	12
D16	C3	D	M	200	61	45	118
A34	C4	A	F	125	66	40	104
A38	C4	A	F	113	69	37	264
A41	C4	A	M	150	72	19	17
B06	C4	B	F	108	67	36	24
X04	C4	D	M	204	73	42	12
X02	C4	D	M	151	71	17	10
B11	C5	B	M	185	68	23	74
X03	C5	B	M	190	71	40	12
C10	C5	B	M	192	74	31	12
B12	C5	B	M	180	75	23	29
X06	C5	C	M	265	73	23	10
C19	C5	C	M	130	62	59	24
D20	C5	D	M	161	72	63	12
D21	C5	D	F	124	66	22	74
A44	C6	A	F	114	63	30	12
D23	C6	D	F	110	69	19	12
C18	C7	B	M	214	71	31	54
C09	C7	C	M	218	72	26	24
B07	T2	B	M	174	73	23	12
A37	T3	A	M	115	74	25	29
A35	T3	A	M	200	73	34	396
A43	T4	A	M	210	74	30	10
A45	T4	A	M	214	72	23	16
D22	T4	D	M	130	68	44	148
A39	T5	A	M	175	70	35	240
C15	T5	C	M	175	72	53	236
D24	T5	D	M	186	73	48	19
D18	T5	D	M	176	72	20	48
A46	T6	A	M	182	73	22	12
X05	T6	D	M	134	64	36	12

Table 2.2: Clinical information from SCI participants including level of injury, AIS grade, gender, weight, height, age and time after injury

a. Recruitment procedures

Participants were recruited from the Frazier Rehab Institute which evaluates approximately 170 SCI patients each year. Our research team has access to a large database of potential participants with spinal cord injury who had manifested their interests in participating in research studies. We identified potential research volunteers based on the characteristics needed for the study (gender, age, level of injury, time since injury, level of physical activity, etc). Additionally, flyers for recruiting both spinal cord injury and non-injured individuals were placed in the different locations at Frazier Rehab Institute and at the University of Louisville campuses.

Once those potential participants were identified we invited them individually for an informational session. All research participants were fully informed of the purposes, risks and potential benefits of participation in the study during the informational session with the research team. The participants signed an informed consent approved by the University of Louisville Institutional Review Board prior to entering the study.

b. Inclusion and exclusion criteria:

i. Non-injured participants:

- had no history of neuromuscular disease, back or joint pain
- were in stable medical condition without cardiopulmonary disease
- were non-smokers

ii. SCI participants:

- were in stable medical condition without cardiopulmonary disease
- had no painful musculoskeletal dysfunction, unhealed fracture, contracture, pressure sore or urinary tract infection that might interfere with training.

- had no clinically significant depression, psychiatric disorders or ongoing drug abuse.
- showed clear indications that the period of spinal shock is concluded determined by presence of muscle tone, deep tendon reflexes or muscle spasms.
- had a non-progressive SCI above T6
- were classified as International Standards for the Neurological classification of Spinal Cord Injury (ISNCSCI) A,B,C or D
- were not ventilator dependent for respiration
- had a sustained SCI at least 6 months prior to entering the study
- were at least 18 years of age

c. Exclusion criteria:

Spinal cord injured and non-injured participants were excluded if: they showed a presence of major cardiovascular or pulmonary disease, endocrine disorders, malignancy, marked obesity, lower extremity deep vein thrombosis, or major gastrointestinal problem such as swallowing or other major medical illness contraindicated for respiratory tests.

3. Equipment

a. Locomotor training

Participants received locomotor training on a Biodex Treadmill and Innoventor Body Weight Support system that includes a force transducer in series with the suspension cable and a pneumatic lifting mechanism to adjust the level of support.

b. Spirometry

Spirometry was performed by using a preVentTM pneumotach with BreezeSuite System (MedGraphics, St. Paul, MN).

c. Maximal inspiratory and expiratory pressure

A differential pressure transducer (MP45-36-871-350) with UPC 2100 PC card and software from Validyne Engineering (Northridge, CA) was used to measure maximal inspiratory and maximal expiratory pressure. Subjects were asked to use a three-way valve system with rubber tube as a mouthpiece (Airlife 001504). The pressure meter incorporated a 1.5 mm diameter leak to prevent glottis closure and to reduce buccal muscle contribution during measurements ¹¹⁷.

d. Electromyography (EMG)

EMG was recorded during the pulmonary functional neurophysiologic assessment testing using an Eclipse Neurological Workstation (Axon Systems Inc., Hauppauge, NY) and RMA100 (MicroDirect, Lewiston, ME) with pair of FE9 silver-silver chloride surface electrodes (Grass Instruments, W Warwick, RI) . The incoming EMG signal was pre-amplified with a gain of 500 and filtered at 30-1000 Hz. The data was sampled at 2000 Hz and mean rectified; burst duration and integrated values were calculated.

4. Experimental Measures

The examination, characterization, and quantification of respiratory function must be carried out within two domains: physical forces, volumes, and flow; and respiratory

muscle activation patterns. Forced vital capacity measures the total volume of air that can be exhaled during a maximal forced expiration effort and forced expiratory volume in one second quantifies the volume of air exhaled in the first second under force after a maximal inhalation. Maximal inspiratory pressure is the highest atmospheric pressure developed during inspiration against an occluded airway. Maximal expiratory pressure is the highest pressure developed during expiration against an occluded airway ^{91;116}.

a. Respiratory function

i. Spirometry

Predicted spirometry values for each subject was based on non-injured individuals with no known pulmonary complaints and derived from gender, age, or height ¹⁶⁴. The following spirometry parameters were obtained: forced vital capacity and forced expiratory volume in 1 second. To measure forced vital capacity, the patient inhales maximally and then exhales as rapidly and as completely as possible. Forced expiratory volume in 1 second is the volume of air exhaled in the first second. To determine the validity of spirometric results, at least three acceptable spirograms were obtained. At least one minute of rest was allowed between each effort, and at least five minutes between each group of three efforts. The range of measurements was calculated for all three efforts in each set.

ii. Maximal Expiratory and Inspiratory Pressure

Maximal inspiratory pressure was measured during maximal inspiratory effort beginning at near residual volume and maximal expiratory pressure was measured during

maximal expiratory effort starting from near total lung capacity¹⁶⁵. Subjects were asked to use a three-way valve system with rubber tube as mouthpiece (Airlife 001504). The pressure meter incorporated a 1.5 mm diameter leak to prevent glottic closure and to reduce buccal muscle contribution during measurements. The assessment required that a sharp, forceful effort be maintained for a minimum of 2 seconds. The maximum pressure was taken as the highest value that is sustained for 1 second^{91;116}. The maximum value from three maneuvers that varied by less than 20% were averaged.

b. Electromyography

The functional neurophysiologic assessment (FNPA) protocol is a comprehensive multi-channel surface EMG recording used to characterize impaired motor control features of limb muscles in persons with upper motor neuron dysfunction¹⁶⁶. EMG patterns developed in response to voluntary and passive lower-limb motor tasks provide information about motor control that includes amplitude and duration of activation of selected muscles as well as their relative characteristic participation in the performance of those motor tasks while in the supine position¹⁶⁶⁻¹⁶⁸. We implemented an adapted FNPA protocol: the pulmonary functional neurophysiologic assessment procedure which involves the performance of voluntary tasks while recording motor unit output to the muscles involved in respiration with the subject sitting and lying supine on a comfortable mat. Figure 2.1 show the spinal segments represented for the myotomes examined.

Bipolar surface electrodes with inter-electrode distance of 2-3 cm were used to measure activity of sternocleidomastoid, scalene, upper trapezius, pectoralis (clavicular portion), 6th intercostal (external), rectus abdominis, external obliques, latissimus dorsi

and paraspinals. The skin over the target muscles was cleaned with alcohol and prepared using mild abrasive conductive paste for pairs of surface electrodes. EMG signals elicited by motor tasks were recorded under standard conditions. Ground electrodes were placed on the shoulders. The recording began with three trials of standard spirometry in sitting position with back and neck supported, followed by three trials each during maximal expiratory and inspiratory pressure maneuvers while forces and EMG were recorded simultaneously.

For all of these active motor tasks, the subject was cued by an audible tone and encouraged to give their best effort by the examiner. Thereafter, the participants were placed in supine position and were asked to relax for five minutes. Relaxation was followed by the performance of three trials each of supine spirometry followed by three trials each during supine Maximal Expiratory/Inspiratory Pressure maneuvers while forces and EMG were recorded simultaneously.

Figure 2.1: Spinal cord segments represented during the pulmonary functional neurophysiologic assessment. We recorded EMG from sternocleidomastoid, scalenes, upper trapezius, pectoralis, intercostals, rectus abdominus, obliques and latissimus dorsi.

Figure 2.2: Electrode placement: sternocleidomastoid, scalene, upper trapezius, pectoralis, external intercostals, rectus abdominis, external obliques and latissimus dorsi. Inter-electrode distance was 3cm from center to center.

i. EMG electrode placement

We recorded external intercostal activity from the lateral (mid-axillary line) portion of the 6th intercostal space. Electrodes for the sternocleidomastoids were located one-third the length of a line from the mastoid process to the suprasternal notch⁹⁴. The electrode placement for scalenes was in the posterior triangle of the neck, at the level of the cricoid cartilage; location that evidenced phasic inspiratory activity¹⁶⁹. Electrode placement for rectus abdominis was bilaterally mid-clavicular line at each side of the umbilicus. Electrodes for external oblique were located lateral side of abdomen, distal from the rectus abdominis electrode location. We recorded the electrical activity of the pectoralis by placing electrodes in the mid-clavicular line, 3rd intercostal space. Finally, the location of electrodes for EMG recording of the latissimus dorsi was immediately

lateral to the lateral scapular border and parallel to the top of the axillary line. Figure 2.2 show the electrodes placement for EMG recording.

c. Verbal commands

Clear understandable verbal commands are very important in order to succeed in the performance of the respiratory tasks evaluated in this study. These verbal commands should be consistent in all the experiments. The verbal commands used in the experiments for these studies were the following:

i. Verbal commands for spirometry:

To Participant: *You will have this nose clip on your nose and you will breathe through this mouth piece. The following test has 3 phases: breathe in, blow it out and breathe in again. It is important that you complete these three phases without stopping or holding your breath. You will hear an audible tone and my instructions when you have to breathe in or out. First: I am going to ask you to take a deep breath in, blow it out as fast and forcedly as you can until all the air is out of your lungs. Keep blowing out even if you feel you don't have any more air left. Then, I am going to ask you to take a deep breath in until your lungs are full. This is the end of the maneuver. Like this (Examiner demonstrated). Do you understand?* If the participant said no, the examiner rephrased and repeated the commands. *We will repeat this task three times and you will have a break in between trials for about 5 minutes.*

Normal breathing...deep breath in, blow it out (here the examiner used verbal encouragement like blow, blow, blow and keep blowing) and then breathe in. Relax.

ii. Verbal commands for maximal inspiratory pressure

To Participant: *We will now measure how much pressure you can create when you breathe in (or inspire) against resistance. You will have this nose clip on your nose and you will breathe through this mouth piece. This mouth piece has a leak and no resistance for you to blow out, but it does have resistance to breathe in. The following test has 2 phases: first you blow it out until you empty your lungs completely and breathe in again through the mouth piece. Like this (Examiner demonstrated). You will feel resistance when you do so, please try to make maximal effort breathing in, since we will be measuring how much pressure you can create during maximal inspiration. Do you understand?* If the participant said no, the examiner rephrased and repeated the commands. *We will repeat this task three times and you will have a break in between trials for about 5 minutes.*

iii. Verbal commands for maximal expiratory pressure

To Participant: *We will now measure how much pressure you can create when you breathe out (or expire) against resistance. You will have this nose clip on your nose and you will breathe through this mouth piece. This mouth piece has a leak and no resistance for you to breathe in, but it does have resistance to blow out. The following test has 2 phases: first you breathe in until you feel that your lungs are full and then blow it out forcedly through the mouth piece. Like this (Examiner demonstrated). You will feel resistance when you do so, please try to make to maximal effort blowing out, since we will be measuring how much pressure you can create during maximal expiration. Do you understand?* If the participant said no, the examiner rephrased and repeated the

commands. *We will repeat this task three times and you will have a break in between trials for about 5 minutes.*

d. Video recording

Video imaging recording was performed during the entire duration of the studies. The recording camera JVC Everio connected to Society of Motion Picture and Television Engineers (SMPTE) timecode box “AVG-50 Active VITC (Vertical Interval Time Code) Generator, Composite HORITA”. SMPTE timecode is a set of cooperating standards to label individual frames of video or film with a time code defined by the Society of Motion Picture and Television Engineers in the SMPTE 12M specification. SMPTE timecodes are added to the video recording to provide a time reference for data analyses.

5. Statistical Analyses

All hypothesis tests were conducted at the 0.05 significance level. Analyses were conducted using the open-source R software package (R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org>). We used means, standard deviations, medians, minima and maxima per group for all the descriptive statistics. For all the statistical analyses we used non-parametric methods. Parametric statistics assume that data have come from a type of probability distribution and makes inferences about the variables of the distribution. In probability theory and statistics, skewness is a measure of the asymmetry of the probability distribution of a real valued random variable. The skewness value can be positive or negative, or even undefined ¹⁷⁰.

