

Muscle Quantity and Quality after Chronic Spinal Cord Injury: An investigation of calf-muscle cross-sectional area and density after long-term paralysis

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Background/Objectives: Individuals with a spinal cord injury (SCI) experience reductions in lower-extremity muscle mass and increased fatty-infiltration of skeletal muscle, predisposing them to an increased risk of specific secondary health conditions. To date, few investigations have prospectively examined changes in muscle in the chronic stage of SCI, especially in women, the aged, and those with incomplete injuries. Peripheral quantitative computed tomography (pQCT) is an imaging technique capable of measuring lower-extremity skeletal muscle cross-sectional area (CSA) and muscle density, the latter is a surrogate measure of muscle fatty infiltration. The purpose of this project was to a) determine the magnitude of muscle CSA and muscle density reduction in a chronic-SCI population with diverse impairments; b) identify demographic and injury characteristics associated with muscle CSA and density status; and c) determine if muscle CSA and muscle density change over a two-year period following chronic-paralysis and if so, what factors are associated with the changes.

Materials and Methods: Seventy individuals [50/20 m/f, mean (\pm SD) age 48.9 ± 11.5 years; duration of injury 15.5 ± 10.0 years] with chronic (>2 years post-injury) SCI (C1-T12, AIS A-D) were enrolled in a two-year cohort study. Muscle CSA and muscle density values were calculated from pQCT scans of the 66%-site of the calf obtained at baseline and two follow-up visits separated by one year. Possible correlates of muscle CSA and density selected *a priori* included: gender, age, height, weight, waist circumference, age at injury, level of injury, injury duration, leg spasm frequency and severity scale score (SFSS), ISNCSCI calf-muscle lower-extremity motor score (cLEMS), wheelchair use, serum vitamin D level, and physical activity level. Dependent t-tests were used to compare muscle CSA and muscle density values of participants with complete and incomplete-SCI to age, gender, and height matched able-bodied

controls. Multiple linear regression models were used to determine correlates of muscle CSA and muscle density. Repeated measures analysis of variance (rANOVA) were used to examine change in muscle CSA and density over the two-year study duration and multiple linear regression models were created to determine correlates of muscle CSA and density change from baseline.

Results: Individuals with motor-complete SCI had a 45% reduction in muscle CSA and a 32% reduction in muscle density relative to controls. Participants with motor-incomplete SCI had a 17% reduction in muscle CSA and a 14% reduction in muscle density relative to controls. A reduced height, waist circumference, cLEMS, and wheelchair use were associated with a smaller muscle CSA in the best-fitting regression model ($R^2 = 0.66$; $p < 0.0001$). In the best-fitting regression model for muscle density, increased age, a lower cLEMS, reduced SFSS, fewer minutes of daily vigorous physical activity, and wheelchair use were associated with a lower muscle density ($R^2 = 0.37$; $p < 0.001$). A high degree of individual variability in muscle CSA change (mean \pm SD: $-1.9 \pm 6.2 \text{ cm}^2$; range: -22.6 to 8.5 cm^2) and muscle density change (mean \pm SD: $-1.2 \pm 3.28 \text{ mg/cc}$; range: -8.6 to 6.4 mg/cc) was observed in those with both complete and incomplete SCI over the two-year study duration. rANOVA indicated a significant reduction in both muscle CSA and density after controlling for individual variability. A greater waist circumference at baseline was weakly associated with a reduction in muscle CSA ($R^2 = 0.14$, $p < 0.05$), and a lower weight and waist circumference at baseline were associated with a reduction in muscle density ($R^2 = 0.26$, $p < 0.001$ and $R^2 = 0.20$, $p < 0.01$, respectively).

Conclusion: Age, completeness of injury, spasticity, physical activity participation, and ambulation ability were identified as potential clinical predictors of muscle status in individuals with chronic-SCI. Muscle CSA and density does not reach a “steady-state” after chronic-SCI.

Further investigation is needed to determine the mechanisms responsible muscle CSA and density change in order to prevent continued reductions after chronic-SCI.

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CHAPTER 1: INTRODUCTION

Individuals with a spinal cord injury (SCI) experience profound reductions in lower extremity muscle mass and muscle quality (1-7). The average muscle cross-sectional area (CSA) of atrophied lower extremity muscle post-SCI has been observed to be approximately 45-80% of able-bodied peers depending on the muscle examined and the degree of impairment or completeness of injury (4,8-10). In addition to atrophy, there is an accumulation of fatty tissue within and between the muscle groups of the lower extremities to the order of three- and four-fold increases in those with incomplete and complete injuries respectively (4,9,11). These deleterious changes in muscle are a contributing factor to mobility limitations (8) and secondary health conditions common after SCI, including cardiovascular disease and diabetes (4,9,12-14).

The lifespan of individuals with SCI is approaching that of their able-bodied peers (15), and consequently, the incidence of secondary health conditions is increasing as the SCI-population ages (16,17). Skeletal muscle plays a vital role in maintaining systemic health, and muscle atrophy and weakness are implicated in the development of common co-morbidities such as osteoporosis, cardiovascular disease, and diabetes (6,14,18-20). After SCI, there is a rapid decline in bone mineral density such that individuals with SCI can lose up to 70% and 52% of bone mineral content in the distal femur and proximal tibia, respectively (21-24). Skeletal muscle is capable of exerting extremely high forces on bone that can be several times greater than body weight (25), thus, it has been suggested that reduced muscle forces on bone after SCI may contribute to bone loss beyond that associated with reduced weight bearing alone (6,20). Some studies (26,27), but not all (28-31), have demonstrated that electrical stimulation of muscle may be effective in preventing osteoporosis or restoring lower-extremity bone mineral content in

individuals with SCI. As recent evidence has suggested a muscle-bone interaction, examination of muscle status may provide insight into bone loss and fracture risk post-SCI (32).

Cardiovascular disease (CVD) is the number one cause of death after SCI and has a prevalence of 30 - 50%; which is high in contrast to age and gender matched able-bodied populations, where the prevalence of CVD is typically reported to be in the range of 5 - 10% (14). Additionally, diabetes is three times more prevalent in the SCI population and develops at a younger age compared to the able-bodied population (13). A positive energy balance, occurring when energy intake exceeds energy expenditure, increases the risk of obesity and related co-morbidities. Therefore, maintaining a healthy energy balance after SCI is important for preventing metabolic syndrome, cardiovascular disease, and diabetes (33-35).

After SCI, resting metabolic rate, which is a primary determinant of energy expenditure, is 14 - 27% lower compared to those without SCI (36). Lean mass is the single most important predictor of resting metabolic rate in the able-bodied and SCI populations, accounting for 70% of the variation in resting metabolic rate (37-41) due to the numerous high-energy processes which occur in organs and muscles, including ion pumps, synthesis and degradation of cell constituents, biochemical cycles, and leakage of protons across mitochondrial membranes (36). In addition, skeletal muscle is responsible for up to 75% of insulin-stimulated clearance of blood glucose (42,43), and reduced muscle mass is related to impaired glucose metabolism and blood sugar regulation in the able-bodied (44) and SCI population (45-48). Secondly, increased skeletal muscle lipid accumulation observed in parallel with muscle atrophy is related to impaired glucose tolerance, again in able-bodied individuals (49-53) and individuals with SCI (9). As skeletal muscle is a major site of metabolic activity, examining muscle status post-SCI may

provide insight into metabolic disease detection, prevention, and treatment in the chronic-SCI population.

According to the Rick Hansen Foundation, those with motor incomplete SCI make up approximately half of the SCI population (54). People with incomplete SCI often have impaired ambulatory capacity characterized by reduced gait speed, step frequency, and stride length (55,56). Given the array of preserved motor function in those with incomplete SCI, it is presumed that there will be variability in muscle status after incomplete SCI. Additionally, muscle atrophy is associated with reduced muscle strength (57-61) and locomotor ability (62,63). In addition, skeletal muscle atrophy after incomplete SCI is associated with decreased voluntary and electronically evoked force (8). Therefore, an increased understanding of the correlates of muscle change may be beneficial in determining who experiences the most detrimental changes in muscle, to ultimately prevent or reverse reductions in strength, function, and mobility over time.

Lastly, as Shields (6) highlights, it is becoming increasingly realistic that a cure for SCI will be available within the 21st century. An understanding of the changes in muscle post-SCI are imperative as considering individuals must have an intact musculoskeletal system that is not irrevocably impaired by chronic paralysis, in order to support weight bearing and ambulation after re-innervation. There is little information outlining the degree of muscle atrophy in the chronic-SCI population. Therefore an investigation characterizing muscle status based on demographic and injury-related characteristics is warranted to understand who may benefit the most from future cure-related therapies that promote restoration of neurological function.

Quantitative computed tomography (QCT) is a non-invasive imaging technique capable of distinguishing tissue types *in vivo* based on their X-ray attenuation characteristics. On this

basis, QCT can determine the area of muscle, bone, and subcutaneous fat from cross-sectional images. In addition, QCT images of muscle can generate muscle density information that can serve as a surrogate measure for the amount of fatty infiltration into the muscle (64). A number of studies have used QCT to measure lower extremity muscle CSA and density in animals and humans, including those with SCI (65-70). Peripheral quantitative computed tomography (pQCT), a modality using a smaller bore scanner capable of accommodating arms and legs, has been previously utilized to examine the relationships between muscle CSA, muscle density and disease risk (69,70). Unpublished work by Wong et al. has demonstrated an acceptable reproducibility (< 5% precision error) of muscle density across a range of age cohorts, and those with SCI (71) (Appendix A). It is therefore hypothesized the pQCT technology will provide novel insight into the distal lower-extremity muscle status of those with chronic- SCI.

The predominance of literature examining changes in muscle CSA and muscle density after SCI is focussed on the initial changes that occur in the acute and sub-acute stages of injury (2,4,7,8,11). There is a consensus among investigators that a rapid decrease in muscle size and quality occurs immediately after injury, followed by a new steady state that develops within 1-2 years post injury (10). However, the notion of a “steady-state” after 1 - 2 years post-injury has been brought into question (72). Surprisingly, there is a dearth of literature examining muscle characteristics in the chronic stage of SCI despite evidence that a change in muscle status may occur. For example, in a case series, three individuals with SCI experienced reductions in thigh and lower limb muscle CSA, ranging from -2.3% to -16.8% in one year (73). The potential for changes in muscle in the chronic stages of SCI warrants further investigation.

Secondly, the majority of available literature examining muscle status after SCI has employed a homogeneous sample of men, and almost exclusively those with motor-complete

SCI (9,27,72,74-88). This is perhaps in an effort to limit confounding factors, however as a result, changes that occur in females and those with incomplete injuries have not been adequately examined. In fact, no investigation has examined muscle outcomes in individuals with chronic incomplete SCI despite their innate neuroplasticity and preservation of motor function which gives these individuals the potential to progress functionally to a greater extent than those with complete SCI (89).

In summary, the purpose of this study is to determine the extent of muscle change in a chronic-SCI population with diverse impairment. In doing so, potential correlates of muscle mass and muscle density will be identified to increase our understanding of muscle size and quality in individuals with chronic SCI. Identifying those with the most adverse changes in muscle may potentially identify those at most risk for related secondary health complications.

CHAPTER 2: BACKGROUND

2.1 Spinal Cord Injury

As of 2012, approximately 85,500 people live with SCI in Canada (15). SCI can occur: a) when a traumatic event, such as a motor vehicle accident, fall, or violent event causes an external physical impact and damages the spinal cord, or b) as a result of non-traumatic damage to the spinal cord as a result of a tumour, transverse myelitis, aneurysm repair, etc. Of the total prevalence rate, an estimate 51% sustained their SCI as a result of traumatic, and 49% from non-traumatic causes (15). The national incidence rate for SCI is estimated to be 4,259 new cases per year. Of this total, an estimated 42% are the result of traumatic SCI and 58% are from non-traumatic SCI (15). The average age at injury across the country is 37.6 years with the majority of injuries occurring between the ages of 16 and 30 (90). Individuals with SCI have a life expectancy of 25 to 30 years beyond their injury with CVD and diabetes being the most common causes of death for those with chronic-SCI (91).

Recent studies examining SCI incidence rates in Ontario have highlighted an increasing number of older adults sustaining a SCI resulting in a bi-modal age-of-onset distribution, with the incidence of SCI peaking among individuals who are in their third and sixth decade (92-94). This bi-modal age distribution is also reflected in the cause of injury and the level of injury. Younger adults are most often injured in motor vehicle collisions whereas older adults are most often injured by falls. With regard to level of injury, younger adults more commonly sustain an injury at the thoracic or lumbar level, whereas older adults more commonly sustain injuries at the cervical level (94). As the age and etiology distribution changes, it is hypothesized that the

effects of age-related health conditions will have a greater impact on the health, quality of life, and life expectancy of those with SCI.

2.1.1 SCI and Aging

The incidence rate of SCI for older adults is growing faster than any other age demographic (92-94). A study examining 936 incident cases of traumatic SCI in Ontario between the years of 2003 and 2007 reported a marked increase in SCI incidence with age beginning between the ages of 40-49 years in men and 50-59 years in women. Furthermore, the incidence rate for men aged 80+ was three times higher than the incidence for men 40–49 years of age. For women, the incidence rate for the 80+ age group was more than four times higher than the incidence rate for the 50–59 age group. Interestingly, falls surpassed motor vehicle collisions as the leading cause of SCI for men and women in Ontario (47.8% and 54.1%, respectively), with a drastic increase in the number of fall-induced injuries observed in the older age groups. This shift in the age of SCI-onset and cause of SCI indicates a shift in the health considerations for those with SCI, as secondary age-related comorbidities may become more prevalent.

Less healthy and functionally less capable older adults are now surviving to older ages because of more effective health care. Consequently, individuals who sustain SCI at an older age may have pre-existing morbidities or impairments such as: osteoporosis, impaired muscle strength, compromised balance and reaction time, a less active lifestyle, poorer nutritional status, that may exasperate SCI-related impairments (95,96). Therefore, the detrimental effects of aging will have an increasing impact on the already expediated aging process observed in the SCI community.

In comparison to peers of the same age, individuals with SCI have an increased prevalence of cardiovascular disease markers, endocrine disruption, compromised immune function, musculoskeletal degradation, and compromised respiratory function. The evidence of increased age-related morbidity at SCI onset, SCI-induced premature aging (191) and age-related functional declines (195) supports the need for continued investigation of chronic disease risk-factors specific to the SCI population. As reductions in muscle quantity and quality are major risk-factors for chronic disease, investigation of muscle status in the chronic-SCI population may be beneficial for chronic disease prevention and treatment.

2.1.2 Pathophysiology of Traumatic SCI

A traumatic SCI is biphasic; consisting of an initial mechanical trauma (primary injury) which triggers a cascade of deleterious secondary effects (secondary injury) (97). The initial injury most commonly occurs as a result of traction and/or compression forces from a traumatic event such as a car accident or fall. The majority of injuries are the result of blunt trauma; however, penetrating trauma due to knife or gunshot wounds make up a significant percentage of cases. SCI can involve shear, stretch, and more commonly contusive and compressive forces on the cord, and in a small number of cases the cord is completely severed. Investigations using a rat model have shown that the degree of neurological impairment is correlated with the force of the trauma and duration of spinal cord compression (97).

Immediately following injury, neurons and their axons become permeabilized leading to immediate cell disability and death (97). Edema and hemorrhaging occurs in the grey matter and to a lesser extent in the white matter as a result of damaged vasculature. In addition, animal studies have demonstrated that post-SCI, there is damage to the meningeal layers and spinal roots, and bleeding in the subdural and subarachnoid space (97). The spinal cord swells to

occupy the entire diameter of the spinal canal at the injury level and secondary ischemia results when cord swelling exceeds venous blood pressure (98). Autoregulation of blood flow ceases, and spinal neurogenic shock leads to systemic hypotension, thus compounding the ischemia (98). After the effects of the initial trauma, the individual succumbs to a group of complications known as secondary injury which can exasperate the degree of impairment.

During secondary injury, systemic, molecular, and cellular cascades, triggered by the initial trauma expand the debilitating effects of the initial injury to adjacent white and grey matter (97). The severity of the primary injury determines the characteristics of the secondary injury, which progresses in the hours and days after the initial trauma. The hypoperfusion that develops in grey matter extends to the surrounding white matter, slowing or completely blocking the propagation of action potentials along axons. This contributes to a condition known as spinal shock. During spinal shock, damaged neurons and glia undergo necrosis. Edema and hemorrhaging, the magnitude of which corresponds to the injury severity, creates ischemic zones at, and around the injury site and increases the amount of necrotic cell death (97). In addition, damaged cells, axons, and blood vessels release toxic chemicals that attack intact neighbouring cells. Glutamate, which is secreted in tiny amounts from the tips of many axons to stimulate cell impulses, floods out of damaged spinal neurons, axons, and astrocytes, thereby overexciting surrounding neurons. The overexcited cells let in waves of calcium ions that trigger a series of destructive events, including production of free radicals, which can attack membranes and other cell components thereby destroying healthy neurons (98). In addition, secondary injury kills oligodendrocytes, the nervous system's myelin-producing cells, possibly resulting in unsevered axons becoming demyelinated and therefore unable to conduct impulses (97). At six months after the initial trauma, axon degeneration continues and neuropathic pain may develop. By one

to two years post-SCI, it is believed that the lesion has ceased to progress and motor and sensory impairment stabilizes. At this point, an individual's chronic-impairment profile can be classified (97).

The injury progression of non-traumatic injuries is much less defined due the heterogeneity of causes. However, clinical signs, symptoms, and general therapeutic principles apply equally to those with non-traumatic injury (98). The most relevant difference between traumatic and non-traumatic etiologies is the rate at which impairments develop. Whereas traumatic-SCI is the result of a sudden event, impairment as a result of a non-traumatic SCI is more gradual in onset. This study will exclude individuals with non-traumatic SCI in order to limit the variability in the rate of impairment onset.

2.1.3 Injury Classification

The SCI population exhibits a diverse impairment profile dictated by the level of injury and the degree of preserved motor, sensory, and autonomic function. To classify the level and completeness of SCI, the International Standards for Neurological and Functional Classification of Spinal Cord Injury published by the American Spinal Injury Association (ASIA), is commonly used. The neurological level of lesion is the most caudal segment of the spinal cord with normal sensory and motor function on both sides of the body as assessed by dermatome and myotome sensitivity and impairment (99). Some individuals may have a different sensory level and motor level on each side of the body in which case, left and right motor and sensory levels are reported. More generally, injury level can be grouped into two categories: tetraplegia or paraplegia. Tetraplegia refers to impairment or loss of motor and/or sensory function in the cervical segments of the spinal cord and results in impairment of function in the arms, trunk, legs

and pelvic organs, whereas paraplegia refers to impairment or loss of motor and/or sensory function below the cervical level (i.e. in the thoracic, lumbar or sacral segments) of the spinal cord. With paraplegia, arm functioning is spared, but, depending on the level of injury, the trunk, legs and pelvic organs may be affected (99).