Qualitatively, a negative skew indicates that the tail on the left side of the probability density function is longer than the right side and the bulk of the values (including the median) lie to the right of the mean. A positive skew indicates that the tail on the right side is longer than the left side and the bulk of the values lie to the left of the mean. A zero value indicates that the values are relatively evenly distributed on both sides of the mean, typically implying a symmetric distribution.

Non-parametric statistics do not have assumptions about the distribution and it makes it safer than parametric. Since EMG and pressure data are not normally distributed (it actually shows right skewness), it is safer and more powerful to use non-parametric methods. Additionally, non-parametric methods demonstrate to have an efficacy of 95 %

For the two-group comparisons for non-repeated measures data we used Wilcoxon rank sum test ¹⁷¹. Individual comparisons were performed by ranking methods. For the two-group comparisons for repeated measures data we used the “Datta-Satten” rank sum test for clustered data ¹⁷². For the correlations for repeated measures data we used a novel method which is a modification of the non-parametric Kendall correlation for clustered/repeated measures for clustered data ¹⁷³.

CHAPTER III

PULMONARY FUNCTION IN INDIVIDUALS WITH CHRONIC SPINAL CORD INJURY

A. Introduction

Respiratory dysfunction accounts for many of the secondary complications of individuals with chronic spinal cord injury (SCI) ^{38;46;67;70}. In fact, pulmonary complications are the leading cause of death among all SCI individuals, accounting for 21%-24% of deaths ^{68;69}. These respiratory complications include pneumonia, atelectasis, and ventilator-dependent respiratory failure ^{70;71}. Symptoms of respiratory insufficiency are highly correlated with the level of the spinal lesion ⁹⁷⁻⁹⁹. The more rostral and severe the level of injury, the more significant is the respiratory impairment⁴⁶. Decreased values for percent predicted forced vital capacity and forced expiratory volume in one second are associated with increased mortality in chronic SCI patients ⁶⁸. Forced vital capacity and forced expiratory volume in 1 second are increased with descending level of SCI ^{98;99}. In addition, there is a decline in these spirometrical values that is related to aging. Lesions in higher segment of the spinal cord are related to higher prevalence of pulmonary dysfunction, and this detriment occurs exponentially faster in older SCI than in those that are younger ¹⁷⁴.

It is widely acknowledged that patients who are ventilator-independent and suffer from spinal cord lesions in the cervical segments are at risk for developing respiratory

complications⁷³. However, individuals with thoracic injuries are also susceptible to suffer from pulmonary dysfunction. In 2005, Cotton et al., report that 51% of high thoracic (T1-T6) and 28% of T7-12 SCI individuals suffer serious respiratory complications⁷¹. However, other studies suggest that pulmonary function is not affected when the lesion is below the thoracic segment T6^{99;175}; suggesting that impairment on the pulmonary function of individuals in those individuals may be related to other causes like cigarette smoking¹⁷⁶⁻¹⁷⁸ and obesity¹⁷⁹.

The American Spinal Injury Association Impairment Scale (AIS) is a widely accepted tool to determine the neurological level and severity of the injury based on the International Standards for the Neurological Classification of Spinal Cord Injury as AIS A, B, C or D. This scale categorizes the sensory level of spinal cord injury from the perception of light touch and pin prick for dermatomes representing C2 through S5 spinal segments. It also categorizes motor level of spinal cord injury by the evaluation of the strength of contraction of five upper limb and five lower limb muscles representing spinal segments C5 to T1 and L2 to 1 respectively.

There is enough evidence to support that the level of SCI adversely affects pulmonary function^{9;38;46;67;68;70;71;100;103;174;176;177;179}; however, literature on the relationship between severity of the spinal lesion and pulmonary function is limited¹⁷⁵⁻¹⁷⁷. Almenoff et al¹⁷⁶ conducted a survey in a group of 165 SCI individuals to determine influences of smoking and level and completeness of injury. They find that forced vital capacity; forced expiratory volume in 1 second, peak expiratory flow, and maximal voluntary ventilation were inversely correlated with the level of injury. Further, they report that individuals with complete high quadriplegia had significantly lower

pulmonary function than those with incomplete injuries. A key limitation on this study is that the completeness of the injury was not assessed with any of the validated scales. They considered motor complete individuals to those with total loss of motor and sensory function; their incomplete group includes individuals with both sensory and motor function below the injury and those who only preserved some sensory function below the level of the injury. Later Linn et al¹⁷⁷ conducted similar cross-sectional survey¹⁷⁷, corroborating the previous findings. In both studies, individuals with paraplegia motor complete or incomplete showed not to have statistically significant differences in pulmonary function. Again, the main limitation of these studies is the classification of SCI. There is not clear which methods for evaluating sensory or motor function preservation was used.

Maximal inspiratory and expiratory pressures are common clinical procedures used to assess the force production of the respiratory muscles. These values are related to lung volumes and it is a very useful tool used when muscle weakness is suspected to be the cause of low lung volumes¹⁸⁰. To our knowledge, there is only one investigation that was conducted to evaluate maximal respiratory pressures and correlated it with severity of the injury. Mateus et al¹⁷⁵ find that correlations between level of injury and pulmonary function forced vital capacity and forced expiratory volume in 1 second previously reported^{99;176;177} are only corroborated for the spinal motor complete groups (AIS A-B)¹⁷⁵. Further, they report a correlation between maximal respiratory pressures and level of the injury on the AIS A-B, but this relationship is lost in incomplete SCI (C-D) group. These findings are vital in the understanding the importance of severity of the SCI on pulmonary function. However, we are still limited in our ability to identify SCI

individuals who are at higher risk of developing serious pulmonary diseases in chronic phase of SCI.

The aim of this study was to assess pulmonary function in relationship to the *severity* of SCI. We have hypothesized that pulmonary function values in individuals with cervical and upper thoracic motor complete SCI (A-B) were significantly lower than in those with motor incomplete SCI (C-D).

B. Methods

All evaluations were performed in the Neuroscience Collaborative Center at the Frazier Rehab Institute after informed consent was obtained as approved by the Institutional Review Board for human studies of the University of Louisville. Participants with sustained non-progressive cervical (C2-C8) or upper thoracic (T1-T6) SCI were invited to participate in the study. Twenty three chronic cervical and twelve upper thoracic SCI subjects (8 women, 27men) cervical or upper thoracic SCI (C3-T6) were evaluated. Clinical characteristics including AIS grade, age, weight and height are presented in table 2.1. Participants were excluded if they suffered from any cardiopulmonary or musculoskeletal dysfunction. Participants had sustained SCI at least 6 months prior to entering the study and were non ventilator-dependent for respiration.

1. Clinical assessments:

If potential volunteers met the inclusion criteria, they had a medical evaluation with a physician. Later, the American Spinal Injury Association Impairment Scale (AIS) was used by our physical therapist to determine the neurological level and severity of the

injury based on the International Standards for the Neurological Classification of Spinal Cord Injury as AIS A, B, C or D. This scale categorizes the sensory level of spinal cord injury from the perception of light touch and pin prick for dermatomes representing C2 through S5 spinal segments. It also categorizes motor level of spinal cord injury by the evaluation of the strength of contraction of five upper limb and five lower limb muscles representing spinal segments C5 to T1 and L2 to 1, respectively. For this study twelve SCI participants were classified as cervical motor complete (AIS A-B), eleven SCI participants were classified as cervical motor incomplete (AIS C-D), seven SCI participants were classified as thoracic motor complete (AIS A-B) and five SCI participants were classified as thoracic motor incomplete (AIS C-D). Level and severity were confirmed by our physician. Clinical characteristics are presented in Table 3.1.

Group	AIS grade	Gender	Age	Weight (Lb)	Height (in)
Cervical motor complete (n=12)	A-B (n=12)	4F; 8M	37 ($\pm$ 13)	169 ($\pm$ 47)	70 ($\pm$ 3)
Cervical motor incomplete (n=11)	C-D (n=11)	2F; 9M	30 ($\pm$ 8)	184 ($\pm$ 33)	71 ($\pm$ 3)
Thoracic motor complete (n=7)	A-B (n=7)	1F; 6M	37 ($\pm$ 17)	175 ($\pm$ 53)	69 ($\pm$ 5)
Thoracic motor incomplete (n=5)	C-D (n=5)	1F; 4M	40 ($\pm$ 13)	160 ($\pm$ 26)	70 ($\pm$ 4)

Table 3.1: clinical characteristics

2. Equipment:

a. Spirometry

Spirometry testing was performed using a preVentTM pneumotach with BreezeSuite System (MedGraphics, St. Paul, MN).

b. Maximal inspiratory and expiratory pressure

A differential pressure transducer (MP45-36-871-350) with UPC 2100 PC card and software from Validyne Engineering (Northridge, CA) was used to measure maximal inspiratory and maximal expiratory pressure. Subjects were asked to use a three-way valve system with rubber tube as a mouthpiece (Airlife 001504). The pressure meter incorporated a 1.5 mm diameter leak to prevent glottis closure and to reduce buccal muscle contribution during measurements ¹¹⁷.

3 Procedures:

Spirometry and maximal inspiratory and expiratory pressure were performed ^{181;182} while individuals were seated in their own wheelchairs. Instruments were calibrated daily according to the manufacturer's procedure, using a volumetric syringe. Subjects were tested seated on their wheelchair. Since all the subjects in this study met the American Thoracic Society criteria for acceptability, no modifications to guidelines for pulmonary function test in SCI patients were needed ¹⁸³ To determine the validity of spirometric results, at least three acceptable spirograms were obtained and the best attempt was used ¹⁶⁵. At least one minute of rest was allowed between each effort, and at least five minutes between each group of three efforts. Predicted forced vital capacity and forced expiratory volume in 1 second values for each subject are based on data from

non-injured individuals with no known pulmonary complaints and derived from gender, age, and height ⁹¹ .

Maximal inspiratory pressure was measured during maximal inspiratory effort from near residual volume (i.e. the volume of air still remaining in the lungs after a forced expiration). Maximal expiratory pressure was measured during maximal expiratory effort from near total lung capacity (i.e. the volume of air that is in the lungs after a maximal inspiration). The maximum pressure is taken as the highest value that can be sustained for a minimum of one second ⁹¹. The assessment of maximal pressures required a sharp, forceful effort maintained for a minimum of 2 s.

4. Statistical Methods

Forced expiratory volume, forced vital capacity, maximal expiratory and inspiratory pressure were summarized graphically with boxplots and with means and standard deviations by injury type (cervical complete, cervical incomplete, thoracic complete, thoracic incomplete). Comparisons of Forced expiratory volume, forced vital capacity, maximal expiratory and inspiratory pressure between selected pairs of groups were conducted using a nonparametric rank sum test for clustered data ¹³⁰. Nonparametric, clustered data tests were required due to the triplicate measurements of maximal expiratory and inspiratory pressure for each subject and to guard against violations of assumptions required for parametric tests (e.g. normality).

5. Results:

a. Differences in pulmonary function based on severity of the SCI

Figure 3.1: Mean group values with standard deviation for forced expiratory volume in 1 second (FEV1%) and forced vital capacity (FVC %) expressed as percentage of predicted values in individuals with motor complete (gray) and motor incomplete (black) SCI. Cervical motor incomplete group have significantly higher FEV1 % ($p=0.016$) and FVC % ($p=0.006$) than cervical motor complete group. FEV1 % for the thoracic motor incomplete group is not significantly different than in the motor incomplete. FVC % in thoracic motor incomplete shows a trend towards higher than in motor complete, however the difference is not statistically significant ($p=0.07$). The red-dashed lines represent normal predicted pulmonary function values for non-injured individuals. Mean values for both, cervical and, thoracic motor incomplete groups fall at or above 80% of predicted values. However, there is more variability on the cervical group.

Level	FEV1 Actual		FVC Actual	
	Complete	Incomplete	Complete	Incomplete
Cervical	2.1 ± 0.8	2.7 ± 0.9	2.6 ± 0.9	3.7 ± 1.2
Thoracic	3.0 ± 1.0	3.4 ± 0.6	3.9 ± 1.1	4.8 ± 0.7

Table 3.2: Mean group values with standard deviation for forced expiratory volume in 1 second (FEV1 Actual) and forced vital capacity (FVC Actual) expressed in liters in individuals with motor complete and motor incomplete SCI.

Figure 3.2: Maximal expiratory and maximal inspiratory pressure in cervical motor complete spinal cord injury and cervical motor incomplete spinal cord injury expressed in cm of H₂O. There were not significant differences between cervical motor complete and cervical motor incomplete groups. Similarly, there were not significant differences between thoracic motor complete and thoracic motor incomplete groups in any of the respiratory pressures.

Figure 3.3: Forced vital capacity and forced expiratory volume in one second in thirty-five SCI participants. Percentages of predicted values overlap in the cervical complete, cervical incomplete, thoracic complete and thoracic incomplete groups. There is an important variability on the percentage of predicted values that are independent of level and severity of the spinal cord injury.

Figure 3.4: Relation between forced vital capacity and the American Spinal Injury Association Impairment Scale (AIS) motor scores. Pulmonary function is variable even in individuals with the same level and severity of the injury, indicating that assessment of motor preservation based on the AIS is not sufficient to characterize the pulmonary dysfunction that occurs after a neurological injury.