Secondly, SCI is classified into complete and incomplete categories. An individual with a complete SCI has an absence of sensory and motor function in the lowest sacral segment of the spinal column (89). An individual with an incomplete injury will have partial preservation of sensory and/or motor function below the neurological level of injury including the lowest sacral segment. Preserved sacral sensation includes sensation at the anal mucocutaneous junction as well as deep anal sensation. The test for preserved motor function at the lowest sacral segment is the presence of voluntary contraction of the external anal sphincter upon digital examination (99). As would be expected, those with complete injuries have greater muscle atrophy compared to those with incomplete injuries (7).

The majority of individuals with SCI have damage to both the upper and lower motor neurons (98). However, in some instances, only the upper or lower motor-neurons are affected. An upper motor neuron lesion is a lesion of the neural pathway above the anterior horn cell of the spinal cord, whereas a lower motor neuron lesion affects only nerve fibers traveling from the anterior horn of the spinal cord to the relevant muscle. Upper motor neuron lesions result in spastic paralysis, whereas lower motor unit lesions result in flaccid paralysis (98). Spasticity, characteristic of involuntary muscle tone, is prevalent in an estimated 40-70% of individuals with SCI (100). Spasticity is related to both negative and positive health outcomes and function post-SCI. For example, negative symptoms include weakness, fatigability, and loss of co-ordination of the extremities (100). However, positive symptoms of spasticity include preservation of

muscle mass and protection from metabolic disorders (101). Because of the positive effect on muscle, spasticity may be an important determinant of muscle quality and quantity in those with chronic-SCI.

Understanding an individual's impairment is important for assessing prognosis and rehabilitation goals. In a research environment, reporting impairment profile is vital for maintaining the external validity of the study results. One of the most widely used impairment classification tools is the ASIA impairment scale (AIS) which uses a single label (letters A-E) to summarize an individual's motor and sensory impairment (Table 1) (99). The AIS is a standard grading system with one level for complete (AIS A), and three for incomplete injuries (AIS B-D). The AIS is based on a systematic neurological examination of sensory and motor function. The sensory level is examined by testing dermatome sensitivity to pin prick and light touch. Each dermatome is scored as having either normal, impaired, or absent sensation. Likewise, the motor impairment level is determined by a manual muscle exam to test the strength of ten key muscle groups on a six-point scale where zero represents no function, and five represents normal function.

Table 1 ASIA Impairment Scale (AIS)

AIS Grade	Completeness	Description
A	Complete	No motor or sensory function is preserved in the sacral regions 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	Incomplete	Motor and sensory function is normal.

2.2 Skeletal Muscle

2.2.1 Physiology

There are three kinds of muscle tissue in the human body: skeletal muscle, cardiac muscle, and smooth muscle. Skeletal muscle makes up 40% of total body weight in able-bodied individuals. Skeletal muscle is made up of long muscle fibers surrounded by a connective sheath. Groups of adjacent sheaths are bundled together and called fascicles. Between each fascicle are collagen, elastic fibers, nerves, and blood vessels which are surrounded in a connective tissue which holds the muscle body to the bone (102).

Skeletal muscle fibers are classified in two ways: contraction speed and resistance to fatigue after repeated stimulation. Fiber groups include: slow-twitch (type I), fast-twitch oxidative-glycolytic (type IIa), and fast-twitch glycolytic fibers (type IIb). Fast-twitch muscle fibers develop tension 2 - 3 times faster than slow-twitch muscle fibers. The isoform of myosin ATPase, which is responsible for adenosine triphosphate (ATP) breakdown, dictates the rate of ATP degradation, and subsequently the speed at which the muscle fiber contracts. Fast-twitch fibers have a fast isoform of ATPase, whereas slow-twitch muscle fibers have the slow isoform

of ATPase. The duration of contraction also varies according to fibre type. Fast and slow-twitch fibers also have a corresponding isoform of sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) which dictates the speed at which calcium can be transferred out of the cytosol, and therefore the speed of contraction. Fast-twitch fibers have a fast isoform of SERCA, and therefore can cycle calcium at a faster rate than slow-twitch fibers. Secondly, muscle fibers can be grouped by their fatigue resistance. Glycolytic fibers (fast-twitch type IIb) rely on anaerobic metabolism to produce ATP, and therefore are susceptible to acidosis which is implicated in the development of fatigue. Oxidative fibers (slow-twitch and fast-twitch type IIa fibers) rely on oxidative phosphorylation to produce ATP, and have increased mitochondrial density and blood supply, and therefore are more fatigue-resistant. Oxidative fibers are smaller in diameter compared to glycolytic fibers and have an increased capillary density. Human skeletal muscle has a mix of fibre types depending on the muscle, genetic predisposition, and training/deconditioned status. The postural muscles of the lower extremities are predominately comprised of type I muscle fibers due to their prolonged periods of contraction (102).

The basic unit of contraction is referred to as a motor unit, which is composed of a group of muscle fibers and a corresponding somatic motor neuron that initiates their contraction. Skeletal muscle is unique in that it contracts in response to a signal from a somatic motor neuron. The number of muscle fibers activated by a motor neuron varies. In muscles responsible for fine motor movements, a motor unit may contain only a few fibers, whereas motor units involved in gross movements contain hundreds to thousands of muscle fibers activated by a single neuron. All muscle fibers in a motor unit are the same type; consequently, there are fast and slow-twitch motor units. The speed of a motor unit is determined by the neuron during embryonic development as a result of growth factors secreted by the neuron (102). After SCI, motor unit

and fiber-type disruption shifts towards a faster, predominantly type IIB phenotype which can impact function and the use and efficacy of rehabilitation interventions and individuals' health status (103,104).

2.2.2 Muscle Plasticity

As with many systems in the human body, muscle has the remarkable ability to adapt to fluctuating stimuli including physical activity, injury, disuse, and illness. The homeostasis of muscle tissue is controlled by a complex and interconnected network of endocrine and neurological signalling cascades that regulate the balance of anabolic and catabolic processes. As individuals mature from childhood to adulthood, myoblasts undergo proliferation and differentiation into myofibrils which increase in size and density in response to intrinsic muscle formation mechanisms associated with growth and maturation, and external incitements such as increased loading (105). When exposed to increased nutritional, hormonal or mechanical stimuli, adult skeletal muscle tissue can respond by increasing the size of the muscle and the force produced during contraction (106). Adaptation occurs mainly by an increase in the size of individual muscle fibers (hypertrophy) (107,108) with a possible increase in fiber number (hypersplasia) (109,110) due to a net accumulation of both sarcoplasmic and myofibrillar proteins (111). The most extensively documented pathway and primary regulator of protein synthesis involved in muscle hypertrophy is the PI3K/Akt/mTOR signaling cascade which enhances protein turnover and synthesis in favour of a net increase in the rate of protein synthesis over degradation (112,113). This pathway is regulated by external influences such as mechanical strain, growth factors, nutrition, and energy balance to maintain optimal muscle status (114). Conversely, muscle tissue also adapts to reduced nutrient availability and/or activity status by decreasing the size and number of muscle fibers.

Muscle atrophy is prevalent in a variety of conditions including cast immobilization (59,60), unilateral lower limb suspension (57,115), bed rest (116,117), space flight (118,119) and inactivity as a result of injury or disease (120,121), and is the result of a net protein balance in favour of degradation. This negative balance is regulated by two major degradation pathways including the ubiquitin-proteasome system and the autophagy-lysosome pathway (122).

During embryonic development, somatic motor neurons secrete growth factors that dictate the type of muscle fiber; subsequently, adult muscle has a relatively fixed distribution of fiber types (102). However, it must be noted that the response to atrophic and hypertrophic stimuli varies depending on the muscle and muscle fiber type composition. For example, with regards to nutrient deprivation, predominantly slow-twitch muscle fibers, such as the soleus, are less sensitive to starvation compared to fast muscles (123). Secondly, the catabolic effects of aging affect type II muscle fibers to a greater extent than type I, whereas paralysis affects type I fibers to a greater extent than type II; a concept that will be elaborated further in section 2.2.4 of this report.

2.2.3 Glucose Metabolism and Skeletal Muscle Insulin Resistance

Skeletal muscle is a metabolically active tissue that accounts for approximately 85% of total insulin-stimulated glucose uptake (124-126). Therefore, skeletal muscle is the predominant tissue responsible for whole body insulin-dependent glucose utilization, and reduced muscle quantity and quality is implicated in insulin resistance and related morbidities (49,127,128). Insulin is produced by beta-cells in the pancreas and circulates the body dissolved in blood plasma. Insulin is released in response to a plasma glucose concentration above 100 mg/dl and initiates glucose uptake in the muscle with the effect of decreasing plasma glucose concentration.

At the cellular level, insulin-initiated signalling increases glycogen synthesis, aerobic metabolism of glucose, and protein and triglyceride synthesis (102). In skeletal muscle, GLUT4 is the primary transporter that mediates insulin-stimulated glucose uptake (129). In the basal state, GLUT4 is located in intracellular membrane vesicles, and upon insulin stimulation, GLUT4 is translocated from the intracellular pools to the plasma membrane to facilitate glucose entry into the cell (130).

Insulin resistance in skeletal muscle is exemplified by the decreased ability of insulin to cause translocation of the GLUT4 receptors to the muscle cell surface and subsequent impaired glucose uptake (131-133). Several potential mechanisms are responsible for the development of insulin insensitivity. Macrophage-derived proinflammatory cytokines such as tumor necrosis factor alpha (TNF- α), and interleukin -1b, -4, and -6, which reduce IRS-1 (a tyrosine residue expressed on the skeletal muscle cell surface and believed to mediate GLUT insulin signaling) phosphorylation are likely involved in reduced GLUT4 activity. Additionally, increased fatty acid uptake by muscle cells coupled with diminished mitochondrial lipid oxidation can lead to insulin resistance causing intramyocellular accumulation of lipids and fatty acyl metabolites (134). These excess fatty acids are esterified and either stored or metabolized to various molecules that may interfere with normal cellular signaling, particularly insulin-mediated signal transduction; altering cellular and whole-body glucose metabolism (135). Recently, an association between impaired insulin action and intramuscular lipid accumulation has been observed.

The mechanisms linking intramuscular lipid accumulation with glucose intolerance remains unclear; however, it has been suggested that intramuscular lipid stores may provide a source of fuel that competes with glucose as an oxidative substrate, which secondly contributes

to the synthesis of other lipid-derived entities that directly interfere with insulin signal transduction (136). Secondly, decreased muscle glycogen synthesis due to insulin resistance increases carbohydrate flow to the liver and causes elevated lipogenesis (137) which supports the notion that insulin resistance in skeletal muscle has a major impact on whole-body insulin sensitivity (134). Studies have found that fat infiltration in skeletal muscle correlates more closely with systemic insulin resistance than with other important factors such as body mass index (BMI), waist-to-hip ratio, and total adiposity in the able-bodied (138). As those with SCI have an altered body composition that decreases the accuracy of traditional metabolic disease screening tools, it is proposed that the examination of muscle fatty infiltration may offer a novel screening method for glucose intolerance post-SCI.

2.2.4 Age-Related Changes in Muscle

It is well established that there is an age-related reduction in muscle mass and quality as the result of neurological, hormonal, nutritional and physical inactivity related changes associated with the biological aging process (139-145). Maximum muscle mass is reached by approximately 25 years of age (146) at which point there is a gradual decrease as individuals age. A reduction to the order of 25 - 40% in lower extremity muscle CSA in older men and women compared to young adults has been observed (140,147-149). Longitudinally, in a cohort of 1678 older adults between the ages of 70-79 years of age, it was observed that on average, older men lose approximately 1% of their thigh muscle CSA per year, and older women lose approximately 0.65% of their thigh muscle CSA per year (150).

It is suggested that the age-related loss in muscle mass is a result of a reduction of type II muscle fiber size, and to a lesser extent, a decrease in muscle fiber number. However, there is a high amount of variability perhaps due to the general heterogeneous composition of skeletal

muscle. A preferential decrease in the number of type II fibers, beginning at approximately age 25 has been observed, such that the total fiber number decreases by about 39% by age 80 (146). There is also an age-related decline in fiber size that is most prominent in type II fibers, whereas type I fibers are often less affected (144,151-155). Aging is also associated with a loss of both fast and slow motor units with a preferential loss of fast motor units (156), and as a result, there is a net conversion of type II to type I fibers, as type II fibers are recruited into slow motor units (157). Ultimately, the age-related decrease in type II fiber number and CSA, as well as a transition from type II to type I fibers due to motor unit switching results in a predominantly type I phenotype in aged muscle.

In accordance with the muscle size and phenotype changes associated with aging, there is a decrease in muscle strength, a slowing of contractile properties, and an increase in fatigue resistance. Summarizing reviews by Vandervoort and Doherty (140,145) report the following three characteristics of age-related strength decline: 1) the average decrease in muscle strength measured in the seventh and eighth decade is 20 - 40% of younger populations with strength attenuation increasing upwards of 50% in the ninth decade; 2) declines in strength are similar in proximal and distal limb muscles; and 3) relative strength losses appear similar for men and women; however, because men typically start from higher baseline values, their absolute losses of strength are greater. In addition, age-related decreases in muscle torque have been observed to be 2 - 5 times greater than the loss of muscle CSA, suggesting that other factors besides decreased muscle mass contribute to the loss of strength (150).

Changes in contraction speed that occur with aging are consistent with the fiber type changes observed. Aged muscle reaches tetanus at lower fusion frequencies, and shows a slowing of electrically evoked muscle contraction speeds (158-160). In aged muscle, reduced

force output and contraction speed is coupled with an increase in fatigue resistance (161). It has been suggested that increased fatigue resistance in older adults is the result of lower maximal motor unit discharge rates, slower contractile properties, and a relatively greater reliance on oxidative metabolism (162), presumably as the result of increased expression of type I fibers (161).

An association between age and fatty infiltration of muscle has also been observed (150,163-165). One study reported an age-related increase in fatty infiltration of mid-thigh skeletal muscle in aged (70 - 79 years) men (36-75%) and women (17-50%) over a five year period (150). Interestingly, skeletal muscle fat content increased in those who lost weight, gained weight, and remained weight-stable. Additionally, evidence suggests that skeletal muscle lipid content influences muscle strength and mobility function (63,165), as well as increases the risk of future mobility loss in older men and women (61). Lastly, as alluded to previously, increases in fatty infiltration within the muscle are associated with impaired glucose metabolism and insulin resistance in the aged population (126,166-168). Fatty infiltration has been proposed as a contributing factor to the functional declines and morbidities associated with aging; however the causal pathway is not fully understood. It is hypothesized that as the SCI population ages, age-related changes in muscle will have a greater impact on health outcomes.

2.3 Skeletal Muscle after SCI

2.3.1 Overview

After SCI there is a transition in skeletal muscle that is characterized by: a loss of muscle mass, a transition to an almost exclusively type II muscle phenotype, an increase in speed of contraction properties, and fatty infiltration of the muscle. There is a reduction in muscle size in all muscles below the level of lesion, with greater atrophy observed after complete compared to

incomplete SCI (2,4,7,9,75,169). There is also evidence that SCI induced atrophy is the result of a decrease in fiber size and a decrease in fiber number (3,5,81-83,85,170,171). Secondly, there is a transition from a mixed muscle phenotype to an almost exclusive type II fiber type expression in all muscles below the level of injury beginning approximately six months post-SCI (3,79,85,172-174). In addition, fast fiber isoforms of both SERCA and myosin heavy chain (MHC) isoforms are elevated disproportionately, resulting in fibers that are mismatched for SERCA and MHC isoforms and consequently are more susceptible to fatigue (175). In accordance with the observed atrophy and fiber-type transition, paralyzed muscle demonstrates a decrease in force output following electrical stimulation, an increase in speed of contraction properties and decreased fatigue resistance. Electrically evoked muscle torque and peak twitch forces produced by the quadriceps of individuals with SCI were 35% and 62%, respectively of the values of able-bodied controls (86,176), and maximal rate of force development was approximately 50% faster, and half-relaxation time about 20% quicker in paralyzed quadriceps muscle compared with normal control muscle (80).

In general, there is a reduction in the number of motor units in both completely and partially paralyzed muscles of individuals with chronic-SCI. However, as the size of the remaining motor units increases, and there is an inverse correlation between the number of surviving motor units and the average twitch force of single units (88,177,178). All muscles below the level of injury become less fatigue resistant such that a reported force loss and slowing of relaxation speeds following repeated fatiguing contractions is greater than those observed in able-bodied controls (80,84,174,176,179,180). Additionally, the degree of fatigue resistance after chronic-SCI is negatively correlated with the duration of injury (80,181). Lastly, as discussed in more detail below, there is an increase in adipose tissue accumulation in the

paralyzed muscles of the lower extremities between three and four times that of able-bodied peers (4,9,11). These detrimental changes in muscle have important implications with regards to mobility, independence, and risk for metabolic disease, and highlight the importance of rehabilitation modalities aimed at preserving muscle status such as electrical stimulation therapy.

2.3.2 SCI and Muscle CSA

Muscle after SCI is most prominently characterized by atrophy. In comparison to other disuse models, which display a 15 - 32% decrease in muscle size; atrophy of paralyzed muscles following SCI is markedly greater. Post-SCI, there is a reduction in muscle CSA observed as soon as six weeks post injury in all muscles below the level of injury, with greater atrophy observed after complete SCI compared to incomplete SCI. Castro et al. (2) reported an overall 46% decline in average CSA of the lower extremity muscles of individuals 24 weeks after complete-SCI, with decreases in the soleus (68%), gastrocnemius (54%), tibialis anterior (20%), quadriceps femoris (42%), and hamstring (44%) muscles reported relative to matched controls. Differential rates of atrophy of leg muscles during the first six months have been observed. For example, the gastrocnemius average CSA decreased by 24% between six and 24 weeks post-injury, while the soleus declined 12%, and the average CSA of the tibialis anterior did not change (2). These results suggest that atrophy is not solely dependent on muscle fiber type composition as the soleus and tibialis anterior each have a high percentage of slow-twitch fibers. In chronic-SCI (> two years post injury), mid-thigh fat free soft tissue mass, assessed by dual X-ray absorptiometry (DXA) was observed to be 39.7% lower (75), and muscle CSA assessed by magnetic resonance imaging (MRI) to be 38-44% lower (9,75) in those with complete SCI compared to age, height, and weight matched controls.