Figure 3.5 shows the relationship between AIS motor score and forced vital capacity as percentage predicted. Individuals with the same level and severity of injury show variable values of forced vital capacity. In particular, individuals with thoracic motor complete lesions who receive the same motor grade show a wide range predicted values ranging from severe and mild pulmonary dysfunction to normal pulmonary function values. Similar trend is observed in figure 3.6 in which forced expiratory volume in 1 second and AIS motor scores are plotted. Maximal expiratory and inspiratory pressures show a similar trend. Individuals with the same level and AIS classification have very different pressure values. This is evident for all the groups, but in particular among those who had suffered from a cervical and thoracic motor complete SCI. The AIS score cannot be used as a predictor of pulmonary function.

Forced Expiratory volume in 1 sec. vs. AIS motor score

Figure 3.5: Relation between forced expiratory volume in 1 second and the American Spinal Injury Association Impairment Scale (AIS) motor scores. Pulmonary function is variable even and sensory preservation based on the AIS is not sufficient to characterize the pulmonary dysfunction that occurs after a neurological injury.

Figure 3.6: Relation between maximal expiratory pressure and the American Spinal Injury Association Impairment Scale (AIS) motor scores. Maximal expiratory pressure is variable even in individuals with the same level and severity of the injury, indicating that assessment of motor preservation based on the AIS is not sufficient to characterize the pulmonary dysfunction that occurs after a neurological injury.

Figure 3.7: Relation between maximal inspiratory pressure and the American Spinal Injury Association Impairment Scale (AIS) motor scores. Maximal inspiratory pressure is variable even in individuals with the same level and severity of the injury, indicating that assessment of motor preservation based on the AIS is not sufficient to characterize the pulmonary dysfunction that occurs after a neurological injury.

6. Discussion

We compared pulmonary function among 4 groups: cervical motor complete SCI (C2-8 AIS grades A-B), cervical motor incomplete SCI (C2-8 AIS grades C-D), upper thoracic motor complete SCI (T1-6 AIS grades A and B) and upper thoracic motor incomplete SCI (T1-6 AIS grades C-D). Previously, a number of investigations using linear regression analysis suggested that there is a negative correlation between level of the injury and pulmonary function (most precisely forced expiratory volume in one second and forced vital capacity). Later, there was a valid argument suggesting that severity or completeness of the injury might play a role on pulmonary dysfunction after SCI. The results from a large transversal study in chronic spinal cord injured patients¹⁷⁵ revealed that there is a positive correlation between maximal respiratory pressures and level of the injury only in the motor complete SCI (AIS grades A and B). However, this was not the case for the motor incomplete SCI group (AIS grade C-D).

We tested the hypotheses that individuals with motor incomplete SCI (AIS grades C and D) have significantly higher forced vital capacity, forced expiratory volume in one second and maximal respiratory pressures than those who have motor complete injuries (AIS grades C and D). Our findings are to some extent in agreement with the findings of Mateus et al¹⁷⁵. We compared pulmonary function in cervical motor complete and cervical motor incomplete SCI; the latter showed significantly higher values for both forced vital capacity and forced expiratory volume in one second. On the other hand, we found no statistically significant differences on pulmonary function between the thoracic motor complete and thoracic motor incomplete SCI groups.

Assessment of motor and sensory preservation based on preservation scales are not sufficient to characterize the pulmonary dysfunction that occurs after a neurological injury. There is a large variability on values of forced expiratory volume in 1 second, forced vital capacity and respiratory pressures within each neurological level group. Data from the present study and from others ⁷¹ suggest that some individuals with thoracic SCI are also at a high risk for developing pulmonary dysfunction. Similarly, as shown in figure 3.4, some individuals with cervical complete or incomplete injuries have pulmonary function values close to normal or normal.

The American Spinal Injury Association Impairment Scale (AIS) is the most widely implemented scale in both acute and chronic SCI evaluations. A key limitation is that the motor evaluation does not evaluate thoracic segments of the spinal cord. Multiple thoracic spinal nerve segments innervate the external and internal intercostals, rectus abdominis and external and internal obliques. Therefore, this scale does not provide any information about the preservation of motor function on these respiratory muscles. Another limitation is the subjective characteristics of the muscle grading.

Spinal nerves have overlapping supply; this is a protective mechanism against injury to assure innervation to a given muscle. Each respiratory muscle in particular is innervated by multiple spinal nerve segments. The AIS scale muscle grading ranges from 0 (total paralysis) to 5 (able to exert against resistance in full range of motion). Let consider the case that a patient receives a grade of 0 for the C5 myotome because she or he is unable to perform elbow flexion and there is not visible muscle contraction. It does not necessarily mean that other muscles that are supplied by the same spinal nerve segment (diaphragm, pectoralis major and scalenes) are also paralyzed.

In view of this controversy, level and severity of SCI should not be used as a predictor of risk for developing pneumonia and other respiratory diseases after SCI. It is necessary to understand the neural mechanisms responsible for these differences in pulmonary function. These findings lead us to aim 2, in which we investigated the role of neuromuscular activation in maximal respiratory pressures in individuals with SCI and compared them to non-injured individuals.

CHAPTER IV

RESPIRATORY MUSCLE ACTIVATION DURING MAXIMAL INSPIRATORY AND EXPIRATORY PRESSURE IN INDIVIDUALS WITH CHRONIC SPINAL CORD INJURY

A Introduction

Respiratory complications are the leading cause of death among spinal cord injured (SCI) individuals ^{68;69}. Respiratory complications include: pneumonia and atelectasis ^{70;71} and are related to abnormal respiratory muscles activity ^{44;128;129}. In individuals with intact diaphragmatic activity, respiratory function may be affected when respiratory muscles below the level of the injury are paralyzed or weak. Spinal cord injury can result in neuromuscular paralysis of the respiratory muscles resulting in decreases in pulmonary function.

Muscle weakness is another feature frequently observed in SCI individuals. In contrast to uninjured individuals, the upper anterior rib cage moves inward during inspiration in persons with cervical or high thoracic SCI ¹⁰⁷. This inappropriate rib-cage movement is the result of the excessive compliance of the abdominal wall ^{96;180}, and is more frequent in cervical than thoracic SCI. The paradoxical rib cage movement does not occur uniformly across all the cervical SCI individuals ^{108;109;111}. It depends on the rib cage elasticity and on the electrical activity of the accessory muscles for inspiration.

When electromyographic (EMG) recording from scalenes is silent (i.e. paralyzed scalene muscles), paradoxal movement of the rib cage is consistently present ¹⁰⁸.

In healthy non-injured individuals, pulmonary function is associated with the neural activation of muscles involved in respiration ^{44;128;129}. EMG of intercostal muscles show significant increases in root mean square values with incremental respiratory loads in inspiratory muscle endurance tests in healthy female individuals¹²⁸. Scalene, sternocleidomastoid and transversus abdominis EMG activity exhibit a significant linear correlation during gradual production of expiratory and inspiratory mouth pressure to maximal. Trapezius demonstrated to have a non-linear correlation with maximal inspiratory mouth pressure but it was recruited at 90% of the maximal ¹²⁹. Further, lower and upper abdominal muscles, external obliques and transverses abdominis are electrically active when expiration occurs ⁷⁴. Studies in animals also suggest that respiratory capacity is related to EMG of respiratory muscles. EMG activity of parasternal intercostals, triangularis sterni and scalene muscles in anesthetized dogs increases as lung volume increases during inspiration ¹³¹.

When a neurological injury occurs, like in the case of lesions in the spinal cord, the neural activation of muscles is being compromised. We suggest that there is plasticity after SCI that may change the respiratory muscle activation pattern in order to compensate for the loss of motor function below the level of the lesion. The aim of this study is to investigate the role of neuromuscular activation underlying the differences in pulmonary function in individuals with SCI. We hypothesize that lower maximal

expiratory and inspiratory pressure in SCI individuals are related to lower expiratory and inspiratory muscle activity. Further, we hypothesize that lower maximal expiratory and inspiratory pressure values are related to higher accessory activity of the pectoralis and latissimus dorsi and the sternocleidomastoid, scalenes and upper trapezius respectively.

B Methods and materials

1. Participants:

Twenty four individuals with chronic cervical or thoracic SCI and fifteen individuals without SCI (non-injured) participated in this study. We assessed the respiratory muscle strength in both non-injured and individuals with SCI by using maximal expiratory and inspiratory pressure evaluations and surface EMG. For this study six SCI participants were classified as cervical motor complete (AIS A-B), seven SCI participants were classified as cervical motor incomplete (AIS C-D), seven SCI participants were classified as thoracic motor complete (AIS A-B) and four SCI participants were classified as thoracic motor incomplete (AIS C-D). Level and severity were confirmed by our physician. Clinical characteristics are presented in Table 4.1.

Group	Neurological level	AIS grade	Gender	Age	Weight (Lb)	Height (in)
Cervical motor complete (n=6)	C	A (n=3) B (n=3)	2F; 4M	33 ($\pm$ 11)	158 ($\pm$ 46)	70 ($\pm$ 3)
Cervical motor incomplete (n=7)	C	C (n=4) D (n=3)	2F; 5M	44($\pm$ 16)	170 ($\pm$ 50)	68 ($\pm$ 6)
Thoracic motor complete (n=7)	T	A (n=6) B (n=1)	1F; 6M	30 ($\pm$ 8)	184 ($\pm$ 34)	71 ($\pm$ 4)
Thoracic motor incomplete (n=5)	T	C (n=2) D (n=2)	4M	34 ($\pm$ 14)	167 ($\pm$ 25)	71 ($\pm$ 2)
Non-injured (n=15)	N/A	N/A	7F; 8M	39 ($\pm$ 10)	165 ($\pm$ 34)	68 ($\pm$ 3)

Table 4.1: Clinical characteristics of spinal cord injured and non-injured participants

2. Clinical assessments:

If potential volunteers met the inclusion criteria, they had a medical evaluation with our physician. Later, the American Spinal Injury Association Impairment Scale (AIS) was used by our Physical Therapist to determine the neurological level and severity of the injury based on the International Standards for the Neurological Classification of Spinal Cord Injury as AIS A, B, C or D. This scale categorizes the sensory level of spinal cord injury from the perception of light touch and pin prick for dermatomes representing C2 through S5 spinal segments. It also categorizes motor level of spinal cord injury by the evaluation of the strength of contraction of five upper limb and five lower limb muscles representing spinal segments C5 to T1 and L2 to 1 respectively.

3. Equipment:

a. Maximal inspiratory and expiratory pressure

A differential pressure transducer (MP45-36-871-350) with UPC 2100 PC card and software from Validyne Engineering (Northridge, CA) were used to measure maximal inspiratory and maximal expiratory pressure. Subjects were asked to use a three-way valve system with rubber tube as a mouthpiece (Airlife 001504). The pressure meter incorporated a 1.5 mm diameter leak to prevent glottis closure and to reduce buccal muscle contribution during measurements¹¹⁷.

b. Electromyography

EMG was recorded during the pulmonary functional neurophysiologic assessment testing using an Eclipse Neurological Workstation (Axon Systems Inc., Hauppauge, NY) and RMA100 (MicroDirect, Lewiston, ME) with pair of FE9 silver-silver chloride surface electrodes (Grass Instruments, W Warwick, RI) . The incoming EMG signal was pre-amplified with a gain of 500 and filtered at 30-1000 Hz. The data were sampled at 2000 Hz and mean rectified. Burst duration and integrated values were calculated.

4. Procedures:

Once that level and severity were established, participants were asked to come to our pulmonary function laboratory where standard spirometry and maximal inspiratory and expiratory pressure were performed ^{181;182}. Instruments were calibrated daily according to the manufacturer's procedure, using a volumetric syringe. Subjects were tested seated on their own wheelchair. Since all subjects in this study met the American Thoracic Society criteria for acceptability, no modifications to guidelines for pulmonary function test in SCI patients were needed ¹⁸³ To determine the validity of spirometric results, at least three acceptable spirograms were obtained with the result of the best attempt was used ¹⁶⁵. At least one minute of rest was allowed between each effort, and at least five minutes between each group of three efforts. Predicted forced vital capacity and forced expiratory volume in 1 second values for each subject were based on data from non-injured individuals with no known pulmonary complaints and derived from gender, age, and height⁹¹ .

Maximal inspiratory pressure was measured during maximal inspiratory effort from near residual volume. Maximal expiratory pressure was measured during maximal expiratory effort from near total lung capacity. The maximum pressure is taken as the highest value that can be sustained for a minimum of one second⁹¹. The assessment of maximal pressures required a sharp, forceful effort maintained for a minimum of 2 s.

5. Statistical Methods

All measured outcomes – forced vital capacity (FVC), forced expiratory volume within 1 minute (FEV1), maximal expiratory pressure (MEP), maximal inspiratory pressure (MIP), and integrated EMG – were summarized with means and standard deviations, medians, minima, and maxima. Summary statistics were calculated by study group (cervical complete, cervical incomplete, thoracic complete, thoracic incomplete). Outcomes data were also summarized graphically with boxplots for univariate data and scatterplots for bivariate data.