After six weeks of incomplete SCI, thigh muscle CSA assessed by MRI was reported to be 33% less than matched controls after accounting for intramuscular fat, and did not significantly change after an additional three months' time (4). Similar results were observed by Shaw et al. (7) who observed between a 24% and 31% decrease in CSA in incompletely paralyzed muscles of the lower extremity including soleus, medial gastrocnemius, lateral gastrocnemius, tibialis anterior, quadriceps femoris, and hamstrings approximately one year post-injury. In addition, subjects who used a wheelchair had significantly smaller muscle CSA values for all of the antigravity muscles (i.e., soleus, medial gastrocnemius, lateral gastrocnemius, and quadriceps femoris) relative to those who were ambulatory (7). Muscle mass is positively related to the degree of preserved motor function post-incomplete SCI however, the magnitude of this relationship has yet to be explored. The decrease in lower-extremity muscle CSA has been examined in the acute and sub-acute stage of SCI, however little investigation has examined the magnitude of muscle loss in the chronic-SCI population. Further investigation regarding the association between the degree of preserved motor function and muscle status is warranted.

2.3.3 SCI and Intermuscular and Intramuscular Adipose Tissue

In addition to atrophy, there is increased infiltration of fat within skeletal muscle post-SCI. Intramuscular adipose tissue is commonly defined as the lipid deposits contained in the intra- and extra- myocellular compartments, whereas intermuscular adipose tissue is defined as the adipose tissue located beneath the fascia lata and between individual muscles (182). Four studies have used magnetic resonance imaging (MRI) to quantify thigh intramuscular adipose tissue post-SCI. The first study was completed by Elder et al. (9) and observed an almost fourfold increase in percentage intramuscular adipose tissue in individuals with chronic complete

SCI compared to matched controls. In addition, fatty infiltration was a good predictor of plasma glucose metabolism in individuals with SCI, such that lipid content around skeletal muscle accounted for about 70% of the variance in plasma glucose 120 min after ingesting 75 grams of glucose. Gorgey et al. (4) observed similar results after six weeks of incomplete SCI in that participants had a relative intramuscular adipose tissue content three times higher compared to able-bodied controls. After an additional three months, the intramuscular adipose tissue content increased an additional 26%. Shah et al. (11) obtained similar results reporting that intramuscular adipose tissue content of the soleus muscle was 3.2 times higher in individuals with acute incomplete SCI compared to able-bodied controls, and that the increase in lipid content was due to an increase in both intramyocellular lipid and extramyocellular lipid content. In addition, both ambulatory and non-ambulatory individuals with incomplete-SCI showed elevated intramuscular adipose tissue content compared to corresponding controls (11). Lastly, Mojtahedi et al. (183) observed that athletes with SCI have greater absolute and relative thigh intermuscular adipose tissue accumulation relative to able-bodied controls. Ultimately, regardless of injury completeness and physical activity level, there are profound increases in skeletal muscle lipid accumulation post-SCI, predisposing those with SCI to increased risk of metabolic disease.

2.3.4 SCI and Glucose Homeostasis

Thanks to advances in SCI rehabilitation, the leading cause of death after SCI has shifted from SCI-related complications to secondary conditions (e.g. cardiovascular disease [CVD] and diabetes). CVD is currently the number one cause of death after SCI and is more prevalent, and presents earlier in life in those with SCI compared to the able-bodied population (14,184). Additionally, those with SCI have an increased susceptibility to virtually all CVD risk factors

including insulin resistance, dyslipidemia, overall obesity, diabetes, a sedentary lifestyle, and reduced physical activity (48,48,184,185). Impaired glucose regulation is indicated by elevated blood glucose and insulin levels is one of the earliest warning signs preceding the development of diabetes mellitus (185). Skeletal muscle is responsible for up to 75% of insulin-stimulated clearance of glucose from blood (42,43), and studies have confirmed a relationship between skeletal muscle fat infiltration and insulin resistance in healthy persons, as well as those with obesity and diabetes mellitus (128,138,186-190). In the SCI population, intramuscular adipose tissue accumulation has been observed to account for 70% of the variance in plasma glucose tolerance (9) and, as skeletal muscle fat infiltration and muscle atrophy are coupled, it is suggested that both play a role in impaired glucose metabolism (4,9). Accordingly, as traditional metabolic risk factors do not adequately identify those in the SCI population at risk for metabolic syndrome (191), quantification of muscle CSA and lipid content has the potential to enhance the identification of individuals at risk of insulin resistance and Type II diabetes.

2.3.5 Summary Skeletal Muscle after SCI

The changes in skeletal muscle post-SCI include reduced muscle mass, a transition towards a fast-twitch phenotype, increased speed of contraction, and increased fatty infiltration. In addition, adverse changes in muscle mass and lipid content have been observed to be associated with metabolic disease. The body of literature examining changes in skeletal muscle post-SCI is focused on the initial changes that occur immediately following injury and has predominantly investigated a small and select sample of the SCI population. Therefore, changes in muscle size and composition in the diverse chronic-SCI population are relatively unknown.

Further investigation as to the degree of muscle change post-SCI is warranted, especially in the chronic-SCI population who are most affected by metabolic dysregulation.

2.4 A Model of Skeletal Muscle Change after SCI

SCI results in a diverse spectrum of functional impairment and health conditions. As skeletal muscle changes in adaptation to the loads and environment it is exposed to, it is proposed that patient demographics (e.g., age, gender, fitness, etc.) and the characteristics of an injury (completeness, level of lesion, etc.) will determine the extent of muscle loss and fatty-infiltration post-SCI. It is hypothesized that a combination of SCI-specific and traditional risk factors for muscle loss and fatty infiltration will determine an individual's muscle status post-SCI, and therefore it is proposed that patient characteristics may be used to form a predictive model of muscle status. As muscle loss and fatty infiltration are associated with each other, it is hypothesized that they will have similar predictive variables. A predictive model of muscle status comprised of SCI-related variables (e.g., duration of injury, level of injury, completeness of injury, spasticity, etc.) and traditional factors influencing muscle status (e.g., age and gender) would be beneficial to: a) describing who experiences the greatest reductions in muscle status, and consequently, who are at the most risk for metabolic disease; b) identifying modifiable risk factors to prevent or treat reductions in muscle mass and fatty-infiltration, and c) determining who may benefit most from muscle-specific rehabilitation modalities.

A model for muscle status for those with chronic-SCI should include determinants of muscle status observed across able-bodied and clinical populations. First, it is hypothesized that gender will be a determinant of muscle status in those with incomplete injuries, as females have less muscle mass and more fatty infiltration prior to injury, and therefore females will have less muscle mass and greater fatty-infiltration post-injury. However, complete paralysis will negate

any gender differences. Secondly, it is hypothesized that older-adulthood is a risk-factor for reduced muscle status. In the able-bodied population, muscle status is negatively correlated with age after peak muscle mass is reached, and the rate of reduction in muscle status increases as individuals reach older-adulthood. Third, some evidence suggests that vitamin D deficiency is negatively associated with muscle status and function in older-adults (192). Therefore, it is hypothesized that vitamin D deficiency may be a determining factor of muscle status in those with chronic-SCI. Fourth, blood sugar dysregulation and systemic inflammation are associated with impaired protein production and decreased muscle mass. As systemic inflammation and blood sugar dysregulation have increased prevalence in the SCI population, it is hypothesized that these two factors will be important determinants of muscle status in those with chronic-SCI. Last, in those with motor-incomplete injuries who retain some degree of voluntary lower-extremity muscle activation, it is hypothesized that 1) low levels of physical activity, 2) the regular use of a wheelchair for ambulation, and 3) periods of lower-limb immobilization or prolonged bed rest will be associated with reduced muscle status. It is proposed that these traditional determinants of muscle status will interact with or compound the effects of paralysis on muscle for those with chronic-SCI.

SCI-specific factors that may interact with traditional determinates of muscle status include completeness of injury, level of injury, duration of injury, age at injury, and the presence of spasticity. Those with complete injures have greater adverse changes in muscle compared to those with incomplete injuries. In addition, having an incomplete injury will introduce other potential determinants of muscle status related to immobilization, ambulation, and physical activity. Secondly, level of injury is proposed as a determinant of muscle status. Those with SCI experience autonomic interruption which can lead to impaired blood flow to lower extremity

muscle (185), atrophy of cardiac muscle, reduced exercise capacity, and exercise-induced decreases in blood pressure leading to critically low perfusion pressure in working muscle (193). It is hypothesized that the extent of autonomic disruption can be determined by the level of injury and consequently, level of injury may be a determining factor of status. For example, muscle status may be reduced in those with autonomic disruption to the heart compared to those with intact cardiovascular innervation. Thirdly, it is hypothesized that the duration of injury will be negatively correlated with muscle status, as extended disuse in combination with SCI-related catabolic morbidities will result in adverse muscle changes. Fourth, it is hypothesized that age of injury will be correlated with muscle status because those injured later in life will have a reduced muscle status at the time of injury, and thus a reduced muscle status post-SCI. Lastly, spasticity results in preserved muscle tone and has been observed to be positively associated with muscle status, therefore spasticity is proposed as a determinant of muscle preservation in those with chronic-SCI. In combination with traditional risk factors, it is hypothesized that SCI-related characteristics will be determining factors with regards to muscle status.

It is worth stressing that the above factors are meant to inform clinical decision making, and therefore include only observable or modifiable factors related to muscle status. It is acknowledged that non-modifiable factors like genetics would have an impact on muscle status. Secondly, it is hypothesized the completeness of injury may modify the effect of factors related to lower-extremity muscle loading and disuse. The magnitude of association between the above factors and muscle status has yet to be determined; therefore, one of the goals of this study is to investigate factors associated with muscle status in the chronic-SCI population. Associated factors could be used to form predictive models of muscle mass and fatty-infiltration following long-term paralysis. A predictive model of muscle status for those with chronic-SCI would be

beneficial to the implementation of rehabilitation interventions and clinical decision making with respect to the treatment and prevention of metabolic disease.

2.5 Peripheral Quantitative Computed Tomography (pQCT)

After SCI, there is increased adipose tissue accumulation around the muscles of the lower extremities concomitant with reduced muscle CSA. Previously, the changes in adipose and lean tissue mass have been quantified independently (47,75,194), and only recently has magnetic resonance (MR) and computed tomography (CT) technology been used to characterize the composition of muscle itself (4,9,11). Quantitative computed tomography (QCT) has the ability to differentiate tissue types on the basis of X-ray attenuation characteristics (195). For example, QCT can differentiate fat from muscle because fat displays negative attenuation values, whereas muscle attenuation values are positive. Attenuation characteristics can therefore provide information regarding adipose tissue interspersed within and around muscle (64). In the last two decades, QCT has been used to observe increased fatty-infiltration of skeletal muscle and its relationship to metabolic disease and functional declines in populations such as young, obese and older adults, and those with Duchenne muscular dystrophy (61,63,63,70,165,196).

Peripheral quantitative computed tomography (pQCT), a modality utilizing a smaller bore scanner capable of obtaining images of the lower extremities, is advantageous as it is less expensive as full-body CT and allows individuals to remain in their wheelchair. A pQCT scanner consists of two components: a source that emits an X-ray beam, and a detector a short distance away that measures the intensity of the radiation and X-ray attenuation. During a pQCT scan, the X-ray beam passes perpendicular to the axis of the leg. The angular distance of the twelve detectors relative to the X-ray source is one degree. After each transverse scan, the gantry is rotated twelve degrees such that 15 rotations of the gantry yield the 180 projections

necessary to construct a cross-sectional slice (197). The field scanned is divided into three-dimensional cubic units known as voxels. The number of voxels in each field is a characteristic specific to each scanner; therefore the size of the field of view can be changed, but not the number of voxels (198). The smaller the field selected, the smaller the voxel dimensions and the greater the resolution of the image. An integration algorithm calculates the attenuation co-efficient value of each voxel which corresponds to the type of absorbing matter (tissue). Through a mathematical folding process known as filtered back projection, the raw data from the different angular positions is used to form a cross-sectional image of the limb. By calibration with phantoms of a specified hydroxyl-apatite concentration the attenuation co-efficient values can be transformed to density values measured in mg/cm^3 (197).

The possibility of image artifacts must be considered when examining pQCT images. Beam hardening artifacts are caused by the polychromatic X-ray beam spectrum (ranging from 20 to 120 keV), and the energy-dependent nature of X-ray attenuation values (195). Most materials absorb low-energy X-ray photons better than they absorb high-energy X-ray photons. As a material's attenuation co-efficient is dependent on the X-ray energy, not correcting for beam hardening can result in inconsistent results and image artifacts.

Two types of artifacts can result from beam hardening: cupping artifacts and the appearance of dark bands or streaks between dense objects in an image (199). Cupping artifacts occur when X-rays passing through the center of a cylindrical-shaped object are hardened more than those passing through the edges. As the beam becomes harder, the rate at which it is attenuated decreases and therefore the beam is more intense when it reaches the detectors than it would if it had not been hardened (199). Streaks or dark band can appear between two dense objects in an image. This is because the portion of the beam that passes through one of the

objects at a certain tube position is less hardened than when it passes through both objects at another tube position. This results in streaks or bands appearing when the image is reconstructed.

Stratec provides no information describing how beam-hardening correction is performed. However, techniques have been outlined by other sources (195). Filters on pQCT are available to compensate for some of the beam hardening effects by first attenuating the lower energy photons with a metal filament- a technique known as “pre-hardening” the beam. pQCT systems are also pre-calibrated to cortical bone of the tibia to adjust for potential beam hardening artifacts within a physiological range of bone densities. However, how correcting for the effects of beam hardening on bone affects the accuracy of muscle results is unknown. According to the manufacture, the beam-hardening correction techniques used in the software are sufficient to attain accurate muscle results (197).

Few studies have reported the direct relationship between muscle density of the lower leg and glucose tolerance. The predominance of literature has examined the relationship between muscle density and glucose tolerance at the mid-thigh site using whole body QCT. The relatively small muscle depot at the calf compared to the thigh may be a possible limitation to extrapolating the results of this study to the assessment of metabolic risk. However, this limitation has been addressed in unpublished data comparing muscle CSA derived from pQCT scans to muscle CSA derived from clinically used spiral CT scans of 18 able-bodied adults (9 men, 9 women), and reported that pQCT is as reliable as a clinical scanner when determining muscle CSA (Appendix A). Secondly, a recent study has shown that QCT muscle density of the mid-thigh is significantly correlated with pQCT derived muscle density values of the calf (64). Calf-muscle density was reported to be lower in diabetics compared to non-diabetics after

adjusting for age, gender, and BMI or age, gender, and waist circumference (70). Additionally, pQCT analysis of calf muscle density was positively related to physical activity and negatively associated with markers of fat distribution and risk for type 2 diabetes in a group of 82 premenopausal women (69). Given the good correlation between thigh- and calf-muscle CSA and density values, calf muscle density measured by pQCT is proposed as a valid assessment of muscle CSA and fatty-infiltration and related disease risk. In addition, it is proposed that cautious interpretation of muscle outcomes with regards to metabolic disease may be possible across studies using peripheral and whole-body scanners to scan the upper and lower leg.

The literature base examining lower-extremity muscle post-SCI has used magnetic resonance imaging (MRI) to examine tissue content from cross-sectional slices. There are some disadvantages of QCT in comparison to MRI that must be noted. First, QCT technology lacks the resolution to distinguish sub-fascial from intermuscular fat, especially in individuals with extensive atrophy. However, QCT derived muscle density has been shown to be valid in relation to skeletal muscle lipid content and glucose tolerance (64). As both sub-fascial and intermuscular fat are in close proximity to the muscle body, and increase after SCI, it is proposed that both are important for determining metabolic health. pQCT is also unable to distinguish between extramyocellular lipids stored in adipocytes, and intramyocellular lipids stored in the myoplasm (IMCL and EMCL, respectively) (200,201). The inability to distinguish fat deposition inside compared with outside the muscle fiber is a possible shortcoming, as recent studies using MRI have suggested that IMCL and EMCL may have independent influences on insulin resistance (186). It is worth noting however, that in a sample of 51 healthy volunteers, QCT-derived muscle attenuation values of the soleus were moderately but significantly associated with IMCL and EMCL ($r = -0.64$ for IMCL and -0.37 for EMCL; $p < 0.01$) assessed by MRI. A multiple

regression analysis showed that muscle attenuation values were mostly explained by IMCL ($p < 0.001$) rather than EMCL ($\beta = -0.010$, $p = 0.94$) (202). The significant association between QCT derived muscle density and MRI outcomes is an important consideration when comparing literature using the two modalities. Lastly, QCT scanning requires participants to be exposed to ionizing radiation. However, the exposure is minimal in comparison to other X-ray techniques and environmental exposures (197). Limitations considered, pQCT is proposed as a robust method to assess distal lower-extremity muscle size and fatty infiltration, and related disease and disability risk, in the chronic-SCI population.

2.5.1 Muscle CSA

Quantifying muscle CSA in those with chronic-SCI may provide valuable information regarding health outcomes. Muscle CSA can be measured using pQCT by identifying pixels corresponding to lean tissue from a cross-sectional image. The validity of pQCT-derived (Stratec XCT3000) quadriceps-muscle CSA has been reported previously in comparison to MRI imaging. Validity measures reported for two investigators comparing thigh-muscle CSA between MRI and pQCT from four women and six men were as follows: coefficients of determination = 0.979 and 0.983, standard errors of estimate = 3.677 and 3.297 cm², constant errors = 1.993 and 2.133cm², total errors = 4.058 and 3.735 cm², and the bias \pm 95% confidence intervals for limits of agreement = -1.405 ± 6.815 and -2.391 ± 5.895 cm² (203). Muscle CSA from pQCT has also been compared to muscle CSA measured by whole body spiral CT in 18 able-bodied adults (nine men, nine women), with the conclusion that pQCT is as reliable as a clinical CT scanner when determining muscle CSA (unpublished abstract (204), Appendix A). These results suggest that pQCT is a valid measure of muscle CSA.

The repeatability of pQCT derived muscle measures from healthy adults have been published, with precision errors ranging from <1% to 3% for lower-leg muscle CSA within the lower-leg (204-208). Most recently, a study investigated pQCT repeatability values from a series of six scans of the 66% site of the calf from 30 healthy participants. Scans were conducted on a Stratec XCT 2000 scanner on two separate days by two different testers and analyzed with Stratec software (209). The influence of different testers, time between repeat scans, and subject anthropometric characteristics on repeatability was explored. The absolute (RMS-SD) and relative (RMS-CV) root mean square (RMS) for muscle CSA from six repeated scans from 30 individuals was 1.01 cm² and 1.41%, respectively and the absolute and relative least significant change (LSC) at the 95% confidence for muscle CSA was 2.79cm² and 3.92%, respectively(209). Interestingly, the precision of muscle CSA was negatively correlated with pQCT derived lower extremity total tissue CSA, suggesting that an increase in subject size was associated with a less precise CSA measurement (209). This is important considering the marked decrease in tissue CSA post-SCI.