To evaluate our study hypotheses, we used a set of recently developed nonparametric test statistics for clustered data. Nonparametric methods were required due to skewness in the distributions of our outcome measures, which violates the normality assumption required for parametric methods. Clustered data methods were required due to the triplicate measures of FVC, FEV, MEP, MIP, and integrated EMG taken on each subject. Repeated measurements on subjects are one type of clustered data, and typically violate the independence assumption required of standard parametric and nonparametric statistical methods. The clustered data methods we applied can be considered to be nonparametric analogues of test statistics for independent (unclustered)

data. Comparisons among injury groups of FVC, FEV1, MEP, MIP, and integrated EMG were conducted using nonparametric rank sum tests for clustered data¹⁸⁴. These tests are clustered data analogues of the well-known Wilcoxon rank sum test and Kruskal-Wallis test for two and two or more groups, respectively. The relationship between muscle-specific integrated EMG and MEP and MIP were assessed by the calculation of nonparametric marginal Kendall correlation coefficients for clustered data¹⁷³. This correlation coefficient is the clustered data analogue of the often-used nonparametric Kendall correlation coefficient. Asymptotic 95% confidence intervals for the correlation coefficients were calculated using the variance formulas published in the source work. All hypothesis tests were conducted at the 0.05 level, and all analysis were conducted using the open-source R software environment (R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, v. 2.12.2).

Figure 4.1: EMG activity of right upper trapezius (RUT), right pectoralis (RPEC), right 6th intercostals (RIC₆), right rectus abdominis (RRA), right obliques (ROB), left upper trapezius (LUT), left pectoralis (LPEC), left 6th intercostals (LIC₆), left rectus abdominis (LRA), left obliques (LOB) in a non-injured (NI) and a cervical motor complete AIS A SCI (A33) during maximal expiratory pressure. NI show higher pressures during the task with activation of right and left obliques and intercostals. SCI individual shows silent activity of expiratory muscles and asymmetrical activation of the pectoralis (RPEC) as a compensatory strategy for the paralyzed muscles below the level of injury.

Figure 4.2: EMG activity of right upper trapezius (RUT), right pectoralis (RPEC), right 6th intercostals (RIC6), right rectus abdominis (RRA), right obliques (ROB), left upper trapezius (LUT), left pectoralis (LPEC), left 6th intercostals (LIC6), left rectus abdominis (LRA), left obliques (LOB) in a non-injured (NI) and a cervical motor complete AIS A SCI (A33) during maximal inspiratory pressure. NI show higher pressures during the task with activation of right and left obliques and intercostals. SCI individual shows silent activity of expiratory muscles and asymmetrical activation of the pectoralis (RPEC) as a compensatory strategy for the paralyzed muscles below the level of injury.

Figure 4.3: Correlations between maximal expiratory pressure (MEP) expressed in cm of H₂O and integrated EMG ($\mu\text{V} \cdot \text{sec}$). Higher MEP is correlated with higher integrated EMG of the rectus abdominis 0.43 (95 % confidence interval 0.28, 0.58; $p < 0.0001$) and obliques 0.48 (95 % confidence interval 0.27, 0.68; $p < 0.0001$) in the all SCI group.

i. Rectus abdominis:

Figure 4.1 shows the correlation between maximal expiratory pressure (MEP) and integrated EMG of the rectus abdomini and obliques. Integrated EMG of the rectus abdominis (uV* sec) correlated with MEP for all the SCI subjects with a correlation value of 0.43, with a significance $p < 0.0001$. Similarly, when non-injured individuals were added, there is still a correlation between integrated EMG and MEP (0.46, $p < 0.0001$). In the non-injured group there is no correlation between integrated EMG and MEP (0.21, $p = 0.13$).

When considering all the cervical SCI individuals, there is a correlation between integrated EMG of the rectus abdominis and MEP (0.55, $p = 0.002$). Further, when cervical incomplete only also showed a significant positive correlation between integrated EMG of the rectus abdominis and MEP (0.51, $p < 0.0001$). Cervical motor complete group show no correlation between integrated EMG and MEP (0.13, $p = 0.73$). When considering all the thoracic SCI individuals, there is a correlation between integrated EMG of the rectus abdominis and MEP (0.28, $p = 0.006$).

ii. Oblique Abdominis

Integrated EMG of the oblique abdominis (uV* sec) correlated with maximal expiratory pressure (MEP) for all the SCI subjects with a correlation value of 0.48, with a significance $p < 0.0001$. Similarly, when non-injured individuals were added, there is a positive correlation between integrated EMG and MEP (0.50, $p < 0.0001$). Further, there is a positive correlation between integrated EMG and MEP in the non-injured group (0.37, $p = 0.005$).

When considering all the cervical SCI individuals, there is a correlation between integrated EMG of the oblique abdominis and MEP (0.52, $p=0.01$). Further, when cervical incomplete only also showed a significant positive correlation between integrated EMG of the oblique abdominis and MEP (0.66, $p<0.0001$). There is no correlation between integrated EMG and MEP in the cervical motor complete group (0.04, $p=0.92$). When considering all the thoracic SCI individuals, there is a correlation between integrated EMG of the oblique abdominis and MEP (0.32, $p=0.001$). Similarly, there is a strong positive correlation between integrated EMG of the obliques and MEP in the thoracic incomplete group (0.70, $p=0.002$). However, there is no correlation observed between integrated EMG and MEP in the thoracic motor complete group (0.30, $p=0.11$).

ACCESSORY MUSCLES FOR EXPIRATION

- **Figure 4.4:** Correlations between maximal expiratory pressure (expressed in cm of H₂O) and integrated EMG (μV* sec). Integrated EMG of the Latissimus dorsi and pectoralis correlate negatively with MEP in all subjects.

iii. **Latissimus dorsi:**

Integrated EMG of the latissimus dorsi (uV* sec) did not correlate with maximal expiratory pressure (MEP) in all the SCI ($-0.21, p=0.24$). However, when non-injured individuals were added, there was a negative correlation between integrated EMG and MEP ($-0.37, p< 0.0008$). There was no correlation between integrated EMG and MEP when only non-injured individuals were considered ($-0.13, p=0.58$).

When considering only all cervical SCI individuals, there is a negative correlation between integrated EMG of the Latissimus dorsi and MEP ($-0.43, p=0.05$). Further, when cervical motor complete only were considered, there was a strong negative correlation ($-0.78, p=0.001$). In the cervical motor incomplete group, the negative correlation between integrated EMG of the latissimus dorsi and MEP was insignificant ($-0.56, p=0.054$).

When considering the entire thoracic SCI individuals, there was not correlation between integrated EMG of the latissimus and MEP ($0.14, p=0.57$). Neither is there a correlation when the thoracic motor complete ($0.39, p=0.07$) or thoracic motor incomplete ($-0.56, p=0.22$) were considered.

iv. **Pectoralis:**

There was a negative correlation between integrated EMG of the pectoralis and MEP ($-0.24, p=0.007$) for the entire SCI+ non-injured group. There is no correlation between integrated EMG and MEP when only non-injured ($0.10, p=0.56$) were considered. Further, there is not correlation in the entire SCI group ($-0.03, p= 0.84$), nor on cervical ($0.07, p=0.73$), cervical motor complete ($0.36, p=0.10$), cervical motor incomplete ($-0.20, p=0.66$). Neither were there correlations of integrated EMG of the

pectoralis and MEP for the thoracic SCI group ($-0.09, p=0.66$), for the thoracic motor complete ($0.39, p=0.06$) or thoracic motor incomplete groups ($0.48, p=0.15$).

b. **Inspiratory Muscles: Correlations between Integrated EMG and Maximal Inspiratory Pressure**

Figure 4.5: Correlation between integrated EMG (uV *sec) of the intercostals and Maximal Inspiratory Pressure (cmH20)

i. Intercostals:

Integrated EMG of intercostals (uV* sec) has a strong correlation with maximal expiratory pressure (MEP) in the non-injured group (0.30, $p < 0.0001$), but not in the SCI group (0.05, $p = 0.56$). When we considered the cervical SCI, again, there was no correlation between variables (0.15, $p = 0.26$). However, when we considered the severity of the injury, the cervical motor incomplete subset showed a strong correlation between integrated EMG of the intercostals and MEP (0.23, $p < 0.0001$). This correlation, however, was not observed in the cervical motor complete group (0.27, $p = 0.11$).

When the entire thoracic group was considered (complete + incomplete), there was a negative correlation between integrated EMG of intercostals and MEP (-0.27, $p = 0.01$). In addition, thoracic motor complete group showed also a strong negative correlation between these variables (-0.41, $p < 0.0001$), but not in the thoracic motor incomplete group (-0.19, $p = 0.63$).

Figure 4.6: Correlation between integrated EMG (uV *sec) of the upper trapezius and Maximal Inspiratory Pressure (cmH2O)

ii. Upper Trapezius:

There were no correlations between integrated EMG (uV * sec) of the upper trapezius and MEP in any of the following groups: all subjects (0.09, $p=0.36$), non-injured (0.10, $p=0.56$), the entire SCI group (0.09, $p=0.49$), cervical SCI (0.27, $p=0.07$) or thoracic SCI groups (0.22, $p=0.24$). The only subset showing a strong positive correlation was the cervical motor incomplete SCI (0.53, $p=0.002$)

Group	Scalenes	Sternocleidomastoid
Non-injured	105 ($\pm$ 76)	145 ($\pm$ 59)
Cervical Complete	241 ($\pm$ 196)	176 ($\pm$ 122)
Cervical Incomplete	212 ($\pm$ 109)	221 ($\pm$ 93)
Thoracic Complete	200 ($\pm$ 134)	226 ($\pm$ 189)
Thoracic Incomplete	132 ($\pm$ 37)	183 ($\pm$ 104)

Table 4.2: Means and standard deviations of integrated EMG of the sternocleidomastoid and scalenes for the non-injured, cervical complete, cervical incomplete, thoracic complete and thoracic incomplete groups. Neither, integrated EMG of the scalenes or sternocleidomastoid correlated with maximal inspiratory pressures.

iii Scalenes:

No correlation was found between integrated EMG (uV *sec) of scalenes and MIP in any of the SCI or non-injured groups.

iii. Sternocleidomastoid:

No correlation was found between integrated EMG (uV *sec) of the sternocleidomastoid and MIP in any of the SCI or non-injured groups.

7. Conclusion

After SCI there is a plasticity of muscles involved in respiration that compensate for other weak or paralyzed muscles that were unable to contract efficiently to keep up with the respiratory demands. However, higher expiratory pressures were related with a higher neural activation of the rectus abdominis and obliques. These muscles are activated during maximal expiratory tasks. Integrated EMG of the latissimus dorsi showed a negative correlation with maximal expiratory pressures in the cervical SCI individuals, especially in the cervical motor complete group. In other words, higher EMG of this muscle are related with lower pressure, which suggest that this muscle is being recruited to compensate with a maximal expiratory tasks when the expiratory muscles below the level of the lesion (rectus abdominis and obliques) were unable to do so. This confirmed our hypothesis that latissimus dorsi acted as a compensatory muscle for maximal expiration, but only for cervical SCI individuals.

Surprisingly, EMG amplitude of the pectoralis was not correlated to maximal expiratory pressure. De Troyer et al^{86;87} report that expiration in SCI is an active process that involves the activation of the pectoralis. Our findings add to the findings of De

Troyer et al⁸⁷ that the activation of the clavicular portion of the pectoralis as a compensatory action during maximal expiratory tasks is not consistent in all the cervical or upper thoracic SCI.

Integrated EMG of intercostals has a strong positive correlation with maximal inspiratory pressure in the non-injured group and cervical motor incomplete. The thoracic motor complete group shows a negative correlation. In other words: lower inspiratory pressures are related to higher EMG, suggesting that more motor pools were being recruited for doing the task, but it was not enough to compensate for the lower intercostal segments innervated below the level of the injury.

CHAPTER V

EFFECTS OF LOCOMOTOR TRAINING ON PULMONARY FUNCTION IN INDIVIDUALS WITH CHRONIC SPINAL CORD INJURY

A. Introduction

Different therapies have been implemented to improve pulmonary function in individuals with SCI. These therapies include abdominal binders¹³²⁻¹³⁴, respiratory muscle training^{97;117;154-157}, functional electrical^{144;147;148 101;141;144;148} and magnetic^{149;150;152;153} stimulation and exercise^{86;135-137}. More recently locomotor training has been suggested as another rehabilitation intervention to improve metabolism, heart rate and pulmonary ventilation in SCI individuals¹³⁹. Locomotor training (LT) is an activity-based therapy in which muscles below the level of the lesion are activated and strengthened¹⁸⁵. The effects of other activity-based therapies has been studied, demonstrating its value in maintaining muscle mass, decrease osteoporosis, diminishing the number and severity of infections in SCI individuals¹⁸⁶. Locomotor training improves metabolic and cardiorespiratory function in SCI individuals¹³⁹. Locomotor training is a task-specific rehabilitation, in which patients are placed on a treadmill with partial body weight support supported by a harness, and therapists manually facilitate the patient to accomplish movements of gait^{139;161;162}.