The results of tester and timing on pQCT precision error from Swinford et al. are reported in Appendix A. Two testers were equally precise at performing muscle CSA measurement. Precision error increased when scans were repeated one week apart as opposed to on the same day (209), however precision values were still within acceptable limits. Unpublished data from our lab has shown acceptable muscle CSA test-retest reliability based on young and older adults and those with SCI. The RMS-SD and RMS-CV for muscle CSA analyzed with two techniques: watershed- and threshold-based tissue segmentation, are presented in Appendix A. Based on the outlined precision and repeatability values, pQCT is proposed as robust technique for the assessment of muscle CSA in able-bodied individuals and those with SCI.

2.5.1.1 Normative Values for Calf-Muscle CSA

Normative calf-muscle CSA data has been previously reported. Unpublished data by Gordon et al. reported the overall mean (SD) of muscle CSA among 18 healthy participants (9 men, 9 women) to be 7156.8 (1112.5) mm² measured at the 66%-site of the tibia using pQCT with a voxel size of 0.4mm (Appendix A) (204). Unpublished data from our lab using pQCT to assess muscle CSA at the 66% site of the tibia from twelve able-bodied persons of Caucasian descent (three men, nine women) with an average age of 25.5 (2.54) years, reported an overall mean muscle CSA (SD) of 7019.6 (1331) mm². Female muscle CSA was 6918.4 (933.53) mm², and male muscle CSA was 7323.0 (2464.46) mm². In another study using pQCT to measure calf-muscle CSA of the 50%-site of the tibia from a sample of 1703 males and 2243 females with an average age of 17.8 years, a muscle CSA (SD) of 5809.7 (954.8) mm² and 4911.4 (799.7) mm² were reported for males and females, respectively (210).

Using magnetic resonance imaging, Castro et al. examined muscle CSA values of two females and twelve males between 18 and 45 years of age 24 weeks post-complete SCI. CSA values of the 890 (70) mm², 125(10) mm² and 580(5) mm² were reported for the gastrocnemius, soleus and tibialis anterior, respectively. To date, our team is the only group to report pQCT-derived muscle CSA values for the SCI population (211). Average total muscle CSA of 4586.08 (1966.29) mm² was observed for males and females with chronic-SCI. There was also a difference in muscle CSA between observed between those with complete (3386.32 [1284.79] mm²) versus incomplete (5823.34 [1779.22] mm²) injuries. The objective of this study is to investigate factors, in addition to completeness of injury that may influence muscle CSA.

2.5.2 Muscle Density

Recent evidence has observed an increase in fatty infiltration of muscle in the acute stage of SCI, and a link between fatty infiltration and metabolic disease. Therefore, the pQCT outcome of muscle density, which is a surrogate measure of fatty infiltration, may provide valuable information related to metabolic risk post-SCI. Muscle density is derived using a scanner specific linear calibration equation that converts X-ray attenuation (1/cm) to density values (mg/cm^3), such that a lower muscle attenuation value correlates to a decreased density value, is indicative of greater adipose tissue infiltration. QCT derived muscle attenuation has been validated by Goodpaster et al., who observed a good correlation between attenuation value and lipid concentration ($r^2 = 0.995$) from single-slice CT scans performed on phantoms of varying lipid concentrations. Increasing the phantom's lipid concentration by 1g/100 ml decreased its attenuation by approximately one Hounsfield Unit (a measure of X-ray attenuation) (64).

The validity of X-ray attenuation as a surrogate for fatty infiltration was assessed *in vivo* at the mid-calf in 45 healthy and obese men and women, including 10 individuals with Type 2 diabetes mellitus (64). Reduced muscle attenuation was associated with increased muscle fiber lipid content determined by histological staining ($P = -0.43$, $P < 0.01$). In a subset of these volunteers ($n = 19$), triglyceride content in percutaneous biopsy specimens from the vastus lateralis was also associated with muscle attenuation ($r = -0.58$, $P = 0.019$). Therefore, it is proposed that muscle density is a valid measure of fatty-infiltration.

The repeatability of muscle density measurement has also been examined previously. The test-retest coefficient of variation for two QCT scans performed in six healthy volunteers was 0.85% for the mid-calf, indicating low methodological variability (64). Another study using

the Stratec XCT 2000 pQCT scanner, with threshold-based Stratec software reported the absolute and relative RMS (i.e., RMS-SD and RMS-CV) for muscle density based on six scans from 30 individuals to be 0.41 mg/cm³ and 0.60%, respectively. The absolute and relative LSC at the 95% confidence level for muscle density were 1.14mg/cm³ and 1.65%, respectively (209). There was no significant effects of tester and timing detected (Appendix A) (209). In unpublished data from our lab, acceptable test-retest reliability data for muscle density values from young and older adults and those with SCI have been observed. The RMS-SD and RMS-CV observed for muscle density, as analyzed by watershed and threshold-based tissue segmentation are presented in Appendix A. Therefore, based on previously published reliability and validity values, pQCT is proposed as a valid and reliable, non-invasive method of assessing fatty infiltration of muscle.

Muscle density is an emerging topic of interest as recent evidence has observed an association between reduced muscle density, metabolic disease and functional declines. Studies have demonstrated that muscle density is associated with impaired blood sugar regulation, serum lipid and lipoprotein levels, and bone and muscle strength (62,63,190,212). One study observed that elderly men and women with normal body weight may be at risk for metabolic abnormalities, including type 2 diabetes, if they possess a high amount of muscle fat (213), and muscle density has been observed to be negatively associated with markers of fat distribution and risk of type 2 diabetes (69). Thigh muscle attenuation has been observed to be independently correlated with insulin-stimulated glucose disposal in a group of sedentary healthy men and women ($r = 0.48$, $p < 0.05$) (49) and in a subsequent study, lean individuals had significantly higher ($p < 0.01$) muscle attenuation values (49.2 ± 2.8 HU) than did obese non-diabetic individuals (39.3 ± 7.5 HU), and obese Type 2 diabetic individuals (33.9 ± 4.1 HU) (64).

Additionally, in a multi-regression model, fatty-infiltration of calf-muscle was observed to be negatively correlated with low-density lipoprotein cholesterol, and positively correlated high-density lipoprotein cholesterol in men with African ancestry (212). This relationship is independent of total and central adiposity skeletal muscle adiposity. Therefore, muscle density is highlighted as a useful measure of metabolic health and disease risk.

The relationship between muscle density and disease continues beyond metabolic disorders. Correlations between skeletal muscle density and muscle and bone strength have been observed in young and older adults, and those with sub-acute and chronic lower limb hemiparesis (32,65-67,214,215). In a group of older adults aged 70-79 years, mid-thigh muscle attenuation values were positively associated with muscle force production (165) and increased lower extremity performance (63). In a study examining side-to-side differences in muscle and bone in those with hemiparesis as a result of stroke, muscle density, but not muscle mass, explained attributes of muscle and bone strength. Based on evidence from other populations, muscle density assessed by pQCT is proposed as a valid measure of adverse health outcomes, and further work is warranted to observe if this relationship exists in those with chronic-SCI.

2.5.2.1 Normative Values for Calf-Muscle Density

Previous studies have reported pQCT derived muscle density values at the 66% site of the tibia by measuring muscle density as a surrogate. Muscle density assessed by pQCT at the mid-calf have been reported from a sample of 1703 males and 2243 females aged approximately 18 years of age. Muscle densities (SD) of 83.4 (3.1) mg/cm³ and 82.8 (3.1) mg/cm³ were reported for males and females respectively (210). A calf muscle density of 72.4 mg/cm³ for women and 75.2 mg/cm³ for men was observed in a group of 471 individuals of African American ancestry aged 18–103 years (mean age 43 years). An age-effect on muscle density was reported such that

a 10% and 12% difference in muscle density among men and women was found between the youngest group (18-29 years) and the oldest group (≥ 60 years), respectively (70). In a sub-set of the same population > 40 years of age, muscle density was lower ($p < 0.001$) in those with diabetes (69.5 mg/cm^3) than those without (74.3 mg/cm^3) and the difference remained significant after adjusting for age, gender, and BMI ($p = 0.005$), and age, gender, and waist circumference ($p = 0.01$) (70). In a group of 80 premenopausal women age (SD) 38.6 (4.7) years, it was observed that calf-muscle density was related to total body fat percentage ($r^2 = -0.53, p < 0.05$), trunk fat percentage ($r^2 = -0.48, p < 0.01$), physical activity level ($r^2 = 0.29, p < 0.01$) and insulin resistance ($r^2 = -0.31, p < 0.05$) (69). Lastly, in 3,075 black and white men and women between 70–80 years of age, muscle density was significant in a model for predicting disability risk (65). pQCT analysis of calf muscle density (reported as $77.8 [2.3] \text{ mg/cm}^3$) was positively related to physical activity and negatively associated with markers of fat distribution and risk for type 2 diabetes in a group of 82 premenopausal women (69) and associated with diabetes, independent of overall and central obesity in families of African heritage (216). Based on muscle density data from other populations, and evidence of fatty infiltration in acute SCI as assessed by MRI, it is proposed that pQCT will aid in the examination of fatty infiltration of skeletal muscle in those with chronic-SCI.

2.6 Potential Correlates of Muscle CSA and Muscle Density

Muscle CSA and muscle density have been selected as outcome variables for this analysis because of their established change as a result of paralysis, and relationship to function and disease. As highlighted previously, muscle CSA and muscle density can be accurately assessed non-invasively via pQCT. Examining muscle CSA and density will aid in describing the muscle status of those living with chronic SCI. Secondly, determining the correlates of

muscle CSA and density status will aid in the identification of those with SCI who experience the greatest reductions in muscle CSA and density and may therefore be at most risk for related co-morbidities. Lastly, this investigation may identify modifiable risk factors for muscle loss and fatty-infiltration. Variables possibly associated with muscle status that will be included in this analysis are discussed below.

2.6.1 Injury Timeline

The life expectancy of individuals with SCI is increasing, as is the number of older adults experiencing an injury (217-219). Consequently, the effect of age on health outcomes and secondary conditions is increasingly relevant as individuals with SCI are susceptible to premature aging and age-related functional declines (16,17,220-226). In addition, age at SCI-onset may have important consequences with respect to morbidities concurrent to SCI. There are a number of studies showing that persons who incur a SCI at later ages have more co-morbidities and poorer functional outcomes than those injured at younger ages (222,227,228). A cross-sectional approach may be limiting for advancing knowledge on the topic of aging with SCI, and additional longitudinal studies investigating the interrelation of changes inherent of aging and changes attributed to a SCI are needed (16,17).

There are detrimental effects of aging on muscle observed in the able-bodied population including reduced muscle mass and muscle quality. Therefore, it is proposed that older adults with SCI will have a reduced muscle status compared to younger adults with SCI. Whether an age-related decline in muscle status continues in the chronic-SCI population is unknown. Secondly, it is hypothesized that age at injury may affect muscle as older adults may have a reduced muscle status prior to injury. For example, the muscle of an individual injured in their twenties may have a different trajectory compared to an individual injured in their fifties.

Surprisingly, muscle status years after SCI has not been adequately investigated either cross-sectionally or longitudinally. Therefore, this project will investigate if chronological age, time post injury, and age at injury are associated with muscle size and composition in those with chronic-SCI.

2.6.2 Level of Injury

A higher level of injury is associated with decreased mobility, independence, and autonomic function, which in turn is related to an altered body composition and metabolic profile (124,229,230). Therefore, it is proposed that level of injury may be a determinant of muscle status. Because of reduced activity levels, lean mass, and altered sympathetic activity, individuals with tetraplegia have fewer daily caloric requirements compared to persons with paraplegia (36,231). This caloric imbalance predisposes those with tetraplegia to obesity and related metabolic complications (232,233). Therefore, it is hypothesized that level of injury may be a determinant of body composition in persons with SCI. Preliminary work by Gorgey and Gater (234) suggests that level of injury does not appear to influence the distribution of visceral and subcutaneous adipose tissue at the trunk. However, it is proposed that the association between level of injury and lower-extremity muscle status warrants further investigation, and thus it is included in this project.

Secondly, level of injury is a primary determinant of the degree of autonomic impairment following SCI. Autonomic dysfunction is characterized by abnormal blood pressure, heart rate, temperature regulation, and ultimately exercise intolerance (100). Depending on the level of SCI, parts of the sympathetic nervous system will be disconnected from supraspinal control, which results in altered sympathetic activity below the level of the injury. No sympathetic autonomic tracts exit the spinal cord above T1, and therefore those with cervical injuries often

sustain decentralization of the sympathetic nervous system (235). Sympathetic innervation to the heart is from T1 to T4, and therefore those with injuries between T1-T4 may have partial innervation to the heart and those with innervation below T4 will have normal cardiac innervation (235). Sympathetic outflow to the splenic organs originates from T5-T9, and those with injuries above T5 may have impaired ability to vasodilate splenic beds. In addition, individuals with impaired innervation to the heart have reduced stroke volumes, reduced muscle mass in the left ventricle, reduced heart rates, and impaired vasodilatory responses to working muscle, and thus may be limited in the amount of exercise they can perform (185). As muscle relies on adequate blood flow to function, it is hypothesized that impaired autonomic function may be a determinant of muscle status post-SCI.

2.6.3 Spasticity

Individuals with upper motor neuron lesions often present with altered sensori-motor control that results in intermittent or sustained involuntary activation of muscle (236). Known as spasticity, this motor disorder is characterized by velocity-dependent increases in muscle tone with exaggerated tendon jerks (237). Despite its negative effects, spasticity has been observed to maintain or improve skeletal muscle size, body composition, metabolic profile (101,238-242), ambulation, and peripheral circulation (243,244). In a SCI rat model, spasticity has been observed to preserve the slow-twitch properties of paralyzed muscle and mitigate the slow to fast fiber transition (245); an observation that has yet to be investigated in humans. However, it is suggested that spasticity contributes to the sparing of slow fibers in some individuals with SCI (103). Six weeks post-incomplete SCI, thigh muscle spasticity accounted for 54% of the variability in muscle CSA (238). There is no evident relationship between skeletal muscle fat deposition and spasticity; however, mechanisms have been proposed that indirectly link

spasticity to the prevention of intramuscular adipose tissue accumulation post injury (238). In chronic complete SCI, knee extensor spasticity was negatively correlated to waist circumference, greater total percent fat free mass, lower percent fat mass, and lower fat mass to fat free mass ratio (239). Most recently the protective effects of spasticity on muscle size have been attributed to circulating insulin growth factors (246).

The Penn Spasm Frequency Scale (PSFS) is a self-report measure of the frequency and severity of muscle spasms that will be used in this investigation (247,248). There are two parts to the scale: the first component is a five point scale assessing the frequency with which spasms occur ranging from zero = no spasms to four = spontaneous spasms occurring more than ten times per hour. The second component is a three point scale assessing the severity of spasms ranging from one = mild to three = severe. The second component is not answered if the person indicates they have no spasms in part one. The PSFS has been used to evaluate spasticity in the SCI population previously (249-251). Reliability has not been established for the PSFS in SCI. Validity for the PSFS has been partially established through correlations with other clinical tools, such as the Ashworth Scale and the Spinal Cord Assessment Tool for Spasticity (SCATS) (195). The PSFS had a Spearman Rank-Order Correlation between 0.40 - 0.51 with the Ashworth Scale, and between 0.40 – 0.59 with SCATS (252). The PSFS only correlated significantly with SCATS clonus scores suggesting that the PSFS may not adequately record flexor and extensor spasms, which may only be triggered during specific activities of daily living (252). Additionally, weak correlations were observed between PSFS and self-report scales of interference with function (0.407) and painful spasms (0.312), and no clinical examination score correlated with self-report scores greater than 0.4 (247). However, for this investigation, spasticity will be treated as a dichotomous variable, and despite the limitations the PSFS is

suggested as a tool to adequately discern between those with, and those without spasms. The dichotomous outcome of spasticity is proposed as a possible variable associated with the muscle status.

2.6.4 Vitamin D

There is evidence to suggest that vitamin D deficiency is highly prevalent in the SCI population. Studies measuring serum 25-hydroxyvitamin D (25(OH)D) levels in individuals with SCI report levels to be significantly lower than controls (253). One study estimated one third of individuals with chronic-SCI have serum 25(OH)D levels less than the normal range suggested for the non-SCI population (254), and more recently, the prevalence of vitamin D deficiency in the SCI population has been estimated as high as 93% (255). Bischoff-Ferrari (192) summarizes four lines of evidence supporting the role of vitamin D with regards to muscle health: 1) proximal muscle weakness is a prominent feature of vitamin D deficiency; 2) the vitamin D receptor is expressed in human muscle tissue, and receptor activation may promote *de novo* protein synthesis in muscle; 3) several observational studies highlight an association between 25(OH)D and muscle strength and lower extremity function in older persons; and 4) there is evidence from several double-blind randomized-controlled trials that vitamin D supplementation increases muscle strength and balance, and reduces the risk of falling in community-dwelling individuals and institutionalized adults. Additionally, it was observed that 1,25(OH)₂D level is associated with physical performance and thigh muscle CSA in a group of individuals with chronic kidney disease (256). In another study, serum 25(OH)D insufficiency was associated with increased fat infiltration in the muscle of healthy young women (257). Lastly, in a sample of 686 community-dwelling older adults, 25(OH)D insufficiency was associated with lower percent lean mass, leg strength, leg muscle quality, and physical activity

level. Based on the evidence from other populations, it is hypothesized that vitamin D may be associated with muscle quantity and quality post-SCI (258).

It has been suggested previously that a 25(OH)D level of 60nmol/l is the threshold at which desirable benefits for muscle health plateau in older adults. However, the validity of this threshold has not been established for the SCI population (259). In fact, with regards to parathyroid hormone regulation, it has been suggested that the threshold for optimal vitamin D levels in the chronic-SCI population may be higher than in the non-SCI population (260). Therefore for this proposed study, vitamin D status will be treated as a continuous variable to gain an understanding of the correlation between vitamin D level and muscle status post-SCI.

2.6.5 Lower Extremity Motor Score (LEMS)

Those with incomplete SCI make up approximately half of the SCI population (54). Traditionally, studies investigating muscle outcomes have grouped participants dichotomously as either having a complete or incomplete injury. This grouping is warranted as those with incomplete injuries may have different or additional factors contributing to muscle status compared to those with complete injuries. Published data from our group has reported a significant difference in muscle CSA between those with complete (AIS AB) and incomplete AIS CD) injuries (211). It is hypothesized that physical activity level and ambulation status may have a greater influence on the degree of muscle atrophy and fatty-infiltration in those with incomplete injuries compared to those with complete SCI. This study will separate the SCI population into those with complete and incomplete injuries. However, within the incomplete injury group there can be a wide range of muscle function not adequately examined with a dichotomous grouping. Therefore, it is hypothesized that lower-extremity motor score (LEMS)

may provide additional precision to determine the effect of voluntary muscle function on muscle status.