The effect of locomotor training on neural plasticity is well documented in both animal and human studies ^{159;187}. This activity-dependent plasticity occurs at the level of interneuronal and propriospinal systems ¹⁸⁷. The lumbar propriospinal neurons project to motoneurons that innervate lower limbs ^{14;29}. Reciprocal connections between cervical and lumbar motor circuits also exist ^{29;32}. Studies performed in animals demonstrate that there is coupling of the cervical and lumbar circuits ^{34;35}. Central pattern generators in the cervical and lumbar ¹⁸⁸ enlargements bring about locomotor activity in the forelimbs and hindlimbs respectively in quadrupeds ²⁹. In humans, this coupling allow switching from locomotor interlimb coordination to more fine motor control tasks ³⁶. Long propriospinal neurons can project six or more spinal segments. If lumbar propi neurons show plasticity as a result of locomotor training, it may be possible that long ascending lumbar propi neurons can excite motoneurons in the thoracic segments, which supply respiratory muscles, with the concomitant improvements in pulmonary function.

To our knowledge, no previous studies have been conducted to investigate the effect of locomotor training on pulmonary function and maximal respiratory pressures in individuals with SCI. Anecdotal reports from individuals with SCI undergoing locomotor training suggest that it may be beneficial in improving pulmonary function as participants increase their phonation and ability to cough and decreased their breathlessness episodes. We have hypothesized that there is a significant improvement in pulmonary function following locomotor training in individuals with cervical or upper thoracic SCI.

B. Methods

All evaluations were performed in the Neuroscience Collaborative Center at the Frazier Rehab Institute after informed consent was obtained as approved by the Institutional Review Board for human studies of the University of Louisville. Eight participants with sustained non-progressive cervical (C2-C8) or upper thoracic (T1-T6) SCI were invited to participate in the study. Participants were excluded if they suffered from any cardiopulmonary or musculoskeletal dysfunction. Participants had sustained SCI at least 6 months prior to entering the study and were non ventilator-dependent for respiration.

1. Clinical assessments:

If potential volunteers met the inclusion criteria, they had a medical evaluation by a physician. Later, the American Spinal Injury Association Impairment Scale (AIS) was used by a physical therapist to determine the neurological level and severity of the injury based on the International Standards for the Neurological Classification of Spinal Cord Injury as AIS A, B, C or D. Level and severity were confirmed by a physician. Clinical characteristics are presented in Table 5.1.

Subjects (n = 8)	Age (years)	Gender	Height (in)	Weight (Lbs)	Level of SCI	AIS Grade	Time after SCI (mo)	Number of LT sessions
A33 (C3A)	51	M	74	198	C3	AIS-A	24	70
A41 (C4A)	19	M	71	181	C4	AIS-A	17	56
B06 (C4B)	37	F	67	123	C4	AIS-B	24	85
B07 (T2B)	23	M	73	174	T2	AIS-B	12	58
C19 (C4C)	59	F	61	130	C4	AIS-C	36	62
C09 (C7C)	26	M	72	218	C7	AIS-C	24	51
D20 (C5D)	63	M	72	161	C5	AIS-D	12	82
D18 (T5D)	20	M	72	176	T5	AIS-D	48	60
Mean ± SD	37 ± 18	N/A	70 ± 4	170 ± 31	N/A	N/A	24 ± 12	65 ± 12

Table 5.1: Clinical characteristics of the SCI individuals who participated in locomotor training.

2. Equipment

a. Spirometry

Spirometry testing was performed using a preVentTM pneumotach with BreezeSuite System (MedGraphics, St. Paul, MN) and MP45-36-871 ± 350 cmH₂O Differential Pressure Transducer with UPC 2100 PC card and software (Validyne Engineering, Northridge, CA).

b. Maximal inspiratory and expiratory pressure

A differential pressure transducer (MP45-36-871-350) with UPC 2100 PC card and software from Validyne Engineering (Northridge, CA) were used to measure maximal inspiratory and maximal expiratory pressure. Subjects were asked to use a three-way valve system with rubber tube as a mouthpiece (Airlife 001504). The pressure meter incorporated a 1.5 mm diameter leak to prevent glottis closure and to reduce buccal muscle contribution during measurements ¹¹⁷.

c. Locomotor Training

A harness (Robertson Harness, Henderson, NV 89009-0086) worn by the subject and was connected to a pneumatic lift suspended over the treadmill to provide the body weight support. Biodex Treadmill and Innoventor Body Weight Support system that includes a force transducer in series with the suspension cable and a pneumatic lifting mechanism to adjust the level of support and computer controlled. The level of weight support was adjusted to maximize bilateral limb loading without the knee buckling during stance.

3. Procedures

a. Baseline and post-locomotor training assessments

Once that level and severity were established, participants were asked to come to our laboratory where standard spirometry was performed^{181;182} while the individual was seated in his or her own wheelchair. Instruments were calibrated daily according to the manufacturer's procedure, using a volumetric syringe. Since all subjects in this study met the American Thoracic Society criteria for acceptability, no modifications to guidelines for pulmonary function test in SCI patients were needed¹⁸³. To determine the validity of spirometric results, at least three acceptable spirograms were obtained with the result of the best attempt was used¹⁶⁵. At least one minute of rest was allowed between each effort, and at least five minutes between each group of three efforts. Predicted forced vital capacity and forced expiratory volume in 1 second values for each subject are based on data from non-injured individuals with no known pulmonary complaints and derived according to gender, age, and height⁹¹.

Additionally, maximal respiratory pressures were assessed. The individuals performed maximal expiratory pressure followed by maximal inspiratory pressure maneuvers. At least one minute of rest was allowed between each effort, and at least five minutes between each group of three efforts. Maximal inspiratory pressure was measured during maximal inspiratory effort from near residual volume (i.e. the volume of air remaining on the lungs after a full forced expiration). Maximal expiratory pressure was measured during maximal expiratory effort from near total lung capacity (i.e. the volume of air in the lungs after a full inspiration). The maximum pressure is taken as the

highest value that can be sustained for a minimum of one second⁹¹. The assessment of maximal pressures required a sharp, forceful effort maintained for a minimum of 2 s.

b. Locomotor training (LT)

Research participants received LT (average of 65 sessions; 60 minutes/session, 5 sessions/ week). During the session, subjects were placed on the treadmill in an upright position and suspended in a harness by an overhead pulley at the maximum load at which knee buckling and trunk collapse were avoided. A trainer positioned behind the subject aided in pelvis and trunk stabilization, as well as appropriate weight shifting and hip rotation during the step cycle. The trainer ensures that the trunk and pelvis were not flexed or hyper-extended during stepping. Trainers positioned at each limb provide manual assistance by using a customized technique developed by this research team that facilitated knee extension during stance and knee flexion and toe clearance during swing. Trainers promote knee extension by applying gentle pressure at the tibial tuberosity and stimulation of the patellar tendon. Trainers promoted knee flexion and toe clearance by applying a gentle force at the medial hamstring tendon. Manual assistance at the trunk-pelvis and at legs was used only when needed.

During the session, the treadmill speed was adjusted to promote the best stepping pattern at the given body weight load. Speeds were maintained within a normal walking speed range (0.89-1.34 m/s). Body weight support was continuously reduced over the course of the training sessions as subjects increased their ability to bear weight on the lower limbs.

4. Statistical analysis

Forced vital capacity (FVC), forced expiratory volume (FEV) both actual and predicted, and maximal expiratory and inspiratory pressures (MEP, MIP) were summarized with medians, minima, and maxima. Differences between pre-training and post-training measurements were tested with the Wilcoxon signed rank test.

5. Results

a. Pulmonary function (group mean values)

Forced expiratory volume in 1 second (actual) and maximal expiratory pressure in sitting significantly increased after locomotor training. Forced expiratory volume in 1 second ($p=0.099$) and maximal inspiratory pressure in sitting ($p=0.078$) were not significant. No other comparisons were significantly different to baseline values.

Time	FVC Actual	FVC Pred.	FEV Actual	FEV Pred.	MEP Sit	MIP Sit	MEP Supine	MIP Supine
Pre-LT	4.1 [0.8, 5.9]	85 [20, 119]	3 [0.7, 4.6]	76 [21, 112]	59.8 [22.6, 83.9]	68 [21.7, 93.5]	51 [20.1, 71.7]	58.8 [23.9, 87.1]
Post-LT	4.3 [2.2, 6.2]	91 [47, 124]	3 [1.2, 4.6]	77 [36, 113]	73 [26, 104.6]	80 [26.8, 98.3]	47.6 [21.1, 98.7]	60.3 [23.8, 82.8]
Change	0.2 [-0.8, 1.4]	3 [-16, 33]	0 [0, 0.5]	1 [-1, 15]	17.2 [-2.1, 23]	7.3 [-12.5, 44]	3.4 [-6.7, 27]	5.5 [-6, 15.4]
P-value	0.129	0.173	0.014	0.099	0.021	0.078	0.195	0.219

Table 5.2: Summary statistics (median [min, max]) and p-values (Wilcoxon signed rank tests) actual forced vital capacity (FVC actual), forced vital capacity as percentage of predicted values (FVC pred.), forced expiratory volume in 1 sec actual (FEV1 actual) and predicted (FEV1 pred.), maximal expiratory pressure in sitting (MEP sit), maximal inspiratory pressure in sitting (MIP sit), maximal expiratory pressure in supine (MEP supine) and maximal inspiratory pressure in supine (MIP supine). FEV1 actual and MEP sitting were significantly higher after locomotor training.

Eight individuals with chronic cervical or upper thoracic SCI participated on this study. As shown in figure 5.1, three of the eight participants presented decreased pulmonary function, all of them suffered from a cervical SCI (C3 AIS A, C4 AIS A and C7 AIS C). Percentage of predicted values for forced vital capacity increased in the majority of the participants after locomotor training (average of 65 sessions; 60 minutes/session, 5 sessions/ week). Figure 5.2 indicates that the forced expiratory volume in one second is below normal in the majority of the participants, with the exception of one cervical motor incomplete (C 5 AIS D) and one thoracic motor complete (T2 AIS B); indicating a decreased expiratory function.

Figure 5.3 and 5.4 illustrate changes on maximal expiratory and inspiratory pressures respectively. Compared to baseline, all the participants show higher post-locomotor training values on respiratory pressures. Even though the magnitude of these changes is variable, the trend towards higher values as a result of locomotor training is consistent.

Figure 5.1: Percentage of predicted values for forced vital capacity (FVC) in eight individuals with cervical or upper thoracic spinal cord injury. Values at or above the red-dashed line indicate normal pulmonary function. There is trend for improvement in the majority of the participants from pre-locomotor training baseline values (gray) to the post locomotor training values (black).

Figure 5.2: Percentage of predicted values for forced expiratory volume in one second (FEV1) in eight individuals with cervical or upper thoracic spinal cord injury. Values at or above the red-dashed line indicate normal pulmonary function. There is trend for improvement in the majority of the participants from pre-locomotor training baseline values (gray) to the post locomotor training values (black).

Figure 5.3: Maximal expiratory pressure in eight individuals with cervical or thoracic spinal cord injury. There is trend for improvement in the majority of the participants from pre-locomotor training baseline values (gray) to post locomotor training values (black).

Figure 5.4: Maximal inspiratory pressure in eight individuals with cervical or thoracic spinal cord injury. There is trend for improvement from pre-locomotor training baseline values (gray) to post locomotor training values (black) that is present in the participants.

6. Discussion

The aim of this study was to investigate the effect of locomotor training on pulmonary function in individuals with cervical or upper thoracic spinal cord injury. Participants were classified as AIS grades A, B, C or D based on the International Standards for the Neurological Classification of Spinal Cord Injury scale. As we stated in previous chapters, a key limitation of this scale is that the motor evaluation does not evaluate thoracic segments of the spinal cord. Therefore, we did not group participants based their AIS grade, and we did not report findings as a group, but rather as individual observations from eight SCI subjects with different neurological levels of impairment who participated in locomotor training.

We measured forced vital capacity, forced expiratory volume in 1 second and maximal respiratory pressures before and after locomotor training. We found that there was a trend for improvement in all of these respiratory variables. However, we still have to elucidate the neurophysiological mechanisms for such improvements. We suggest that activity-dependent plasticity at or below the level of the injury may bring about changes in the respiratory muscle activation. Further, we propose that long ascending lumbar proprioneurons may excite motoneurons in the thoracic segments that supply respiratory muscles, with the concomitant improvements in pulmonary function. Future directions include the evaluation of the effect of locomotor training with larger number of participants, to compare the effects of locomotor training with more traditional respiratory therapies and to investigate changes in the neural activation patterns to determine if activity-dependant plasticity occurs as a result of locomotor training.

CHAPTER VI

CONCLUSIONS

The respiratory system is under sophisticated neural controls. When the dorsal respiratory group fires, it signals the phrenic nerve to contract the diaphragm and intercostal nerves to contract external intercostals. The diaphragm and the external intercostals are the prime movers for quiet inspiration. As ventilatory demands increase, for example during exercise, other accessory muscles for inspiration are recruited. Expiration is usually passive and occurs due to the elastic recoil of the lungs. However, if it is necessary, expiration occurs by the active involvement of the internal intercostal portion of the internal intercostal and abdominal musculature (i.e. forced expiration).

We investigated the neural role of spinal cord injury on pulmonary dysfunction in individuals with cervical and upper thoracic spinal cord injury. Additionally, we evaluated the effect of locomotor training on pulmonary function on those individuals. First, we compared pulmonary function among groups with different levels and severity of spinal cord injury. We refuted our hypotheses and concluded that the assessment of motor and sensory preservation based on the current validated scales is not sufficient to characterize the pulmonary dysfunction that occurs after a neurological injury. Further, in Chapter III we suggested level and severity of SCI should not be used as a predictor of risk of developing pneumonia. Further, we suggested that respiratory therapies should be

incorporated as prophylaxis in every individual with cervical or upper thoracic SCI. Finally, we suggested that the neural activation of respiratory muscles may play a role in the pulmonary dysfunction that occurs as a result of lesions in the spinal cord; this suggestion led us to aim 2.