LEMS is a component of the ASIA neurological exam completed by manual testing of key muscles on the right and left side of the body in 10 paired myotomes. The strength of each contraction is graded on a six-point scale from zero to five, where zero is total paralysis and five is normal active movement and full range of motion against full resistance (99). It is proposed that motor preservation, quantified by LEMS, may be an important correlate of muscle status after SCI. One study has indicated that LEMS is correlated with gait speed, step length, and walking cadence in tetraplegic and paraplegic patients with SCI (261). Additionally, Shah et al. (7,169) have observed that those with incomplete injuries have preserved muscle CSA and reduced fatty infiltration compared to those with complete injuries, and those who are ambulatory have a greater muscle CSA and less fatty infiltration compared to those who use a wheelchair. However to date, no investigation has assessed muscle size and quality within the spectrum of preserved motor function in those with incomplete SCI. For this investigation, only the LEMS of the ankle dorsiflexors, long toe extensors and plantar flexors will be used to isolate the effect of voluntary muscle contraction on calf-muscle status. Incorporating LEMS will add increased precision to assess the association between the degree of motor function and muscle status in the incomplete SCI population.

2.6.6 Physical Activity

Physical activity is a potent factor in the maintenance of health of individuals living with SCI. Regular physical activity through upper-body training is effective at improving fitness, psychological well-being, and carbohydrate and lipid metabolism disorders in adults with chronic-SCI (262-264). In addition, regular physical activity improves circulation, and reduces

inflammatory cascades related to muscle atrophy, cardiovascular disease, and diabetes (263,265-267). It is likely that physical activity done by those with SCI will be performed with the upper-body; therefore, it is hypothesized that physical activity will affect muscle status by introducing a systemic response characteristic of reduced inflammation and improved blood sugar and fatty-acid metabolism. Observations from able-bodied individuals have shown that physical activity is effective at improving whole-body glucose tolerance, and attenuating the effects of catabolic cytokines related to systemic low-level inflammation associated with muscle wasting and fatty-infiltration of muscle (265,267). Because of its systemic effect, it is possible that upper-body exercise could improve lower-extremity muscle status.

2.6.7 Mobility

SCI results in a diverse array of mobility impairments. The SCI population includes those who use power wheelchairs, manual wheelchairs, assistive gait aids and those with minimally impaired gait (100). With regards to muscle status, differential atrophy rates are observed between those who are wheelchair dependant versus those who are not (7). Differential rates of atrophy are suspected as a result of loading pattern differences associated with weight bearing and functional gait. Therefore, it was hypothesized that those who are able to ambulate without the use of a wheelchair will have an increased muscle status compared to those with greater mobility impairments.

2.6.8 Immobility

Periods of immobilization are associated with muscle atrophy in other clinical populations (57,59,60,117). Individuals with SCI can experience events, such as lower-extremity fracture or prolonged illness that can limit the mobility of individuals for an extended period of time. It is proposed that an immobilized lower-extremity or illness that confines an

individual to bed or results in an ambulatory individual using wheelchair may result in a reduced muscle status. Therefore, it is hypothesized that a period of immobility will be negatively associated with muscle status.

2.7 Summary of background

After SCI, there is a rapid decrease in muscle CSA and increase in fatty infiltration of muscle, predisposing those with SCI to increased risk of secondary health conditions and mobility limitations. To date, few investigations have prospectively examined the changes in muscle status in the chronic stage of SCI, especially in females, the aged, and those with incomplete injuries. pQCT is a non-invasive method that has been employed previously to quantify muscle CSA and muscle density, which is a surrogate measure for fatty infiltration. In other clinical populations, muscle CSA and density have been associated with obesity, impaired blood sugar regulation, serum lipid and lipoprotein levels, and bone and muscle strength. Examining the changes that occur to muscle in a diverse population with chronic-SCI may provide insight as to: 1) what happens to muscle status following the rapid initial changes that occur in the acute stage of SCI; and 2) what descriptors characterize those experiencing the most detrimental changes in muscle status, and consequently may be at the most risk for related morbidities such as CVD and diabetes.

2.8 Clinical Relevancy

The muscle status of the chronic-SCI population has been inadequately examined. There is little information to inform researchers and clinicians of the factors associated with muscle loss and fatty-infiltration, and related health risks. The goal of this study, which is to establish detriments of muscle status and change in muscle status in the chronic-SCI population, will be beneficial to informing care and gauging rehabilitation intervention success. Models of muscle

status and change in muscle status will be created to inform clinical interventions related to metabolic disease such as the initiation of muscle-preserving interventions (e.g., electrical stimulation), or treatment regimens for metabolic disorders (e.g., pharmacotherapy, exercise, etc.).

It is unknown if, and to what extent, individuals with SCI experience reduction in muscle status in the chronic stage of injury. If reduction continues, there could be implications for the design of studies assessing the effect of muscle-preserving interventions. Commonly, an improvement in muscle from baseline is deemed a success. If the natural trajectory of muscle is towards a decrease in size and quality, then a therapy that demonstrates no change may be successful in that it prevents an unfavourable reduction. Understanding the degree, and factors associated with muscle status variability in the chronic-SCI population, may help develop and assess the efficacy of therapies to improve, or slow reductions in muscle size and quality.

Secondly, it has been demonstrated that muscle density is able to distinguish between those with and without diabetes. For example, in the able-bodied population, Miljovic-Gacia et al. observed that those with diabetes have a calf-muscle density that is approximately 5 mg/cm³ lower than those without diabetes (216); and Goodpaster et al. reported that lean subjects had muscle attenuation values ~10HU higher (49.2 ± 2.8 HU) than obese non-diabetics (39.3 ± 7.5 HU), and ~ 16 HU higher than those who were obese and had type 2 diabetes (33.9 ± 4.1 HU) (64). An improved understanding of muscle CSA and density reductions following SCI may improve detection of metabolic disease risk.

To date, there is little evidence as to the degree of muscle change needed to be clinically significant with regards to metabolic disease after SCI. However, studies examining the relationship between electrical stimulation training and metabolic activity have begun to address

this issue. An improvement in both plasma glucose and insulin after ten weeks of electrical stimulation cycling in individuals with SCI was been observed in individuals with paraplegia and tetraplegia (45). The improvements in glucose tolerance occurred along with an increase in whole-body lean muscle mass (~4%) and no significant change in adipose tissue. These results suggest that a 4% difference in whole-body lean tissue mass is sufficient to alter metabolic health. Mahoney et al., observed that a muscle CSA increase of 35-39% did not significantly influence blood glucose or insulin after training (46). However, a trend for a reduction in plasma glucose level was observed ($p = 0.074$). In preliminary case study involving a male with chronic complete-SCI, thigh-muscle hypertrophy ranging from 30% to 112% was detected in cross-sectional MRI images after a twelve week electrically-stimulated knee extensor resistance training protocol (268). Additionally, intermuscular fat decreased by more than 50%. In a subsequent study of nine individuals with motor-complete SCI by the same investigators, twelve weeks of progressive electrically-stimulated knee extensor resistance training, in combination with a healthy diet, increased the muscle CSA of the whole thigh (28%), knee extensor (35%), and flexor (16%) muscle groups, reduced visceral adipose tissue (30%), and improved fatty infiltration of skeletal muscle (3% reduction in exercise group vs. 3% increase in control group) (269). The hypertrophy and reductions in ectopic adipose tissue distribution observed in this study were linked with improved indices of carbohydrate metabolism, insulin resistance, and lipid profiles in men with SCI. Although variability is observed, the results of these studies suggest that a) it is possible to increase muscle size and quality after-SCI, and b) muscle size and quality are determinants of metabolic health.

The results of this study will characterize the muscle status of a diverse sample of the SCI population, and provide baseline data to gauge intervention success and predict who may benefit

the most from rehabilitation therapy. Secondly, the results of this study may highlight pQCT as a novel, accessible clinical tool to improve our understanding of metabolic disease risk to facilitate disease detection in those with chronic-SCI. This information is an important step towards developing individualized, SCI-specific thresholds capable of informing clinical decision making.

CHAPTER 3: RESEARCH QUESTIONS AND HYPOTHESES

3.1 Research Questions

3.1.1 Primary Research Questions

What characteristics are associated with muscle CSA and muscle density for those with complete and incomplete SCI? Possible variables include: gender, age, height, weight, waist circumference, injury duration, age at injury, level of injury, leg spasticity, vitamin D level, calf-lower extremity motor-score (cLEMS), wheelchair use, and daily minutes of mild, moderate, vigorous, and total physical activity.

3.1.2 Secondary Research Questions

- 1) a) What is the magnitude of change in muscle CSA and muscle density over one- and two-year time periods for individuals with complete and incomplete injuries?
- b) What variables are associated with a change in muscle CSA and density over one- and two-year time periods for individuals with chronic complete and incomplete injuries?
Possible variables included: gender, age, height, weight, waist circumference, injury duration, age at injury, level of injury, leg spasticity score, vitamin D level, cLEMS, wheelchair use and mild, moderate, vigorous, and total physical activity levels.
- 2) a) How does the muscle CSA and muscle density of individuals with chronic-SCI compare to gender, age, and height matched able-bodied controls?

b) What is the difference in muscle CSA and muscle density reduction between those with chronic complete and incomplete SCI?

3.2 Research Hypotheses

3.2.1 Primary Research Hypotheses

1. It was hypothesized that in those with incomplete injuries, males would have a greater muscle CSA compared to females. However, in those with complete injuries there would be no gender differences in muscle CSA. Muscle CSA would be inversely related to age and duration of injury, and positively related to age at injury. It is likely that age and time post-injury would co-vary. It was hypothesized that those with an injury below C7 would have a greater muscle CSA compared to those with SCI at or above C7. Muscle CSA would be positively related to vitamin D level, spasticity, physical activity level, and mobility score. It was hypothesized that muscle CSA would be positively related to cLEMS and negatively related to wheelchair use.
2. It was hypothesized that in those with incomplete injuries, females would have a lower muscle density compared to males. However, in those with complete injuries there would be no differences in muscle density at baseline. Muscle density would be inversely related to age and duration of injury, and positively related to age at injury. It is likely that age and time post-injury would co-vary. Muscle density would be positively related to vitamin D status, spasticity, physical activity level, and mobility status. Lastly, it was hypothesized that muscle CSA would be positively related to cLEMS and negatively related to wheelchair use.

3.2.2 Secondary Research Hypothesis

1. It was hypothesized that there would be no difference in muscle CSA and muscle density between baseline, year-1 and year-2 time points in those with complete and incomplete injuries.

2. a) It was hypothesized that males and females with SCI would have decreased muscle CSA and muscle density compared to gender, age, and height matched able-bodied controls.

b) It was hypothesized those with complete injuries would have greater reductions in muscle CSA and muscle density compared to those with incomplete injuries.

CHAPTER 4: METHODOLOGY

4.1 Overview of Study

This observational study was a sub-study of a larger 2-year prospective cohort study entitled Bone Quality in Individuals with Spinal Cord Injury, and incorporates able-bodied control data from the University of Saskatchewan. The primary objective of the Bone Quality in Individuals with Spinal Cord Injury study is to evaluate bone loss in individuals with SCI. This study involves collaborations between the University of Waterloo, McMaster University, University of Toronto, and the University Health Network - Toronto Rehabilitation Institute. Briefly, seventy individuals with SCI were recruited to participate. Participant data was collected at yearly follow-up visits. Data collected include: a) medical history, including etiology and impairment descriptors; b) areal bone mineral density and body composition assessed by DXA; c) volumetric bone mineral density, bone geometry, muscle CSA, muscle density, and trabecular bone structure assessed by pQCT; and d) blood work collected at baseline to measure vitamin D levels. To date, all baseline and year-1 follow up visits have been completed by the cohort, and all but two individuals have completed their year-2 follow-up visit. It is expected that the remaining two participants will attend their year-2 follow appointment by June, 2014 (Recruitment flowchart – Appendix A).

The primary aim of the proposed investigation was to examine muscle CSA and muscle density values from pQCT scans of the 66%-site of the calf at baseline, year-1, and year-2 time points. Baseline scans were used as a cross-sectional sample of the SCI population to determine the correlates associated with muscle status. For individuals who did not undergo a baseline pQCT scan, the scan from the next available time point was used if available. Secondly, muscle status was compared longitudinally between baseline, year-1, and year-2 time points, and

correlates associated with a change in muscle CSA and muscle density were determined.

Thirdly, muscle CSA and muscle density values from baseline were compared to age, gender, and height matched able-bodied controls to assess the change in muscle status as a result of paralysis.

4.2 Recruitment and Screening

Individuals with SCI were recruited in person from outpatient services in Toronto, Ontario and Hamilton, Ontario, and by mail out invitation. Sources of recruitment included: 1) the Jousse long-term follow-up database; 2) outpatient services at the Lyndhurst Centre in Toronto, Ontario; 3) physiatry clinics in Hamilton, Ontario; and 4) the MacWheelers adaptive exercise program at McMaster University, Hamilton, Ontario. The Jousse long-term follow-up database contains the demographic, injury characteristics, health status, and contact information of individuals with SCI who have consented to be contacted regarding ongoing research projects at the Lyndhurst Centre. Participants who were recruited through the Jousse long-term follow-up database or the Hamilton clinics were sent a letter of invitation to participate in this study. The letter informed them that a research coordinator would contact them by telephone to determine their eligibility and interest in participating in the study. For individuals who preferred not be contacted, a phone number was provided in the letter where they could leave a message to exclude themselves from the recruitment call.

Potential participants making use of outpatient services at the Lyndhurst Centre were identified by their respective physicians or therapists. Potential participants were informed of their possible eligibility for the study and were asked if they would be interested in being contacted by a member of the research staff to learn more about the study. The physicians and therapists were required to complete a referral form for eligible participants who expressed

interest in the study and forward it to the research coordinator who contacted the potential participant via telephone. Recruitment through the MacWheelers program was performed by providing brochures to eligible participants. Other recruitment strategies included advertisements on the Spinal Cord Injury Canada website and newsletter, and posters posted throughout the Lyndhurst Centre building.

Potential participants interested in taking part in this research project were provided with a detailed description of the study over the telephone by the research coordinator at the Lyndhurst Center. Individuals who gave verbal consent to participate in the study were assessed to ensure that they met all the inclusion criteria (Table 2). A visit to the Lyndhurst Center was arranged for eligible participants, during which written informed consent was obtained.

Table 2 Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
<p>Able to understand instructions in English</p> <p>A spinal cord impairment (C2-T12 AIS A-D) of sudden onset (< 24hrs) associated with a stable upper motor neuron, neurologic deficit of trauma-like etiology having occurred at least 24 months prior study inclusion</p> <p>Ability to give informed consent</p> <p>Age \geq 18 years</p>	<p>Current or prior known conditions other than paralysis that are known to influence bone metabolism including: oral glucocorticoid use for \geq 3 months, malignancy, known liver or malabsorption condition</p> <p>Weight > 270lbs (limit for bone density machine)</p> <p>Contraindications to pQCT testing</p> <p>Women who are pregnant or planning to become pregnant</p>

4.3 Assessment Overview

Study visits were conducted at the Lyndhurst Center. Each visit involved a series of questions pertaining to participants' medical history and health demographic information. Medical history was obtained by direct patient interview and validated by medical chart review. If the participant had not had an ASIA exam performed previously, an exam was conducted. A second visit to the McMaster University Medical Centre was scheduled within three months for a pQCT scan. The study visit and pQCT scan were repeated twice at one-year time intervals.

4.4 Able-bodied Controls

Age, gender, and height matched able-bodied control scans of the 66% site of the tibia were attained from multiple studies previously conducted at the University of Saskatchewan. Individuals with SCI were matched to controls in a 1:1 ratio to examine the influence of SCI on calf-muscle size and quality across the duration of chronic-SCI. A matching algorithm was designed to prioritize gender, followed by age, followed by height.

4.5 Outcome Measures

4.5.1 pQCT Imaging

Muscle CSA (cm^2) and muscle density (mg/cc) was calculated from pQCT scans at the 66% site of the tibia as measured from the measuring from distal landmark. The 66%-site of the tibia was calculated and marked as 1/3 of the distance measured from the palpated tibiale mediale (most superior point on the medial border of the head of the tibia) and the sphyrion tibiale (most distal palpable prominence of the medial malleolus). The proximal one-third of tibia (66% of tibia length) was chosen because it is the region of the calf with the highest circumference and cross-sectional area (270,271). A standard operating procedure (SOP) for

image acquisition and analysis is contained in Appendix A. Images were acquired using a Stratec XCT 2000 scanner (Stratec Medizintechnik, Germany). The right tibia was scanned except in cases of severe spasticity or other contraindications, such as the presence of metal or fracture in the right leg. Images have a slice width of 2.2mm and voxel size of 0.5mm. Able-bodied scans from the University of Saskatchewan employed an identical protocol with the exception of a voxel size of 0.4mm was used. All pQCT scans of individuals with SCI were acquired by the same X-ray technician at Hamilton Health Sciences using the same scanner. All pQCT analysis (SCI and able-bodied) were performed by a single investigator (Cameron Moore). Scans that had severe movement artifacts were excluded from the analysis based on the visual scale defined by Blew et al. (272). Prior to analysis, pQCT scans were randomized and blinded.

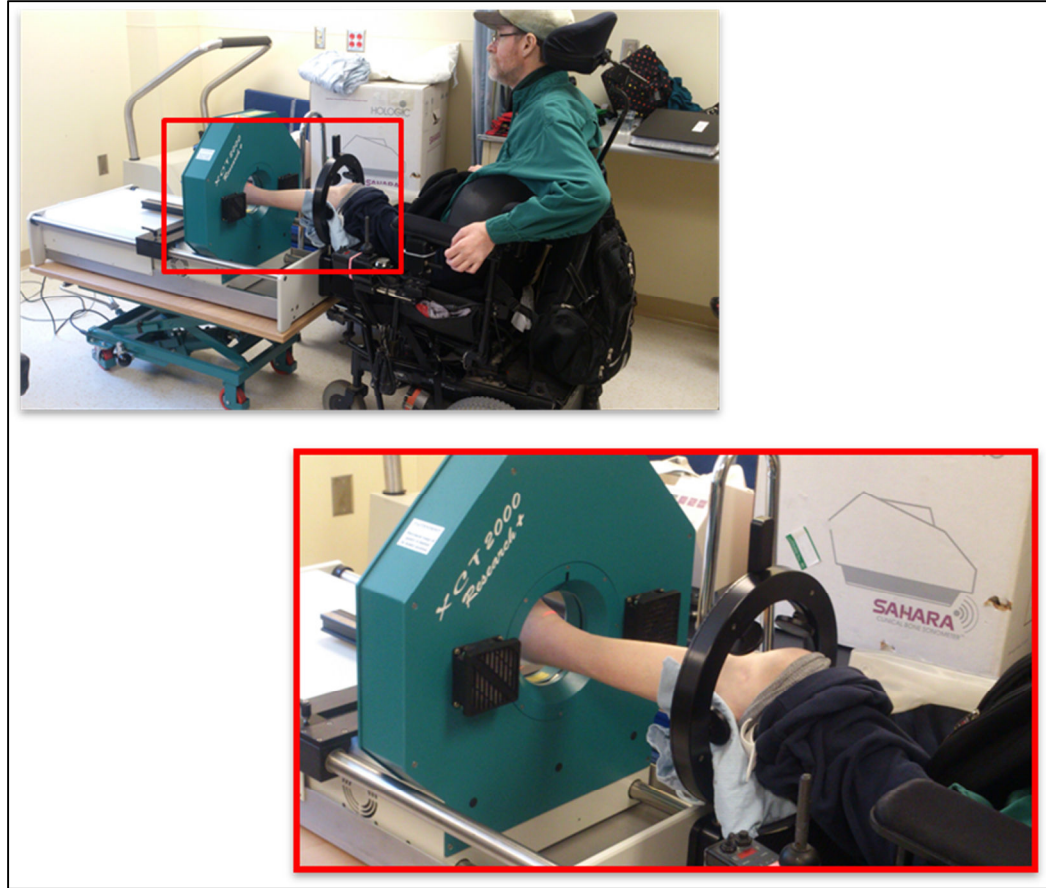


Figure 1 pQCT positioning at the distal tibia

For this analysis, tissue segmentation was performed by manual tracing aided by a Watershed algorithm. Prior pQCT studies have used density-based threshold algorithms to distinguish muscle from fat, bone, and skin. However, unpublished data from our lab reports that manual tissue segmentation has greater reproducibility and tighter re-testing limits (Appendix A). Although manual tracing may be less efficient, its higher reliability makes it a more favourable technique.

Tissue segmentation and the calculation of muscle CSA and muscle density were performed using sliceOmatic software version 4.3 for PC (SliceOMatic; Tomovision, Montreal, Canada). Before image analysis, gamma correction was performed to compensate for variability

in grey-level values between computer screens. After gamma correction, grey level images (GLI) of the pQCT scans were loaded into the software, and the contrast was adjusted to maximize the visibility of the border between the external fascia and subcutaneous fat. SliceOmatic software has the ability to segment and “tag” tissue types within the image, and compute the surface area and density of the tagged pixels. Included in the software package are a number of analysis modes to aid in the segmentation and identification of tissue types based on the properties of the GLI file. “Morpho” mode was used to analyze the pQCT images. Morpho mode uses mathematical morphology to segment the image by computing watershed gradients. Ideally, the borders created by the watershed segmentation correspond to borders between tissue types, and therefore identifying these regions is faster than editing the image one pixel at a time. In some cases, manual edits were needed to correct for watershed spillover. Tissue types were identified in the following order: muscle, subcutaneous fat, bone, and marrow space. For this analysis, muscle tissue was defined as the tissue surrounded by the epimysium, which contains subfacial fat, intermuscular and intramuscular lipid deposits and connective and contractile tissue. Figure 3 shows how bone, muscle and subcutaneous fat were identified via watershed segmentation.

Muscle CSA was calculated by multiplying the number of pixels tagged as muscle by the surface area of one pixel, and the average density value of the pixels tagged muscle represent the muscle density. Muscle density was calculated using a calibration equation that converts GLI units, in this case linear attenuation coefficients ($1/\text{cm}$), into density values (mg/cc). This equation was derived by scanning ten compounds of known densities ranging from zero to 675 mg/cc to attain a calibration curve. The curve for the pQCT scanner at Hamilton Health

Sciences was a linear equation with an r^2 value of 1, suggesting a direct relationship between X-ray co-efficient and density values (Appendix A).

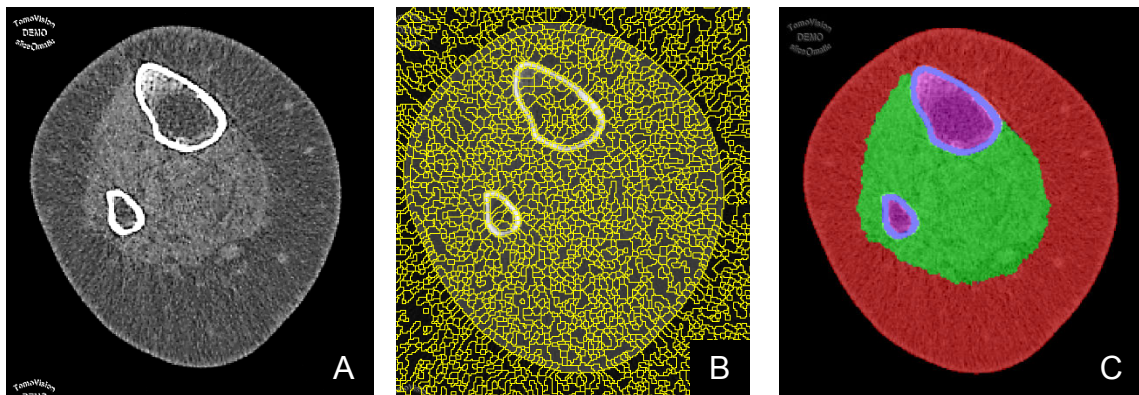


Figure 2 Watershed Analysis of pQCT scans at the 66%-site

A. Grey level image (GLI) B. Watershed segmentation C. Tissue identification

4.5.1.1 Reliability Analysis of Tissue Segmentation

A repeatability analysis for the watershed tissue segmentation technique was performed to determine test-retest reliability. Fifteen pQCT scans were randomly selected and analyzed twice in random order by the same investigator conducting all pQCT image analysis for this study (Cameron Moore). Intra-class correlation coefficients are reported and the root mean square (RMS) method was used to calculate the overall standard deviation (RMS-SD, units) and the coefficient of variation (RMS-CV, %) of the precision error of muscle CSA and density measurements.

4.5.2 Spasticity

Spasticity was assessed using the Penn Spasm Frequency Severity Scale (SFSS). The SFSS is a self-report measure of the frequency and severity of muscle spasms (247,248). There are two parts to the scale: the first component is a five point scale assessing the frequency with

which spasms occur ranging from 0 = no spasms to 4 = spontaneous spasms occurring more than ten times per hour. The second component is a three point scale assessing the severity of spasms ranging from 1 = mild to 3 = severe. The second component is not answered if the person indicates they have no spasms in part 1. The SFSS was administered by a research assistant at three points over the duration of the study: baseline, year-1 and year-2. However, only the baseline scale was used for the cross-sectional and longitudinal analysis. Reliability has not been established for SFSS. For this investigation, spasticity score for the leg scanned was treated as a continuous variable with a maximum score of seven.

4.5.3 Serum vitamin D

Blood collection was performed during the baseline visit by a trained phlebotomist. Participants were required to fast for at least twelve hours prior to blood collection. For those participants unable to fast, a standard breakfast of toast and apple or orange juice was allowed, in which case blood was drawn four hours following food consumption. Participants who were Vitamin D deficient were given a prescription for Vitamin D supplementation with the goal of raising Vitamin D levels to a sufficient level. Participants were then re-tested. In these instances, the result of the first vitamin D test was used for the cross-sectional analysis and the result of the second test was used for the longitudinal analysis.

Two 10mL Vacutainers® of blood were collected per participant. Immediately following blood collection, the blood from one 10 mL Vacutainer® serum separator tube was left to clot for 10-30 minutes, and then was centrifuged at 2800 rpm for 15 minutes. The serum layer was carefully removed and distributed into 1.5mL microcentrifuge tubes and sent to the Research Laboratory at Mt. Sinai Hospital for same-day analysis.

Serum 25(OH)D was determined with a chemiluminescent immunoassay (CLIA) using the DiaSorin LIAISON instrument as the platform (DiaSorin, Stillwater, MN); which detects both 25(OH)D₂ and 25(OH)D₃ to estimate the total 25(OH)D circulating in the body. The DiaSorin LIAISON CLIA uses an antibody to isolate serum 25(OH)D from other materials and metabolites. Reagents are added to the sample to initiate a flash chemiluminescent signal which can then be measured and related to the 25(OH)D concentration (273). The DiaSorin LIAISON® CLIA has demonstrated acceptable assay precision (2.8 – 13%) and inter-assay precision (7.3 – 17.5%) when evaluated against a well-established radioimmunoassay technique (274,275). Additionally, the LIAISON® CLIA exhibits 100% cross-reactivity for both 25(OH)D₂ and 25(OH)D₃ (275). These results suggest that the LIAISON 25(OH)D assay is an accurate and precise tool for the measurement of 25(OH)D.

4.5.4 AIS and Lower-extremity Motor Score (LEMS)

The AIS and LEMS was either obtained by chart abstraction or determined by a physiatrist at baseline. This project examined the muscle status of the calf, and therefore to isolate the effect of voluntary muscle activation on muscle status, only the calf-LEMS (cLEMS) (ankle dorsiflexors, long toe extensors, and plantar flexors) of the leg scanned was used in this analysis. It has been observed that light-touch, pin-prick, and motor scoring have high inter-rater repeatability. The interclass correlation coefficients (ICC) for light-touch, pinprick, and total motor scores were 0.96, 0.88 and 0.97, respectively. For LEMS specifically, an inter-rater reliability (ICC, 95% CI) of 0.98 (0.92 - 1.00) was observed (99). Based on these scores, the AIS including LEMS, is considered a reliable measure.

4.5. The Physical Activity Recall Assessment for People with Spinal Cord Injury (PARA-SCI)

The PARA-SCI was designed to capture information on the type, frequency, duration and intensity of physical activity performed by those with SCI using a wheelchair as their primary mode of mobility (276). The PARA-SCI uses an interview format to capture activities performed over the last three days which are divided into eight periods from morning routine to evening routine. The two routine periods are subdivided to capture activity related to daily living (transfer, bowel and bladder management, dressing, etc.). The number of minutes spent on each specific activity is recorded and the activity is coded into two categories: leisure-time physical activity or lifestyle activity. Physical activity information is reported as an average number of minutes of activity per day (mild, moderate, heavy, total) for the two dimensions (leisure-time physical activity or lifestyle activity) and a cumulative index. Over repeated administrations one week apart, the ICC for the PARA-SCI range from 0.45 to 0.91 for the PARA-SCI activity categories and intensities (276). In a validity study, correlations between PARA-SCI scores and indirect calorimetry estimates of activity ranged from 0.27 to 0.88(277). An important limitation to address is that the PARA-SCI is designed to record wheelchair based physical activity which is predominantly performed with the upper body. Therefore, the PARA-SCI may not adequately capture the activity levels of those with incomplete injuries doing lower-extremity exercise. However, physical activity has a systemic response on the body and therefore, limitations considered; the PARA-SCI provides a tool to gauge if physical activity is a determinant of muscle status post-SCI. Average minutes per day of mild, moderate, vigorous, and total physical activity were used in the cross-sectional and longitudinal analyses.

4.6 Statistical Analysis

Descriptive statistics were used to describe participants' muscle status and demographic, anthropometric, and impairment characteristics. Categorical variables are presented as counts (n) and percentage (%), and continuous variables are presented as means \pm standard deviations (SD). All statistical analyses were performed on SAS 9.2 software (Cary, North Carolina).

To determine the test retest reliability of the manual watershed-guided tissue segmentation technique, the scans of 15 individuals were blinded and analyzed twice in random order. The intra-class correlation coefficients (ICCs) are reported for muscle CSA and density from a one-way random effects model. In addition, the RMS method was used to calculate the overall standard deviation (RMS-SD, units) and the coefficient of variation (RMS-CV, %) of the precision error of muscle CSA and density.

For the primary research question investigating the correlates of muscle status at baseline, two multiple linear regression analyses with the dependant variables of muscle CSA and muscle density were used to identify correlates of muscle CSA or density. It was hypothesized that completeness of injury maybe an effect modifier, such that the magnitude of some correlates of muscle status may affect those with complete and incomplete injures differently. Therefore, each analysis was conducted on the complete sample, and sub-samples separated by motor-completeness of injury (i.e., AIS A and B versus C and D). Potential correlates selected a priori for the primary analysis are listed in Table 3. All independent variables were treated as continuous variables except for gender (m/f), diabetes status (Y/N), and level of injury (C1-T6, T7-S5). Correlates found to be independently significant at $\alpha < 0.20$ in the bi-variate regression analyses were entered into multi-variable regression models using manual model selection based of R^2 and $C(p)$ statistics (278). Multicollinearity between independent variables

was defined as a variance inflation factor greater than five (279). Models have a maximum of ten observations for each independent variable to avoid over-fitting (280). Outlying data points were investigated based on Cook's Distance, which is a measure of the impact an observation on all regression coefficients. A value greater than $4/n$, where n is the number of observations in the model was used to define an outlier (281). To assess model assumptions, the residual plots were examined.

Table 3 Covariates used in the cross-sectional and longitudinal analyses

Variable	Unit
Gender	Male / Female
Age (years)	Years
Height	Centimetres (cm)
Weight	Kilograms (kg)
Waist Circumference	Centimetres (cm)
Age at Injury	Years
Level of Injury	C1-T6 / T7-S5
Time Post Injury	Years
Single Leg Spasticity	No unit (/7)
cLEMS	No unit (/15)
Wheelchair Use	(Yes/No)
Baseline Vitamin D level	Nmol/L
Mild Physical Activity	Minutes per day
Moderate Physical Activity	Minutes per day
Vigorous Physical Activity	Minutes per day
Total Physical Activity	Minutes per day

A mixed-model repeated measures ANOVA (rANOVA) was used to assess whether there were differences in muscle CSA and density between the three study time points in the full sample as well as sub-samples of participants with complete and incomplete injuries. Study time point (baseline, year-1, year-2) was treated as a fixed effect and participants were treated as

random effects in the model. The mixed model approach allowed for missing muscle CSA and density values to be estimated based on a restricted maximum likelihood method which is favourable in the accommodation of data missing at random (282,282-284). Only participants with scans from at least two time points were included in the model. If a difference between baseline and year-1 or baseline and year-2 is detected at a significance level of $p < 0.05$ in the rANOVA analysis, regression models that include muscle CSA and muscle density change scores as the dependant variables were formed to examine variables related to change in muscle status. For the regression analyses, correlates found to be independently significant at $\alpha = 0.20$ in bi-variate analyses were entered into multi-variable models based on R^2 and $C(p)$ statistics (278). Potential variables and covariates that were included in each of the secondary analyses are the same as those used in the cross-sectional analysis (Table 3).

For the final research question, independent t-tests were used to test for significant differences in age and height, and a chi-square test was used to compare gender frequency between SCI participants with SCI and able-bodied controls. Paired t-tests were used to compare the muscle CSA and muscle density of individuals with complete and incomplete SCI to controls. Independent t-tests were used to compare the mean changes in muscle CSA and density values between individuals with complete and incomplete SCI.

4.7 Ethical Considerations

4.7.1 Potential Risks to the Participants

Participants were exposed to small amounts of radiation during the pQCT scans. The total level of radiation exposure associated with the scans is approximately $30\mu\text{Sv}$, which is less than the amount of radiation received during a whole body CT scan ($30\text{-}60\mu\text{Sv}$) or annually from background radiation ($2500\mu\text{Sv}$).

4.7.2 Anonymity

Each participant was assigned a unique identification (ID) number that was used on all forms and in the electronic database. The key file linking participant information to the ID is stored in a separate password protected database. All hardcopy data is stored at the Research Department at University Health Network - Toronto Rehabilitation Institute, Lyndhurst Centre in locked and secured filing cabinets. Additionally, all research data has been inputted and securely stored on an online electronic database, Empower, or on servers at the Lyndhurst Centre.

CHAPTER 5: RESULTS

5.1 Participants

5.1.1 Participant Recruitment

Four hundred nine individuals with SCI were approached to participate in the two-year prospective cohort study. Of the 409 individuals, 188 individuals were unreachable by phone, seven were deceased, and 79 declined to participate, resulting in 135 individuals being pre-screened for eligibility. Twenty four individuals did not meet the inclusion criteria, and 41 individuals declined further participation in the study. In total, 70 individuals were enrolled in the study. One participant died before the first follow-up visit, and two individuals died before the second. Sixty four participants completed the study, and three participants are scheduled to complete their Year-2 follow-up visit by June 2014, and their data is not included here. A recruitment flow diagram can be found in Appendix A.

5.1.2 Participant Characteristics

The sample population included 20 females and 50 males with a mean (\pm SD) duration of injury of 15.5 ± 10.0 years, and an age of 33.7 ± 14.7 years (Table 4). Forty five individuals had motor-complete injuries and 25 individuals had motor-incomplete injuries. Fourteen of the 25 individuals used a wheelchair for ambulation. Thirty six individuals had tetraplegia and 34 individuals had paraplegia. Individuals with motor-complete injuries were younger, injured at an earlier age, and were injured for a longer period of time ($p < 0.001$). There was no difference in the average daily mild, moderate, vigorous, and total minutes of physical activities between those with motor-complete (AIS A and B) and incomplete injuries (AIS C and D).

Table 4 Participant Characteristics

	All participants	Participants with motor-complete Injuries	Participants with motor-incomplete Injuries	p-value
# Participants	70	45 (64.2%)	25 (35.7%)	
Sex, n (%)				0.9371
Female	20 (28.6%)	13 (28.9%)	7 (28.0%)	
Male	50 (71.4%)	32 (71%)	18 (72%)	
Age (years)	48.8 ± 11.5	45.5 ± 9.8	54.9 ± 12.0 *	<0.001*
Duration of Injury (years)	15.5 ± 10.0	17.8 ± 10.0	11.4 ± 8.8 *	<0.05*
Age at Injury (years)	33.7 ± 14.7	28.9 ± 12.4	42.4 ± 14.9 *	<0.01*
Level of Injury, n (%)				0.5684
Tetraplegic	36 (51.4)	22 (48.9%)	14 (56%)	
Paraplegic	34 (48.6)	23 (51.1%)	11 (44.0%)	
Height (cm)	174.5 ± 10.3	173.7 ± 10.5	176.0 ± 9.8	0.4
Weight (kg)	80.1 ± 18.5	78.6 ± 19.2	82.9 ± 14.6	0.37
Waist Circumference (cm) †	97.4 ± 14.8	96.6 ± 15.0	98.9 ± 14.6	0.55
25(OH)Vitamin D (nmol/L)	87.8 ± 35.0	89.2 ± 32.9	85.6 ± 38.9	0.69
AIS, n (%)				
A	42 (60.0%)	42 (93.3%)	-	
B	3 (4.2%)	3 (6.7%)	-	
C	10 (14.2%)	-	10 (40.0%)	
D	15 (21.4%)	-	15 (60.0%)	
LEMS	11.0 ± 15.8	-	29.1 ± 12.5	
Physical Activity (min/day)‡				
Mild	121 ± 133	130 ± 150	105 ± 100	0.44
Moderate	86 ± 114	81 ± 135	94 ± 71	0.66
Vigorous	25 ± 35	26 ± 36	24 ± 35	0.81
Total	232 ± 210	237 ± 245	223 ± 139	0.78

† Indicates n = 68 due to missing data

‡ Indicates n = 61 due to missing data

*Significant difference between motor-complete and incomplete groups (Student's t-test p-value reported for continuous variables, Chi square p-values reported for categorical variables)

5.1.3 Sample Size for pQCT Assessment

The scans of 55, 52, and 46 participants were analyzed at baseline, year-1, and year-2, respectively (Table 5). At baseline, one individual declined to be scanned, one individual was unable to be contacted to schedule their pQCT appointment in Hamilton following their medical assessment in Toronto, three individuals could not fit their calf into the pQCT scanner, seven scans had movement artifacts, two individuals were unable to be scanned because of health complications, and one individual died after enrollment but before a baseline pQCT scan could be performed. At year-one, two individuals declined to be scanned, five individuals were unable to be contacted to schedule their second pQCT scan, four individuals could not fit their calf into the pQCT scanner, three scans had movement artifacts, three individuals were unable to be scanned because of health complications. At year-two, two individuals declined to be scanned, eleven individuals were unable to be contacted or missed their pQCT appointment, three individuals could not fit their calf into the pQCT scanner, two scans had movement artifacts, one individual was unable to be scanned because of health complications, and two additional individuals died. Two individuals are scheduled to complete their year-2 scans in May 2014 and June 2014, respectively, and their year-two data was not included in this report.