Aim 2 was to investigate the role of neuromuscular activation in pulmonary function in individuals with SCI. We selected maximal respiratory pressure maneuvers as respiratory tasks because they provide information about the neural activation of primary and accessory muscles for breathing. We proposed that lower respiratory pressures would correspond with lower EMG activity of the respiratory muscles below the spinal cord lesion, and with a higher EMG activity of the accessory muscles above the level of lesion. We concluded that after SCI there is a plasticity of the muscles involved in respiration that compensate for other weak or paralyzed muscles that are unable to contract efficiently to keep up with the respiratory demands.

We were not surprised to find that higher expiratory pressures correspond with a higher neural activation of the rectus abdominis and obliques. Integrated EMG of the latissimus dorsi had a negative correlation with maximal expiratory pressures in the cervical SCI individuals, especially in the cervical motor complete group. This suggests that the latissimus dorsi is being recruited to compensate during maximal expiratory tasks when the expiratory muscles below the level of the lesion (rectus abdominis and obliques) are unable to do so. We confirmed the hypothesis that latissimus dorsi acts as a compensatory muscle for maximal expiration, but only for cervical SCI individuals.

Surprisingly, EMG amplitude of the pectoralis was not correlated to maximal expiratory pressure. However, several of the cervical motor complete and incomplete SCI

participants, and some thoracic incomplete SCI show higher EMG activity of the pectoralis with lower expiratory pressures. Our findings add to the findings of De Troyer et al.⁸⁷, that the activation of the clavicular portion of the pectoralis as a compensatory action during maximal expiratory tasks is not consistent in all the cervical or upper thoracic SCI. Higher integrated EMG values of the intercostals are related with higher maximal inspiratory pressure in the non-injured group and cervical motor incomplete. On the other hand, lower inspiratory pressures are related to higher EMG in the thoracic motor complete group. Further, we suggest that different EMG patterns of the respiratory muscles may be present after SCI that need to be identified in future studies with larger sample sizes. Finally, we wanted to evaluate the effect of locomotor training on pulmonary function in individuals with cervical or upper thoracic SCI. After locomotor training, individuals with SCI showed higher respiratory function and maximal respiratory pressures than baseline.

The findings of this study have several clinical implications that need to be emphasized. First, pulmonary function after SCI cannot be predicted using the current validated scales for motor or sensory preservation. The majority of the muscles involved in forced expiration and coughing are innervated by thoracic spinal nerve segments that are not evaluated in the AIS scale. Therefore, caution should be used in predicting pulmonary function outcomes based solely on the level and severity of injury based on this grading system. Second, after a neurological lesion, there is plasticity in the nervous system involving new compensatory strategies to counteract the lack of neural activation of the prime mover muscles for inspiration and expiration. For instance, latissimus dorsi is an accessory muscle for forced expiration in individuals with paralyzed rectus

abdominis or obliques. Similarly, upper trapezius compensates the lack of neural activation of the external intercostals.

There are some limitations on this study. First, time since injury should be considered. There is a decline in these spirometrical values that is related to aging. Lesions in higher segment of the spinal cord are related to higher prevalence of pulmonary dysfunction and this detriment occur exponentially faster in older SCI than in those who are younger¹⁷⁴. Future studies investigating the differences in pulmonary function in relation with the time since injury and age at the time of injury should be considered.

Second, the gender distribution was imbalanced between SCI and non-injured groups. There is a high prevalence of young males who suffer from SCI. In fact, males account for 85% of all the SCI cases⁵². We tried to match non-injured participants with SCI participants in gender, age and clinical characteristics. However, in relationship with the gender prevalence in SCI, we had more proportion of non-injured females volunteering for this study. Female participants in both SCI and non-injured groups show lower maximal expiratory and inspiratory pressure than males. This may account for the discrepancy on respiratory pressures previously reported^{44;175}. Finally, fitness status or level of physical activity that could have influenced pulmonary function in the research participants was only controlled in 8 SCI individuals participating on locomotor training; but neither was controlled or recorded in the rest of SCI or non-injured participants.

Future directions include the evaluation of the effect of locomotor training with larger number of participants, to compare the effects of locomotor training with more traditional respiratory or rehabilitation therapies and to investigate changes in the neural

activation patterns to determine if activity-based plasticity occurs as a result of locomotor training.

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APPENDIX A

LIST OF ABBREVIATIONS

SCI : Spinal Cord Injury

ASIA: American Spinal Injury Association

AIS: American Spinal Injury Association Impairment Scale

ISNCSCI: International Standards for the Neurological Classification of Spinal Cord Injury

NASCIS: National Acute Spinal Cord Injury Studies

EMG: Electromyography

μV: microvolt

LT: Locomotor Trainig

FVC: Forced Vital Capacity

FEV1: Forced Expiratory Volume in 1 second

MEP: Maximal Expiratory Pressure

MIP: Maximal Inspiratory Pressure

C1-8: Cervical spinal nerve segments 1-8

T1-12: Thoracic spinal nerve segments 1-12

L1-5: Lumbar spinal nerve segments 1-5

S1-5: Sacral spinal nerve segments 1-5

APPENDIX B
INFORMED CONSENTS

NON-DISABLED SUBJECT INFORMED CONSENT DOCUMENT

RESPIRATORY AND TRUNK MUSCLE ACTIVATION PATTERNS IN INDIVIDUALS WITH SPINAL CORD INJURY

IRB assigned number:
Investigator(s) name & address:
Susan Harkema
Frazier Rehab Institute
220 Abraham Flexner Way, Louisville, KY 40202
Site(s) where study is to be conducted: Frazier Rehab Institute

Phone number for subjects to call for questions: (502) 581-8747

Introduction and Background Information

You are invited to take part in a research study because we are interested in understanding the pulmonary function and trunk muscle function after a spinal cord injury. If you choose to participate, your participation will allow us to determine normal test results for comparison with those of spinal cord injury subjects. **The study is being conducted under the direction of Susan Harkema, PhD. Approximately 20 local subjects (at least 18 years old) will be invited to participate.** Your participation in this study will be for two days within a week for approximately 2.5 hours each session. This study will take place at the Frazier Rehab Institute. If you are pregnant, anticipate pregnancy or are nursing you are not eligible to participate in this study.

Purpose

The purpose of this study is to investigate the activity of the trunk muscles in individuals with spinal cord injury and its effects in pulmonary function and to compare to non-injured control subjects. The results of this study may aid in better understanding the neuromuscular mechanisms affected by a spinal cord lesion and in the development of rehabilitation strategies to help individuals with spinal cord injuries that also have abnormalities in pulmonary function.

Procedures

If you volunteer to participate in this experimental study, and sign this consent form, we will ask you to participate in a series of tests. You will not be required to stay in the hospital.

Testing:

The tests include standard measurements of the pulmonary function and the muscle activity of your neck and trunk. Totally those tests will last approximately 2.5 hours.

- The pulmonary function tests include recording of your chest movements, lung volumes, airflow and airway pressure by using standard equipment. You will be asked to inhale maximally and then exhale as rapidly and as completely as possible in the spirometer.
- We will ask you to make maximum breathing efforts with a nose clip on.

Consent version date_08.25.10

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IRB #: Respiratory muscle activation patterns in non-injured individuals and in spinal cord injured individuals

- We will ask you to breathe to a mouth piece with some resistance to your breathing efforts.
- All these tasks will be done in sitting (with back support) and in supine position while we will be recording the electrical impulses from your neck and trunk muscles by placing self-adhesive recording electrodes over the muscles and elastic belts around your chest and abdomen.
- We will ask you to perform a series of trunk movements including: trunk flexion/extension, trunk rotation, sitting from supine, and sitting balance while we will be recording the electrical impulses from your neck and trunk muscles.

Potential Risks

The study may involve the following physical risks and/or discomforts:

Likely	Less Likely	Rare
Dizziness during pulmonary test (less 50%)		
Skin irritation from electrode placement (60%)		
Changes in blood pressure and heart rate (30%).(Blood pressure and heart rate can decrease slightly when moving from sitting to supine or increase when moving from supine to sitting)		
	Shortness of breath (20%). (after blowing through the mouth piece forcedly)	
		Muscle and joints aches (10 %)

In addition, you may suffer harms that we have not seen before.

- If you have any of these difficulties during testing, the test will stop. There are no reasonably foreseeable psychological risks, social risks, and/or legal risks. This study may involve risks that are current unforeseeable.

Possible Pregnancy Risks

Pregnant women are excluded from this study, as the risk to the fetus is unknown. You should discuss these risks with your doctor before signing this consent form. Talk to your doctor about the best method of birth control to use while you are in this study. If you are pregnant or become pregnant, your unborn child may suffer harms that we have not seen before. It is important that you tell someone on the research team at (502) 581-8747 right away if you become pregnant during the course of this study. If you become pregnant, you will be terminated from the study.

Benefits

The information collected may or may not benefit you directly; however, the information learned in this study may be helpful to others.

IRB #: Respiratory muscle activation patterns in non-injured individuals and in spinal cord injured individuals

Alternatives

You may choose not to participate in this research study.

Research Related Injury

If you are injured by being in this research study treatment is available. Your insurance will be billed for the cost of treatment. If you are injured, there is no money set aside for lost wages, discomfort, disability, etc. You do not give up your legal rights by signing this form. If you think you have a research related injury, please call Dr. Susan Harkema, PhD at (502) 581-8747

Compensation

You will be compensated for your travel to and from Frazier Rehab Institute for your participation in this study based on the current standard rate mileage set by the IRS with a cap of \$20. and you will receive free parking at Frazier parking garage on the days of testing. No other compensation will be provided.

Costs

There will be no additional costs to you for participating.

HIPAA Research Authorization

The Health Insurance Portability and Accountability Act of 1996 (HIPAA) provides federal safeguards for protected health information (PHI). Examples of PHI are your name, address, and birth date. PHI may also include your medical history, results of health exams and lab tests, drugs taken and results of this research study. Your PHI may not be used or shared without your agreement, unless it meets one of the HIPAA exceptions. If you agree to take part in this research you may be required to sign a "Research Authorization" form. This allows the use and sharing of your PHI by those listed in the "Research Authorization."

Confidentiality

Total privacy cannot be guaranteed. We will protect your privacy to the extent permitted by law. If the results from this study are published, your name will not be made public. The following may look at your research and medical records:

- The sponsor and others hired by the sponsor to oversee the research
- The University of Louisville Institutional Review Board, Human Subjects Protection Program Office, Privacy Office and others involved in research administration at the University
- TIRR/Memorial Hermann who will review and score the testing done
- People who are responsible for research and HIPAA oversight at the institutions where the research is conducted
- Government agencies, such as:
 - Office for Human Research Protections,
 - Office of Civil Rights,
- People responsible for billing, sending and receiving payments related to your participation in the study.

Security

Consent version date 08/25/10

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Your data will be kept private by assigning you a coded identification number. All research related files will be kept in a locked cabinet. The only people who will know that you are a research subject are members of the research team and, if appropriate your physicians and nurses. No information about you, or provided by you during the research will be disclosed to others without your written permission, except if necessary to protect your rights or welfare (for example, if you are injured and need emergency care), or if required by law.

When the results of the research are published or discussed in conferences, no information will be included that will reveal your identity.

The video recorded during the study will be used for teaching and research purposes only. Such teaching and research purposes can include lectures, presentations, conferences, seminars, critiques of experimental procedures, and data analysis. Your identity will not be disclosed, as neither your name nor face will be shown. You agree that the tapes shall be kept for these purposes for an indefinite time.

Conflict of Interest

This study does not involve any conflicts of interest for the investigator or the institution.

Voluntary Participation

Taking part in this study is completely voluntary. You may choose not to take part at all. If you decide not to be in this study, you won't be penalized or lose any benefits for which you qualify. If you decide to be in this study, you may change your mind and stop taking part at any time. If you decide to stop taking part, you won't be penalized or lose any benefits for which you qualify.

You will be told about any new information learned during the study that could affect your decision to continue in the study.

Termination

The investigator, Dr. Harkema, or a research team member has the right to stop this study at any point. The investigator or a research team member may take you out of this study with or without your okay. Reasons why this may occur include circumstances that arise which warrant doing so. If you become ill during the research, you may have to drop out, even if you would like to continue. The investigator or a research team member will make the decision and let you know if it is not possible for you to continue. The decision may be made either to protect your health and safety, or because it is part of the research plan that people who develop certain conditions may not continue to participate. If Dr. Harkema or a research team member believes that the pain or discomfort might pose a risk to you, you will be terminated from the study. If you become pregnant you will be terminated from this study.

Participation in Other Research Studies

You may not take part in this study if you are currently in another research study that will interfere with this study. It is important to let Dr. Harkema know if you are in another research study.

Contact Persons

IRB #: Respiratory muscle activation patterns in non-injured individuals and in spinal cord injured individuals

If you have any questions, concerns, or complaints about the research study, please contact Susan Harkema at (502) 581-8747.

Research Subject's Rights

If you have any questions about your rights as a research subject, you may call the Human Subjects Protection Program Office at (502) 852-5188. You may discuss any questions about your rights as a research subject, in private, with a member of the Institutional Review Board (IRB). You may also call this number if you have other questions about the research, and you cannot reach the investigator, Dr. Harkema, or want to talk to someone else. The IRB is an independent committee made up of people from the University community, staff of the institutions, as well as people from the community not connected with these institutions. The IRB has reviewed this research study.

Concerns and Complaints

If you have concerns or complaints about the research or research staff and you do not wish to give your name, you may call the toll free number 1-877-852-1167. This is a 24 hour hot line answered by people who do not work at the University of Louisville.

Acknowledgment and Signatures

This informed consent document is not a contract. This document tells you what will happen during the study if you choose to take part. Your signature indicates that this study has been explained to you, that your questions have been answered, and that you agree to take part in the study. You are not giving up any legal rights by signing this informed consent document. You will be given a copy of this consent form to keep for your records.

Do you want your primary care physician notified that you are a subject in this study? ☐ Yes ☐ No

Printed Name of Subject/Legal Representative	Signature of Subject/Legal Representative	Date Signed
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Printed Name of Person Explaining Consent Form	Signature of Person Explaining Consent Form (if other than the Investigator)	Date Signed
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Printed Name of Investigator	Signature of Investigator	Date Signed
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LIST OF INVESTIGATORS

PHONE NUMBERS

Susan Harkema, PhD

(502) 581-8747

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UNIVERSITY OF LOUISVILLE
INSTITUTIONAL REVIEW BOARD
Date Approved 8/30/2010 Valid Thru 8/29/2011

SUBJECT INFORMED CONSENT DOCUMENT

RESPIRATORY AND TRUNK MUSCLE ACTIVATION PATTERNS IN INDIVIDUALS WITH SPINAL CORD INJURY

IRB assigned number: 09.0321|
Investigator(s) name & address: Susan Harkema
Frazier Rehab Institute
220 Abraham Flexner Way
Louisville, KY 40202

Site(s) where study is to be conducted: Frazier Rehab Institute

Phone number for subjects to call for questions: (502) 581-8674

Introduction and Background Information

You are invited to take part in a research study because you have cervical or thoracic spinal cord injury. We are interested in understanding the pulmonary function, the trunk function and muscle activation patterns after a spinal cord injury. The study is being conducted under the direction of Susan Harkema, PhD. Approximately 20 local subjects (at least 18 years old) will be invited to participate. Your participation in this study will be for two days within a week for approximately 2.5 hours each session. This study will take place at the Frazier Rehab Institute. If you are pregnant, anticipate pregnancy or are nursing you are not eligible to participate in this study.

Purpose

Many individuals with spinal cord injury experience decreases in pulmonary function. This may be due to paralyzed or weak trunk muscles. The aim of this study is to evaluate how pulmonary (breathing) function and trunk and neck muscles are affected by spinal cord injury.

Procedures

If you volunteer to participate in this experimental study, and sign this consent form, we will ask you to participate in a series of tests. You will not be required to stay in the hospital.

Testing

The tests include standard measurements of the pulmonary function and muscle from your neck and trunk. These tests will last approximately 2.5 hours. We have termed this testing the Trunk-Respiratory Motor Control Assessment (TRMCA).

- The pulmonary function tests include recording of your chest movements, lung volumes, airflow and airway pressure by using standard equipment. You will be asked to inhale as much as you can and then exhale as fast and as completely as possible into a mouth piece.

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- We will ask you to breath into a mouth piece with some resistance to your breathing efforts and a nose clip will be on during this task.
- All these tasks will be done while sitting (with back support) and lying down. We will be recording the muscle activity from your neck and trunk muscles by placing self-adhesive recording electrodes over the muscles and elastic belts around your chest and abdomen.
- We will ask you to perform a series of trunk movements including: voluntary coughing, bend forward/backward, turn to the side, try to sit from lying down, and maintain your sitting balance while we will be recording the activity impulses from your neck and trunk muscles. If you are able to do so independently, we will ask you to maintain your standing balance.
- We will ask you repeat each task 3 times.

We will record the entire study with a digital video camera. Videos are used as an evaluation tool for this study. Therefore, in order for you to participate of the study you must agree to allow use of video recording.

Potential Risks

The study may involve the following physical risks and/or discomforts:

In addition, you may suffer harms that we have not seen before. If you have any of these difficulties during testing, the test will stop. There are no reasonably foreseeable psychological risks, social risks, and/or legal risks. This study may involve risks that are currently unforeseeable.

Likely	Less Likely	Rare
Skin irritation from electrode placement (50%)	Dizziness during pulmonary test (less 20%)	Muscle and joints aches (1 %)
	Changes in blood pressure and heart rate (20%).(Blood pressure and heart rate can decrease slightly when moving from sitting to supine or increase when moving from supine to sitting)	
	Shortness of breath (20%) after blowing through the mouth piece forcedly	

Possible Pregnancy Risks

Pregnant women are excluded from this study, as the risk to the fetus is unknown. You should discuss these risks with your doctor before signing this consent form. Talk to your doctor about the best method of birth control to use while you are in this study. If you are pregnant or become pregnant, your unborn child may suffer harms that we have not seen before. It is important that you tell someone on the research team at (502) 581-8686 right away if you become pregnant during the course of this study. If you become pregnant, you will be terminated from the study.

Benefits

The information collected may or may not benefit you directly; however, the information learned in this study may be helpful to others.

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Alternatives

You may choose not to participate in this research study. Choosing not to take part will not affect any treatment you may be receiving at Frazier Rehab Institute.

Research Related Injury

If you are injured by being in this research study treatment is available. Your insurance will be billed for the cost of treatment. If you are injured, there is no money set aside for lost wages, discomfort, disability, etc. You do not give up your legal rights by signing this form. If you think you have a research related injury, please call Dr. Susan Harkema, PhD at (502) 581-8747

Compensation

You will be compensated for your travel to and from Frazier Rehab Institute for your participation in this study based on the current standard rate mileage set by the IRS with a cap of \$20. You will receive free parking at Frazier parking garage on the days of testing. No other compensation will be provided.

Costs

There will be no additional costs to you for participating. However, you or your insurance company will be billed for all office visits and procedures that are part of routine medical care. It is your responsibility to find out what costs, if any, your insurance company will cover before taking part in the study.

HIPAA Research Authorization

The Health Insurance Portability and Accountability Act of 1996 (HIPAA) provides federal safeguards for protected health information (PHI). Examples of PHI are your name, address, and birth date. PHI may also include your medical history, results of health exams and lab tests, drugs taken and results of this research study. Your PHI may not be used or shared without your agreement, unless it meets one of the HIPAA exceptions. If you agree to take part in this research you may be required to sign a "Research Authorization" form. This allows the use and sharing of your PHI by those listed in the "Research Authorization."

Confidentiality

Total privacy cannot be guaranteed. We will protect your privacy to the extent permitted by law. If the results from this study are published, your name will not be made public. The following may look at your research and medical records:

- The University of Louisville Institutional Review Board, Human Subjects Protection Program Office, Privacy Office and others involved in research administration at the University
- TIRR/Memorial Hermann who will review and score the testing done
- People who are responsible for research and HIPAA oversight at the institutions where the research is conducted
- Government agencies, such as:
 - Office for Human Research Protections,
 - Office of Civil Rights,

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- People responsible for billing, sending and receiving payments related to your participation in the study.

Security

Your data will be kept private by assigning you a coded identification number. All research related files will be kept in a secure office. The only people who will know that you are a research subject are members of the research team and, if appropriate your physicians and nurses. No information about you, or provided by you during the research will be disclosed to others without your written permission, except if necessary to protect your rights or welfare (for example, if you are injured and need emergency care), or if required by law.

When the results of the research are published or discussed in conferences, no information will be included that will reveal your identity.

The video recorded during the study sessions will be used for teaching and research purposes only. Such teaching and research purposes can include lectures, presentations, conferences, seminars, critiques of experimental procedures, and data analysis. Your identity will not be disclosed, as neither your name nor face will be shown. You agree that the videos shall be kept for these purposes for an indefinite time.

Conflict of Interest

This study does not involve any conflicts of interest for the investigator or the institution.

Voluntary Participation

Taking part in this study is completely voluntary. You may choose not to take part at all. If you decide not to be in this study, you won't be penalized or lose any benefits for which you qualify. If you decide to be in this study, you may change your mind and stop taking part at any time. If you decide to stop taking part, you won't be penalized or lose any benefits for which you qualify. You will be told about any new information learned during the study that could affect your decision to continue in the study.

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The investigator, Dr. Harkema, or a research team member has the right to stop this study at any point. The investigator or a research team member may take you out of this study with or without your okay. Reasons why this may occur include circumstances that arise which warrant doing so. If you become ill during the research, you may have to drop out, even if you would like to continue. The investigator or a research team member will make the decision and let you know if it is not possible for you to continue. The decision may be made either to protect your health and safety, or because it is part of the research plan that people who develop certain conditions may not continue to participate. If Dr. Harkema or a research team member believes that the pain or discomfort might pose a risk to you, you will be terminated from the study. If you become pregnant you will be terminated from this study.

Participation in Other Research Studies

IRB # 09.0321: Respiratory muscle activation patterns in non-injured individuals and in spinal cord injured individuals

You may not take part in this study if you are currently in another research study that will interfere with this study. It is important to let Dr. Harkema and the research team know if you are in another research study.

Contact Persons

If you have any questions, concerns, or complaints about the research study, please contact Susan Harkema at (502) 581-8674.

Research Subject's Rights

If you have any questions about your rights as a research subject, you may call the Human Subjects Protection Program Office at (502) 852-5188. You may discuss any questions about your rights as a research subject, in private, with a member of the Institutional Review Board (IRB). You may also call this number if you have other questions about the research, and you cannot reach the investigator, or want to talk to someone else. The IRB is an independent committee made up of people from the University community, staff of the institutions, as well as people from the community not connected with these institutions. The IRB has reviewed this research study.

Concerns and Complaints

If you have concerns or complaints about the research or research staff and you do not wish to give your name, you may call the toll free number 1-877-852-1167. This is a 24 hour hot line answered by people who do not work at the University of Louisville.

Acknowledgment and Signatures

This informed consent document is not a contract. This document tells you what will happen during the study if you choose to take part. Your signature indicates that this study has been explained to you, that your questions have been answered, and that you agree to take part in the study. You are not giving up any legal rights by signing this informed consent document. You will be given a copy of this consent form to keep for your records.

Do you want your primary care physician notified that you are a subject in this study? ☐Yes ☐No

Printed Name of Subject/Legal Representative	Signature of Subject/Legal Representative	Date Signed
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Printed Name of Person Explaining Consent Form	Signature of Person Explaining Consent Form (if other than the Investigator)	Date Signed
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Printed Name of Investigator	Signature of Investigator	Date Signed
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LIST OF INVESTIGATORS Susan Harkema, PhD	PHONE NUMBERS (502) 581-8674
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Consent version date 08/25/10

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For IRB Approval Stamp UNIVERSITY OF LOUISVILLE INSTITUTIONAL REVIEW BOARD Date Approved 8/30/2010 Valid Thru 8/29/2011
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APPENDIX C
RECRUITMENT FLYERS

Research Study for People with Spinal Cord Injury

Principal Investigator: Susan Harkema, Ph.D.

Purpose of Research:

Many individuals with spinal cord injury experience decreases in pulmonary function. This may be due to paralyzed or weak trunk muscles. The aim of this study is to evaluate how pulmonary function and trunk muscle activation are affected after spinal cord injury. This study will take place at the Frazier Rehab Institute.

Potential Benefits to Participants:

There are no benefits to the participants in the study.

Potential Risks to Participants:

This study may involve the following risks and/or discomforts: increased respiration; increased heart rate; changes in blood pressure; dizziness; skin irritation or abrasion from electrodes.

Subjects Must:

- Have a spinal cord injury of the neck or back with minimum six months post injury
- Have a clinically complete or incomplete spinal cord injury, usually using a wheelchair
- Be in a stable medical condition
- Be non ventilator-dependent for respiration
- Be free from any cardiopulmonary diseases
- Not be pregnant
- Not have muscle pain or broken bones
- Be 18 years of age or older

Procedures:

Subjects will undergo a series of tests including

- Breathe through a machine with some resistance to your breathing effort
- Perform a series of respiratory and trunk movements
- Self-adhesive electrodes will be placed to record the electrical activity of the muscles of your neck, chest and back (Surface electromyography)
- The entire study will require up to 2 visits to the lab, each visit will last 2.5 hours.

Compensation:

Travel compensation available (in the form of mileage reimbursement) and free parking at Frazier Rehab the days of testing. There is no other compensation.

Contact:

Daniela Terson de Paleville at (502)581-8674

Email: dtdepa01@louisville.edu

Research Study for People with Spinal Cord Injury

Principal Investigator: Susan Harkema, Ph.D.

Purpose of Research:

Many individuals with spinal cord injury experience decreases in pulmonary function. This may be due to paralyzed or weak trunk muscles. The aim of this study is to evaluate how pulmonary function and trunk muscle activation are affected after spinal cord injury. This study will take place at the Frazier Rehab Institute.

Potential Benefits to Participants:

There is no benefit to the participants in the study.