Table 5 Summary of pQCT sample size across study visits

	Scanned	Unable to Contact	Declined Scan	Unable to Fit	Movement Artifact	Health Complications	Deceased	Pending
Baseline	55	1	1	3	7	2	1	0
Year 1	52	5	2	4	3	3	1	0
Year 2	46	11	2	3	2	1	3	2
TOTAL	153	18	5	10	12	6	5	2

Table 6 Case-control matching summary

	Full-sample		Complete-SCI		Incomplete-SCI	
	SCI Participants	Able-bodied Controls	SCI Participants	Able-bodied Controls	SCI Participants	Able-bodied Controls
N	65	65	42	42	23	23
Gender (m/f)	(45/20)	(45/20)	(15/27)	(15/27)	(18/5)	(18/5)
Age (years)	49.0 ± 11.9	47.4 ± 13.8	50.1 ± 12.0	47.8 ± 14.3	46.9 ± 11.6	46.5 ± 13.1
Height (cm)	174.3 ± 10.3	173.8 ± 8.5	173.7 ± 11.5	173.3 ± 9.2	175.5 ± 8.0	174.8 ± 7.0

5.2 Precision of Muscle CSA and Density Measurement

Repeated calculations of muscle CSA and muscle density using the watershed tissue segmentation technique were highly correlated and had a small precision error. For muscle CSA, an ICC of 0.999 ($p < 0.0001$), a RMS-SD of 0.61 cm^2 , and a RMS-CV 1.01% were observed. For muscle density, an ICC of 0.997 ($p < 0.0001$), a RMS-SD of 0.57 mg/cc , and RMS-CV 0.95% were observed.

5.3 Able-bodied Controls

Able-bodied controls had muscle CSA and density values of $78.0 \pm 12.1 \text{ cm}^2$ and $71.0 \pm 2.3 \text{ mg/cc}$, respectively (Table 7). The proportion of males versus females, age, or height were not significantly different between SCI participants and able-bodied controls ($p > 0.05$) (Table 6). Matching was conducted on a 1:1 ratio. Matching for age was prioritized over height, under the assumption that an age difference is likely more relevant to calf-muscle status than a difference in height. Sixty percent of controls had an absolute difference in age of one year or less, 71% of controls had an absolute difference in age of five years or less, and 97% of controls had an absolute difference in age of ten years or less. With regards to height, 46% of controls had an absolute difference of one centimetre or less, 79% of controls had an absolute difference of five centimetres or less and 96% had an absolute difference of ten centimetres or less.

5.4 Muscle CSA and Density after SCI

Muscle CSA and muscle density values were obtained from 65 individuals who had at least one pQCT scan of sufficient quality for analysis (Table 7). Muscle CSA values ranged from 11.2 cm² to 97.7 cm². A mean muscle CSA of 51.8 ± 20.8 cm² was observed. Muscle density values ranged from 17.2 mg/cc to 72.0 mg/cc for the 65 participants with SCI. A mean muscle density of 53.2 ± 13.2 mg/cc was observed for all participants.

Compared to able-bodied controls, individuals with complete and incomplete SCI had significantly reduced calf-muscle CSA and density values (Table 7). Participants with motor-complete SCI had a mean reduction of -34.7±20.2 cm² (45%) in muscle CSA and a mean reduction of -22.7±13.6 mg/cc (32%) in muscle density relative to controls. Participants with motor-incomplete SCI had a mean reduction of -13.0±22.3 cm² (17%) in muscle CSA and a mean reduction of -10.0±9.4 mg/cc (14%) in muscle density relative to controls. The mean reduction in muscle CSA and muscle density was greater for participants with complete SCI compared to those with incomplete-SCI (p < 0.001).

Table 7 Muscle CSA and density values for participants with complete and incomplete SCI and their matched able-bodied controls

	Complete SCI	Incomplete SCI	Able-bodied controls
N	39	25	64
Muscle CSA (cm ²)	43.1 ± 16.7*	65.5 ± 19.3*	78.0 ± 12.1
Muscle Density (mg/cc)	48.8 ± 14.1*	60.2 ± 9.2 [†]	71.0 ± 2.3

* Indicates significant difference between SCI and controls (p < 0.0001)
[†] Indicates significant difference between SCI and controls (p < 0.01)

5.5 Determinants of Muscle Status

5.5.1 Determinants of Muscle CSA

The scans from 65 individuals were included in the cross-sectional analysis of muscle CSA. Gender, age, height, weight, waist circumference, level of injury, duration of injury, age at injury, wheelchair use and cLEMS were associated with muscle CSA in bi-variate analyses (Table 8, $p < 0.2$) and were included as possible independent variables in a multivariable model of muscle CSA. Due to multicollinearity, waist circumference was included in favour of bodyweight, and injury duration was included in favour of age at injury. Based on R^2 and Mallows' $C(p)$ statistic, the best fitting model for muscle CSA indicated that a reduced height, waist circumference, cLEMS and wheelchair use were associated with a reduced muscle CSA (Table 10a; $R^2 = 0.66$, $p < 0.0001$). Height exhibited a trend towards statistical significance ($p = 0.073$), whereas all other variables were significant in the model ($p < 0.05$)

In a subsample of individuals with complete SCI, gender, height, weight, waist circumference, age at injury, SFSS, vitamin D, and daily minutes of vigorous physical activity were associated with muscle CSA (Table 8, all $p < 0.2$). The best-fitting regression model identified by R^2 and Mallows' $C(p)$ statistic indicated that decreased waist circumference, age at injury, and SFSS were associated with a decreased muscle CSA (Table 10b; $R^2 = 0.55$, $p < 0.0001$).

In a subsample of those with incomplete SCI, gender, height, weight, waist circumference, level of injury, age at injury, and cLEMS, were associated with muscle CSA (Table 8, all $p < 0.2$). Waist circumference was included in favour of body weight as multicollinearity was observed. The best-fitting regression model for individuals with incomplete

injuries included the variables of waist circumference and level of injury (Table 10c; $R^2 = 0.63$, $p < 0.0001$). The model showed that a smaller waist circumference and paraplegia were associated with a decreased muscle CSA. In a post hoc analysis, independent t-tests and chi-square tests were performed to determine if there were differences between those with incomplete tetraplegia and paraplegia that may confound the significance of the association between level of injury and muscle CSA. Those with tetraplegia tended to have a higher cLEMS, but the relationship was not statistically significant ($p = 0.07$).

Table 8 Bi-variate analyses for muscle CSA

	Gender (years)	Age (years)	Height (cm)	Weight (kg)	WC (cm)	LOI (para/tetra)	Inj.Dur (years)	Inj.Age (years)	cLEMS (/15)	Wheel-chair use (y/n)	VitD (nmol/l)	SFSS (/7)	Mild (min)	Mod (min)	Vig (min)	Total (min)
N	65	65	65	64	62	65	65	65	65	65	52	64	57	57	57	57
R ²	0.1057	0.1162	0.0921	0.436	0.3669	0.0851	0.0375	0.217	0.3639	0.3079	0.0072	0.0064	0.0234	0.0095	0.0040	0.0023
p-value	0.0088	0.0059	0.0148	<.0001	<.0001	0.0849	0.1251	0.0001	<.0001	<.0001	0.5499	0.5336	0.2472	0.4697	0.6393	0.7209
Complete SCI																
N	40	40	40	39	37	40	40	40	-	-	29	39	34	34	34	34
R ²	0.1308	0.012	0.0635	0.4741	0.4019	0.0126	0.0151	0.1145	-	-	0.1326	0.0439	0.0019	0.0006	0.0769	0.0006
p-value	0.0237	0.5073	0.0827	<.0001	<.0001	0.4958	0.4566	0.0271	-	-	0.0521	0.1833	0.8090	0.8901	0.1124	0.8892
Incomplete SCI																
N	25	25	25	25	25	25	25	25	25	25	23	25	23	23	23	23
R ²	0.1381	0.0634	0.1302	0.511	0.4336	0.2733	0.0034	0.0744	0.3005	0.2865	0.0097	0.004	0.0123	0.0127	0.0613	0.0077
p-value	0.0674	0.2217	0.0764	<.0001	0.0003	0.0073	0.7811	0.1871	0.0046	0.0058	0.6555	0.7743	0.6142	0.6093	0.2548	0.6901

WC: waist circumference; LOI: level of injury; Inj.Dur: Injury Duration; Inj.Age: Age at Injury; VitD: Vitamin D level; SFSS: Spasm Frequency and Severity Score; Mild: Mild Physical Activity; Mod: Moderate Physical Activity; Vig: Vigorous Physical Activity; Total: Total Physical Activity

Table 9 Bi-variate analyses for muscle density

	Gender (years)	Age (years)	Height (cm)	Weight (kg)	WC (cm)	LOI (para/tetra)	Inj.Dur (years)	Inj.Age (years)	cLEMS (/15)	Wheel-chair use (y/n)	VitD (nmol/l)	SFSS (/7)	Mild (min)	Mod (min)	Vig (min)	Total (min)
N	65	65	65	64	62	65	65	65	65	65	52	64	57	57	57	57
R ²	0.0040	0.0295	0.0025	0.0101	0.0071	0.0284	0.0387	0.0085	0.1215	0.1556	0.0015	0.0854	0.0379	0.0135	0.0538	0.0002
p-value	0.6178	0.1751	0.6945	0.4334	0.5161	0.2232	0.0856	0.4692	0.0057	0.0012	0.7691	0.0177	0.1039	0.3899	0.0826	0.736
Complete SCI																
N	40	40	40	40	37	40	40	40	-	-	29	39	34	34	34	34
R ²	0.0006	0.1759	0.0111	0.0502	0.0407	0.0052	0.0246	0.1087	-	-	0.0178	0.1540	0.0123	0.0083	0.0932	0
p-value	0.8815	0.0079	0.523	0.2051	0.0960	0.6614	0.3403	0.0404	-	-	0.4181	0.0148	0.5203	0.5976	0.0792	0.9768
Incomplete SCI																
N	25	25	25	25	25	25	25	25	25	25	23	25	23	23	23	23
R ²	0.0291	0.1139	0.0913	0.0015	0.0009	0.1887	0.0068	0.1104	0.0214	0.222	0.0341	0.0393	0.1574	0.0106	0.0175	0.04
p-value	0.415	0.0990	0.1421	0.8527	0.8861	0.030	0.6954	0.1047	0.4853	0.1885	0.3876	0.3422	0.0645	0.6397	0.5473	0.3605

WC: waist circumference; LOI: level of injury; Inj.Dur: Injury Duration; Inj.Age: Age at Injury; VitD: Vitamin D level; SFSS: Spasm Frequency and Severity Score; Mild: Mild Physical Activity; mod: Moderate Physical Activity; Vig: Vigorous Physical Activity; Total: Total Physical Activity

Table 10 Multivariate regression models for muscle CSA

a. Model 1: Full Cohort, n = 62

Variable	Parameter Estimate	Standard Error	p
Height	0.31	0.167	0.073
Waist Circumference (cm)	0.65	0.118	<0.0001
cLEMS	1.13	0.373	<0.0001
Upright Ambulation	15.8	6.05	0.011

R-Square for model = 0.66, p< 0.0001

b. Model 2: Motor-Complete SCI, n = 36

Variable	Parameter Estimate	Standard Error	p
Waist Circumference (cm)	0.71	0.14	<0.0001
Age at Injury (years)	0.38	0.16	0.025
SFSS	3.96	1.40	0.008

R-Square for model = 0.55, p< 0.0001

c. Model 3: Motor-Incomplete SCI, n = 25

Variable	Parameter Estimate	Standard Error	P
Waist Circumference (cm)	0.80	0.17	<0.0001
LOI (Tetra/Para)	-17.34	4.9	0.0020

R-Square for model = 0.63 p< 0.0001

5.5.2 Determinants Muscle Density

The scans of 65 participants were included in the cross-sectional analysis of muscle density. Age, injury duration, cLEMS, wheelchair use, SFSS and daily minutes of mild and vigorous physical activity were significantly associated with muscle density in the bi-variate analyses (Table 9, p<0.2). Based on R^2 and Mallows' C(p) statistics, the best fitting model for muscle density indicated that increased age, decreased cLEMS, reduced SFSS, a lower amount of daily vigorous physical activity, and wheelchair use were associated with a lower muscle density (Table 11a, $R^2 = 0.37$, $p = 0.0002$). In this model, only SFSS reached statistical significance of $p<0.05$. It is likely that cLEMS and wheelchair use are related. When wheelchair use was removed from the model, the R^2 valued decreased ($R^2 = 0.33$, $p = 0.0003$);

however, cLEMS reached statistical significance and minutes of vigorous physical activity trended towards statistical significance ($p=0.052$).

In those with complete SCI, age, age at injury, waist circumference, and SFSS were associated of muscle density in the bi-variate analyses (Table 9, $p < 0.2$). Multicollinearity was observed between waist circumference and weight, and weight was excluded. The best-fitting multivariate model included age and SFSS (Table 11b; $R^2 = 0.25$, $p < 0.01$). Increased age and decreased SFSS were associated with reduced muscle density; however, SFSS did not reach statistical significance ($p = 0.106$).

In those with incomplete SCI, age, height, level of injury, age at injury, wheelchair use, and daily minutes of mild physical activity were associated with muscle density (Table 9, all $p < 0.2$). Increased age and wheelchair use were associated with decreased muscle density in the best-fitting model (Table 11c, $R^2 = 0.34$, $p < 0.01$). Wheelchair use reached statistical significance in the model ($p=0.011$), and age exhibited a trend towards statistical significance ($p=0.053$).

Table 11 Multivariate regression models of muscle density

a. Model 1: Full sample, n = 57

Variable	Parameter Estimate	Standard Error	p
Age (years)	-0.23	0.14	0.100
cLEMS (/15)	0.76	0.45	0.102
SFSS (/7)	1.94	0.90	0.035
Vigorous Physical Activity (min/day)	0.08	0.05	0.095
Upright Ambulation	10.28	5.62	0.073

R-Square for model = 0.37, p = 0.0003

b. Model 2: Motor-Complete SCI, n = 39

Variable	Parameter Estimate	Standard Error	p
Age (years)	-0.45	0.20	0.036
SFSS	2.27	1.37	0.106

R-Square for model = 0.25, p = 0.005

c. Model 3: Motor-Incomplete SCI, n = 25

Variable	Parameter Estimate	Standard Error	p
Age (years)	-0.27	0.13	0.053
Wheelchair use	8.84	3.16	0.011

R-Square for model = 0.34 p = 0.0090

5.6 Longitudinal Change in Muscle CSA and Muscle Density

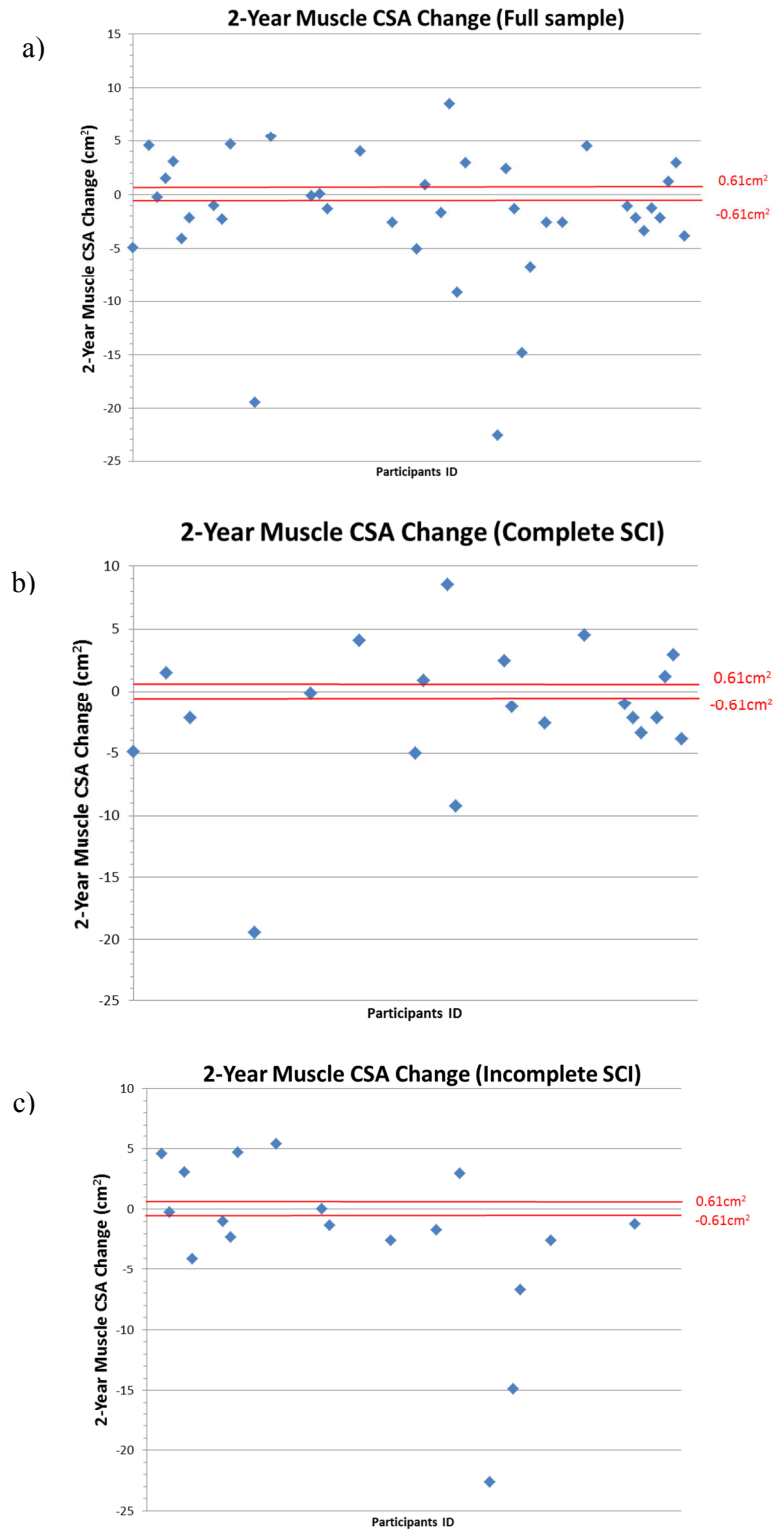
The change in muscle CSA and muscle density was calculated for one- and two-year time periods for the entire cohort, and for subsamples of those with complete and incomplete SCI. Only individuals with at least two scans were included in the analysis. Forty nine individuals had pQCT scans at a one-year time interval, and thirty nine individuals had scans at a two-year time interval. Thirty four individuals had scans at all three time points.