Potential Risks to Participants:

This study may involve the following risks and/or discomforts: increased respiration; increased heart rate; changes in blood pressure; dizziness; skin irritation or abrasion from electrodes.

Subjects Must Have:

- Healthy individuals with no spinal cord injury or other neurological disorder
- A stable medical condition
- Free from any cardiopulmonary diseases
- No muscle pain or broken bones
- At least 18 years old

Procedures:

Subjects will undergo a series of tests including

- Breathe through a machine with some resistance to your breathing effort
- Perform a series of respiratory and trunk movements
- Surface electromyography of your neck, chest and back muscles will be performed during those tasks (*self-adhesive electrodes will be placed to record the electrical activity of your muscles*)
- The entire study will require 2 visits to the lab, each visit will last 2.5 hours.

Compensation:

Travel compensation available (in the form of mileage reimbursement) and free parking at Frazier Rehab the days of testing. There is no other compensation.

Potential Participants:

Contact Briana Dockins/ Daniela Terson de Paleville at (502)581-8674.

Preparation Date: July 2010

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CURRICULUM VITAE

Daniela Terson de Paleville

PERSONAL DATA

Date of Birth: June 07, 1975.
Place of Birth: Lujan de Cuyo, Mendoza, Argentina.
Address: 209 Savannah Nicole Rd, Jeffersonville,
IN, 47130
Telephone: (502) 296-2123
Email: dtdepa01@louisville.edu,
danielaterson@gmail.com

EDUCATION

2006-2011 **Ph.D.** in Physiology and Biophysics under the supervision of Susan Harkema, PhD at the University of Louisville

2006-2008 **M.S.** in Physiology and Biophysics under the supervision of Susan Harkema, PhD at the University of Louisville

2003-2005 **M.S.** in Exercise Physiology under the supervision of Ann Marie Swank, PhD at the University of Louisville

2002-2003 **M.Ed.** (18 credit hours completed) at The Citadel under the supervision of John Carter, PhD

1996-1999 **B.S.** in Exercise Sciences and Physical Education obtained at the Instituto de Educacion Fisica, Jorge Coll, Mendoza, Argentina. The college study program includes 1 year of educational practical internship in primary and secondary schools under supervision of the Ministry of Education of the Province of Mendoza, Argentina.

POSITIONS AND HONORS

- 2006-Present** **Graduate Research Assistant**, Neuroscience Collaborative Center, University of Louisville and Frazier Rehab Hospital, Louisville, KY
- 2006-2008** **Integrated Program in Biomedical Sciences (IPIBS) Graduate Student Fellowship**, Department of Physiology and Biophysics, University of Louisville, KY.

ACADEMIC APPOINTMENTS

- 2011-present** **Instructor**, Department of Health and Sport Sciences, University of Louisville, KY
- 2006-Present** **Research Assistant**, Department of Physiology and Biophysics, University of Louisville, Louisville, KY, USA
- 2005-2006** **Health Promotion, Education and Exercise physiology Lecturer**, Department of Health and Sport Sciences, University of Louisville, KY
- 2003-2005** **Graduate Teaching Assistant**, Department of Exercise Physiology, University of Louisville, KY
- 2005-2006** **Water safety and adapted swimming instructor and coordinator of the program “Aqua-Angels”** (adapted swimming and aqua-therapy program for children with cerebral palsy and other disabilities) Baptist East Milestone Wellness Center Louisville, KY.
- Research Assistant** Harambee Nursing Center “A Community-As-Partner Model to Integrated Mental Health in Vulnerable Populations” University of Louisville (School of Nursing) Louisville, KY
- Clinical Rehabilitation Intern** in cardiac rehabilitation **Cardiovascular Associates** (Norton Audubon Hospital) Louisville, KY.
- Health Education Instructor** American Red Cross Louisville, KY

- 2002-2003** **Graduate Teaching Assistant**, Department of Health, Exercise and Sport Sciences, The Citadel, South Carolina.
- 1996-2001** **Mendoza Government “Different Capabilities” program** instructor and rehabilitation therapist. Adapted track and field, and swimming coach. Municipality of Lujan de Cuyo. Mendoza. Argentina.

PROFESSIONAL MEMBERSHIPS

- Member of the Society for Neuroscience (SFN)
- Member of American College of Sport Medicine (ACSM)
- Member of the Louisville Chapter of the Society for Neuroscience
- Member of the American Physiological Society (APS)

PROFESSIONAL HONORS AND AWARDS

- 2010** **University of Louisville School of Medicine** travel award to attend to the National Neurotrauma meeting, Las Vegas, June 2010
- 2009** **University of Louisville School of Medicine** travel award to attend to the Society for Neuroscience conference, Chicago IL, October 2009
- University of Louisville Department of Physiology and Biophysics** travel award to attend to the Society for Neuroscience conference, Chicago IL, October 2009
- American Physiological Society (APS)** travel award to attend to the Professional Skills Training on "Writing and Reviewing for Scientific Journals" Orlando, FL, January 2009
- Presidential Award for Excellence in Science, Mathematics and Engineering Mentoring/ American Association for the Advancement of Science (PAESMEM/AAAS)** Building Science, Engineering, and Technology Leadership Workshop for Women in Industry, Academia and Government. Washington DC, October 1-2 2009.

- 2008** **Excellence in Neuroscience Research**, Graduate Students Poster Competition Award “Neuroscience Day” Society for Neuroscience Louisville Chapter. April 17th 2008. Title: *“Respiratory Motor Control Assessment in Individuals with Spinal Cord Injury”*
- Research! Louisville 2008** winner in the “Doctoral Basic-Science Student Division” October 2008. Title: *“Effects of Locomotor Training on Pulmonary Function in Individuals with Spinal Cord Injury”*

PRESENTATIONS

- 2011** University of Louisville School of Medicine; Physiology & Biophysics seminar series *“Respiratory Muscle Activation Patterns in individuals with Chronic Spinal Cord Injury: effects of different rehabilitative therapies”*. February 3rd 2011.
- 2010** Kentucky Spinal Cord Injury Center Seminar Series, *“Respiratory Muscle Activation Patterns in individuals with chronic Spinal Cord Injury”*. June 24th 2010.
- University of Louisville School of Medicine; Physiology & Biophysics seminar series *“Respiratory Muscle Activation Patterns in individuals with Chronic Spinal Cord Injury: effects of different rehabilitative therapies”*. February 23rd 2010.
- 2009** Kentucky Spinal Cord Injury Center Seminar Series, *“Altered Respiratory Muscle Activation Patterns in individuals with Spinal Cord Injury”*. May 22th 2009.
- 2008** Kentucky Spinal Cord Injury Center Seminar Series, *“Effects of Locomotor Training in individuals with Spinal Cord Injury”*. September 5th 2008.

MEETING ABSTRACTS

- 2010** **National Neurotrauma Symposium** Respiratory Muscles Activation during Voluntary Respiratory Tasks in Individuals with Chronic Spinal Cord Injury” June 14th- 17th 2010, Las Vegas, NV

- 2009**
- Research! Louisville** October 2009 “*Respiratory Muscles Activation Patterns in Individuals with Spinal Cord Injury*” **Daniela Terson De Paleville**, Alexander V. Ovechkin, William B. McKay, Susan J. Harkema.
- Society for Neuroscience**, Chicago IL, October 2009 “*Respiratory Muscles Activation Patterns in Individuals with Spinal Cord Injury*” **Daniela Terson De Paleville**, Alexander V. Ovechkin, William B. McKay, Susan J. Harkema.
- University of Louisville Graduate Research Symposium**, March 6th 2009. “*Locomotor Training Improves Pulmonary Function in Individuals with Spinal Cord Injury*”. March 18th 2009
- 2008**
- International Respiratory Care Symposium** December 2008, Anaheim, CA. “*Evaluation of Expiratory Muscle Activation Patterns in Individuals with Spinal Cord Injury*” ID: 498420, Alexander V. Ovechkin, Susan J. Harkema, **Daniela G. Terson De Paleville**, William B. McKay.
- Society for Neuroscience**, Washington DC, November 2008. “*Evaluation of Motor Activity for Respiration in Individuals with Spinal Cord Injury*” Alexander V. Ovechkin, Susan J. Harkema, **Daniela G. Terson De Paleville**, William B. McKay
- Research! Louisville** October 2008 “*Neurophysiological Characterization of Motor Activity in Individuals with Acute Spinal Cord Injury*”; Alexander V. Ovechkin, William B. McKay, Susan J. Harkema, **Daniela Terson De Paleville**, Todd W. Vitaz
- Research! Louisville** October 2008 “*Effects of Locomotor Training on Pulmonary Function in Individuals with Spinal Cord Injury*” **Daniela Terson De Paleville**, Alexander V. Ovechkin, William B. McKay, Susan J. Harkema.

PEER-REVIEWED PUBLICATIONS

1. **Daniela Terson de Paleville, MS**; Alexander Ovechkin, M.D., Ph.D.; Susan Harkema, PhD. Pulmonary Function in Individuals with Chronic Spinal Cord Injury. In preparation for submission.

2. **Daniela Terson de Paleville, MS**; Douglas Lorenz, PhD; Susan Harkema, PhD. Respiratory Muscles Activation during Voluntary Respiratory Tasks in Individuals with Chronic Spinal Cord Injury. In preparation for submission.
3. **Daniela Terson de Paleville, MS**; Alexander Ovechkin, M.D., Ph.D.; Susan Harkema, PhD. Locomotor training Improves Pulmonary Function and Respiratory Muscle Strength in Individuals with Chronic Spinal Cord Injury. In preparation for submission.
4. William McKay, B.S.; Alexander Ovechkin, M.D., Ph.D.; Todd Vitaz, M.D.; **Daniela Terson de Paleville, MS**; Susan Harkema, PhD. Neurophysiological Characterization of Voluntary Motor Control Recovery in Acute Spinal Cord Injury. *Spinal Cord*, 2011, 49 (421-429).
5. William McKay, B.S.; Alexander Ovechkin, M.D., Ph.D.; Todd Vitaz, M.D.; **Daniela Terson de Paleville, MS**; Susan Harkema, PhD. Long-lasting involuntary motor activity after spinal cord injury. *Spinal Cord*, (2011) 49, (87-93)
6. Alexander Ovechkin, M.D. PhD, Todd Vitaz, MD.; **Daniela Terson de Paleville, M.S.**; Sevda Aslan, Ph.D.; William McKay, B.S. Evaluation of respiratory muscle activation in individuals with chronic spinal cord injury. In press. *Respiratory Physiology & Neurobiology*, 173, 2010 (171-178)
7. **Daniela Terson de Paleville, MS**; Ann Marie Swank, PhD, Daniel Funk, PhD; Sherly Bradley, MS; Robert Topp, PhD, RN. Adding weights to low intensity exercise increases isometric muscular strength and functional ability in healthy older adults. *Journal International Fitness*. Vol.5, Issue 1, 2009.
8. **Daniela Terson de Paleville, MS**, Robert Topp, PhD; Ann Marie Swank, PhD. Effects of aerobic training prior to and during chemotherapy in a breast cancer patient: a case study. *Journal of Strength and Conditioning Research*, 2007 May; 21(2):635-7.

TEACHING EXPERIENCE

1. Department of Health and Sport Sciences, University of Louisville, Louisville, KY. January 2011- present: Advanced Exercise Physiology; Physiology of Exercise and Neuromuscular Mechanics of Human Movement
2. Department of Health and Sport Sciences, University of Louisville, Louisville, KY. Fall 2005 and Spring 2006: First Aid responding to emergencies.
3. Department of Health and Sport Sciences, University of Louisville, Louisville,

KY. Fall 2005 and Spring 2006: Healthy Lifestyles.

4. Department of Health and Sport Sciences, University of Louisville, Louisville, KY. Fall 2004 and Spring 2005: First Aid responding to emergencies.
5. Department of Health and Sport Sciences, University of Louisville, Louisville, KY. Fall 2003 and Spring 2004: Beginning Swimming.
6. Department of Health and Sport Sciences, University of Louisville, Louisville, KY. Fall 2003 and Spring 2004: Intermediate Swimming.
7. Department of Health and Sport Sciences, University of Louisville, Louisville, KY. Fall 2003 and Spring 2004: Fitness Walking.
8. Department of Health and Sport Sciences, University of Louisville, Louisville, KY. Fall 2003 and Spring 2004: Mini-marathon training.
9. Department of Health and Sport Sciences, University of Louisville, Louisville, KY. Fall 2003 and Spring 2004: Water Fitness.
10. Department of Health and Sport Sciences, University of Louisville, Louisville, KY. Fall 2003 and Spring 2004: Step aerobics.
11. Department of Health, Exercise and Sport Sciences, The Citadel, Charleston, SC. Fall 2002 and Spring 2002: First Aid and CPR.
12. Department of Health, Exercise and Sport Sciences, The Citadel, Charleston, SC. Fall 2002 and Spring 2002: Beginning Swimming.
13. Department of Health, Exercise and Sport Sciences, The Citadel, Charleston, SC. Fall 2002 and Spring 2002: Intermediate Swimming.
14. Department of Health, Exercise and Sport Sciences, The Citadel, Charleston, SC. Fall 2002 and Spring 2002: Dance Aerobics.

FUNDING HISTORY

2006-2008 University of Louisville IPIBS Fellowship (Integrated Programs in Biomedical Sciences)