5.6.1 Muscle CSA Change

Muscle CSA change over a one-year period ranged from a loss of 18.1 cm² to a gain of 7.3 cm². A mean one-year reduction in muscle CSA of 1.24 ± 4.9 cm² was observed for the entire cohort. Over a two-year period, muscle CSA change ranged from a reduction of 22.6cm²

to a gain of 11.2 cm², with an overall mean reduction of 1.62 ± 6.5 cm² observed (Table 12a). Of the 39 participants that had two-year muscle CSA change scores, 23 experienced a reduction in muscle CSA (mean reduction: 5.1 ± 5.9 cm²), 13 experienced an increase in muscle CSA (mean increase: 3.6 ± 2.1 cm²) and three participants experienced a change within the repeatability error of 0.61cm² observed in the precision analysis (Figure 3a). In the subsample of participants with complete injuries, twelve participants experienced a reduction in muscle CSA (mean reduction: 4.8 ± 5.1 cm²) and eight experienced an increase in muscle CSA (mean increase: 2.9 ± 2.5 cm²). One participant experienced a change within the repeatability error of 0.61cm² (Figure 3b). In the subsample of participants with incomplete injuries, eleven participants experienced a reduction in muscle CSA (mean reduction: 5.5 ± 6.9 cm²) and five experienced an increase in muscle CSA (mean increase: 4.1 ± 1.1 cm²). Two participants experienced a change within the repeatability error of 0.61cm² (Figure 3c).

Figure 3 Two-year muscle CSA change score for a) the full-sample b) participants with complete SCI c) participants with incomplete SCI



The results of the rANOVA showed a trend towards a difference in muscle CSA between the three time points ($p = 0.053$), and a significant reduction between baseline and year-2 (Figure 4, Table 13a, $p < 0.05$). The change in muscle CSA from baseline to year-2 was entered as the dependant variable in bi-variate regression models with the independent variables listed in Table 3. Weight ($R^2 = 0.05$, $p < 0.16$) and waist circumference ($R^2 = 0.14$, $p < 0.05$) were associated with the two-year change in muscle CSA, such that a lower weight and waist circumference were associated with an increase in muscle CSA. Only waist circumference reached statistical significance at a $p < 0.05$ (Figure 5). Two participant with a large waist circumference were considered outliers based on a Cook's Distance value $> 4/n$. When these participants were omitted, the association between waist circumference and two-year muscle CSA change did to reach statistical significance.

Figure 4 Distribution of Muscle CSA

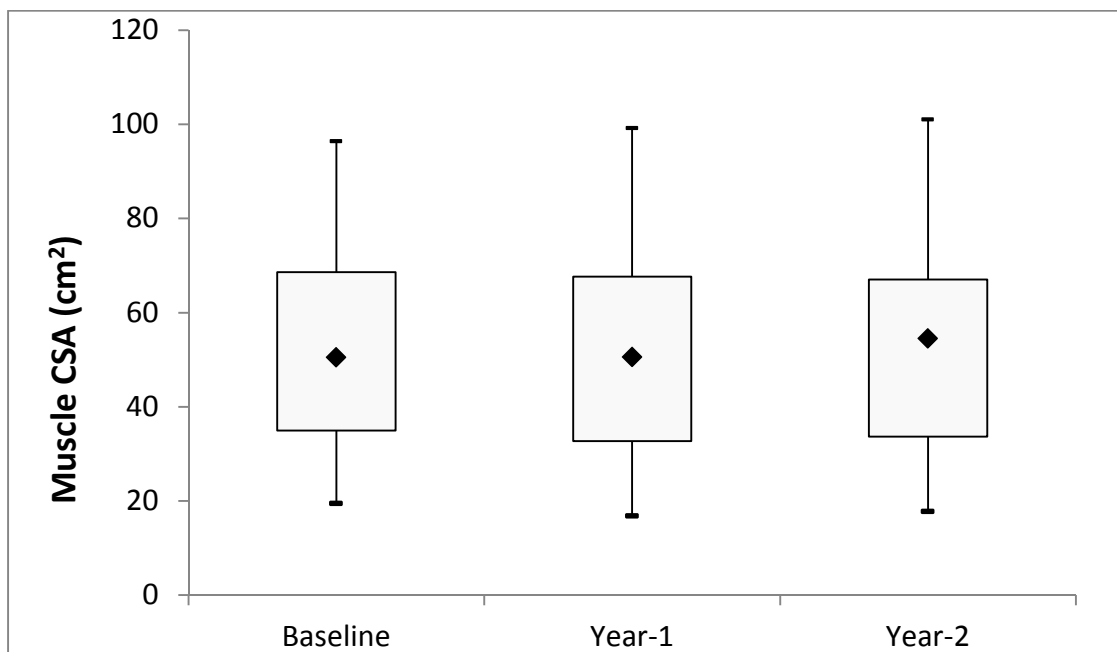
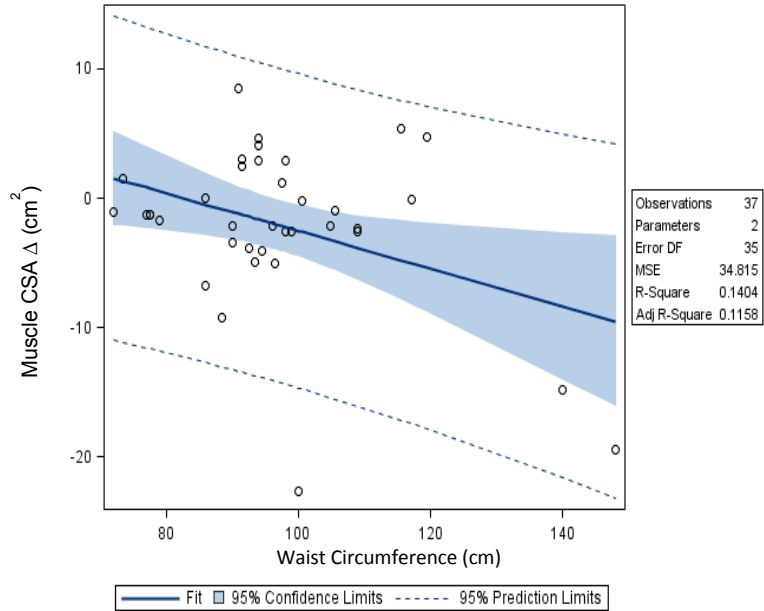


Figure 5 Relationship between Two-year Change in Muscle CSA and Baseline Waist Circumference



In a subsample of individuals with complete SCI, one-year muscle CSA change ranged from a reduction of 10.1 cm² to a gain of 7.34 cm². The mean one-year change was a reduction of 1.3 ± 3.4 cm². Over a two year period, muscle CSA change ranged from a reduction of 19.5 cm² to a gain of 8.52 cm² with a mean reduction of 1.5 ± 5.7 cm² (Table 12b). The results of the rANOVA showed no significant change between the three study visits for those with complete injuries (p = 0.114).

In a subsample of individuals with incomplete SCI, muscle CSA change ranged from a reduction of 18.1 cm² to a gain of 4.5cm², with a mean one-year reduction of 1.1 ± 6.5 cm². Over two years, individuals with incomplete SCI had a muscle CSA change ranging from a reduction of 22.6 cm² to a gain of 5.4cm², with a mean two-year reduction of 2.3 ± 6.9 cm² (Table 12c). The results of the rANOVA showed no significant change in muscle CSA between the three visits for those with incomplete SCI (p = 0.33).

Table 12 One- and Two-year Muscle CSA Change Scores

a. Full Cohort		
	One-year Δ (cm)	Two-year Δ (cm)
N	49	39
Mean	-1.24	-1.85
SD	4.89	6.21
Maximum Reduction	-18.06	-22.64
Maximum Increase	7.34	8.52

b. Complete SCI		
	One-year Δ (cm)	Two-year Δ (cm)
N	26	21
Mean	-1.32	-1.50
SD	3.39	5.67
Maximum Reduction	-10.05	-19.45
Maximum Increase	7.34	8.52

c. Incomplete SCI		
	One-year Δ (cm)	Two-year Δ (cm)
N	23	18
Mean	-1.15	-2.25
SD	6.26	6.94
Maximum Reduction	-18.06	-22.64
Maximum Increase	4.47	5.39

Table 13 rANOVA Results for Between-Visit Change in Muscle CSA

a. Full Cohort, n=65				
Time period	Adjusted mean difference (cm ²)	Standard Error (cm ²)	Df	p
Baseline - Year-1	-1.26	0.71	86	0.081
Baseline - Year-2	-1.8	0.78	86	0.024
Overall Effect of Visit			(2, 86)	0.053

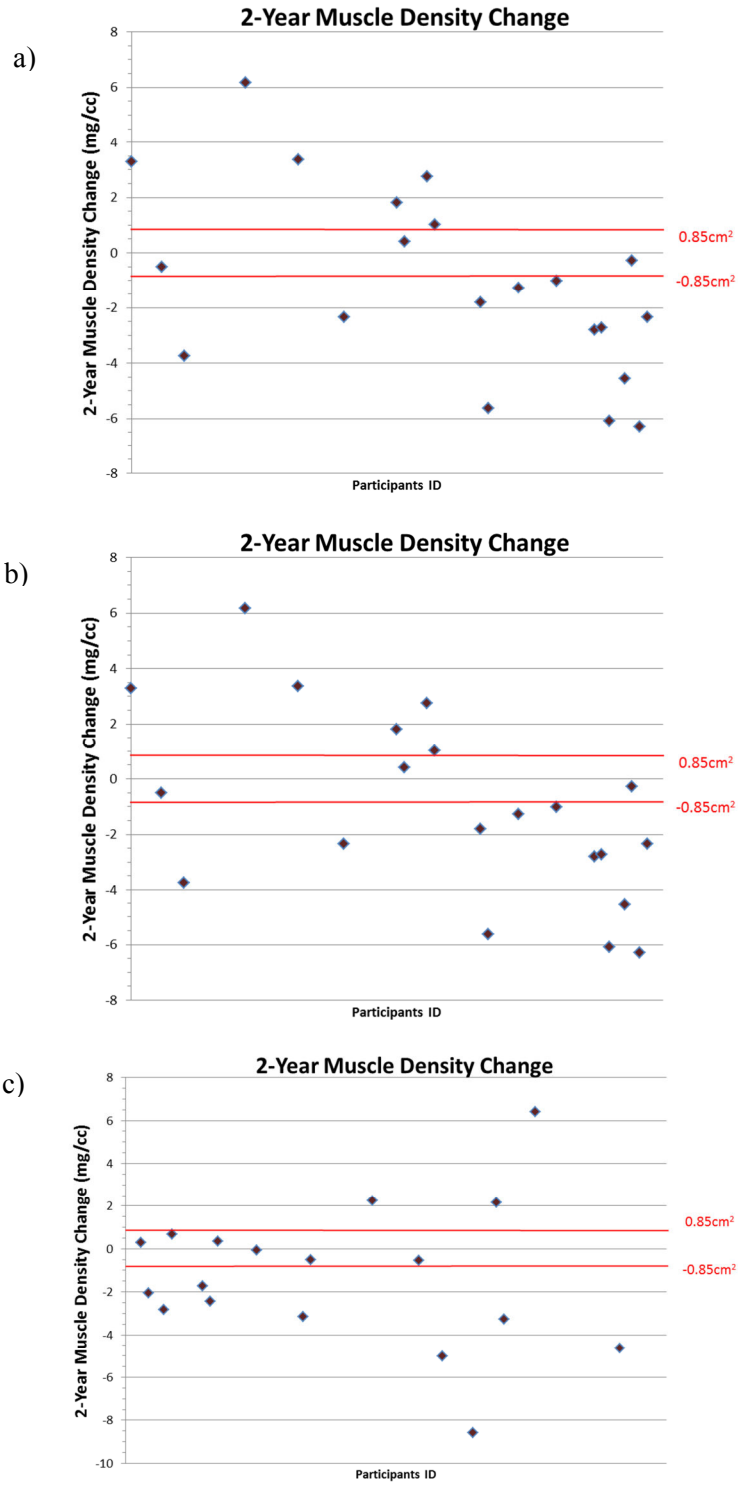
b. Complete SCI, n = 31				
Time period	Adjusted mean difference (cm ²)	Standard Error (cm ²)	Df	p
Baseline - Year-1	1.42	0.82	45	0.089
Baseline - Year-2	1.59	0.89	45	0.078
Overall Effect of Visit			(2, 45)	0.114

c. Incomplete SCI, n= 23				
Time period	Adjusted mean difference (cm ²)	Standard Error (cm ²)	Df	p
Baseline - Year-1	-1.15	1.25	39	0.362
Baseline - Year-2	-2.01	1.37	39	0.142
Overall Effect of Visit			(2, 39)	0.337

5.6.2 Muscle Density Change

Muscle density change over one-year ranged from a loss of 11.3 mg/cc to a gain of 4.3 mg/cc, with a mean one-year reduction in muscle density of 1.09 ± 4.3 mg/cc observed for the entire cohort. Over a two-year period, muscle density change ranged from a reduction of 8.6 mg/cc to a gain of 6.4 mg/cc, with a mean reduction of 1.2 ± 3.3 mg/cc (Table 14a). Of the 39 participants that had two-year muscle density change scores, 21 experienced a reduction in muscle density (mean reduction: 3.5 ± 1.9 mg/cc) and nine experienced an increase in muscle density (mean increase: 3.2 ± 1.9 mg/cc). Nine participants experienced a change within the repeatability error of 0.85mg/cc reported by Wong et al. (71) (Figure 6a). In the subsample of participant with complete injuries, twelve participants experienced a reduction in muscle density (mean reduction: 3.4 ± 1.9 mg/cc) and six experienced an increase in muscle density (mean increase: 3.1 ± 1.8 mg/cc). Three participants experienced a change within the repeatability error of 0.85mg/cc (Figure 6b). In the subsample of participant with incomplete injuries, 18 participants experienced a reduction in muscle density (mean reduction: 3.7 ± 2.1 mg/cc) and three experienced an increase in muscle density (mean increase: 3.6 ± 2.4 mg/cc). Two participants experienced a change within the repeatability error of 0.85mg/cc (Figure 6c).

Figure 6 Two-year muscle density change scores for a) full-sample b) participants with complete SCI c) participants with incomplete SCI



A significant difference was detected between baseline, year-1, and year-2 muscle density values (Figure 7, Table 15a, $p < 0.05$). In bi-variate regression models, injury duration was positively associated with a change in muscle density between baseline and year-1 ($R^2 = 0.050$, $p = 0.12$); however, it was not significant at a $p < 0.05$. No other variables were associated with one-year change scores. The variables of weight ($R^2 = 0.26$, $p < 0.001$), waist circumference ($R^2 = 0.20$, $p < 0.01$), and wheelchair use ($R^2 = 0.063$, $p < 0.2$) were associated with two-year muscle density change scores, such that decreased weight and waist circumference, and wheelchair use were associated with a reduction in muscle density. Because weight and waist circumference are likely related, their relationship with muscle density was looked at separately (Figure 8). Adding wheelchair use to each model did not significantly improve the fit of the regression line. One participant with a large weight and waist circumference was considered an outlier based on a Cook's Distance value $>4/n$. When this participant was omitted, the association between weight and two-year muscle density change remained statistically significant ($p < 0.05$) and the association between waist circumference and two-year muscle density change trended towards statistical significance ($p = 0.058$).

Figure 7 Distribution of Muscle Density

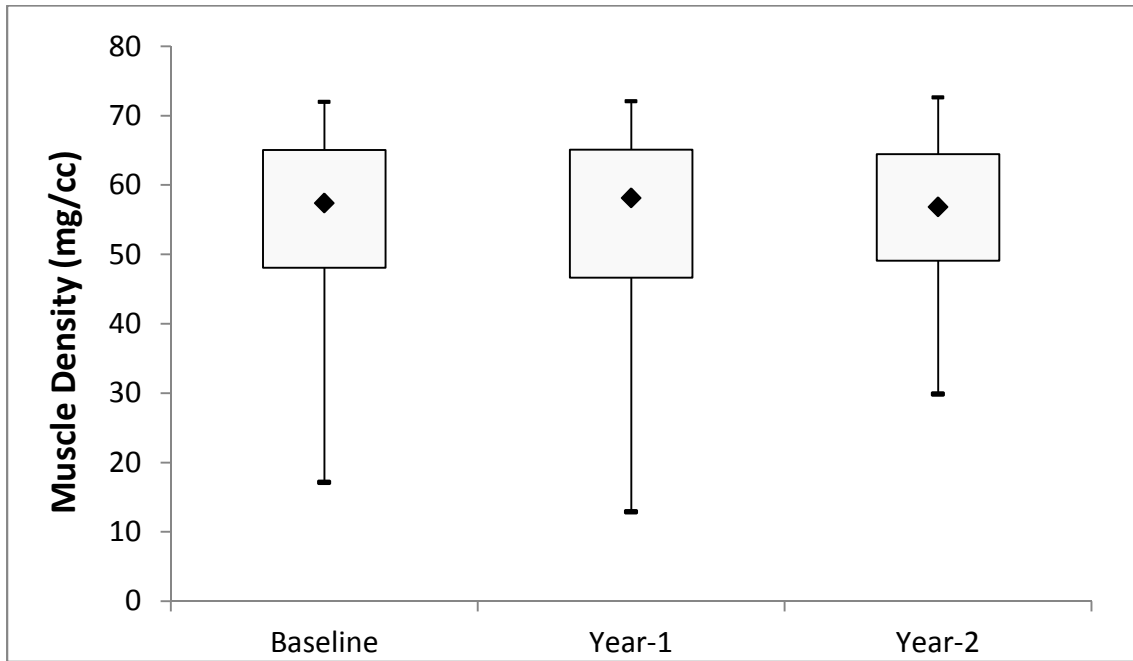
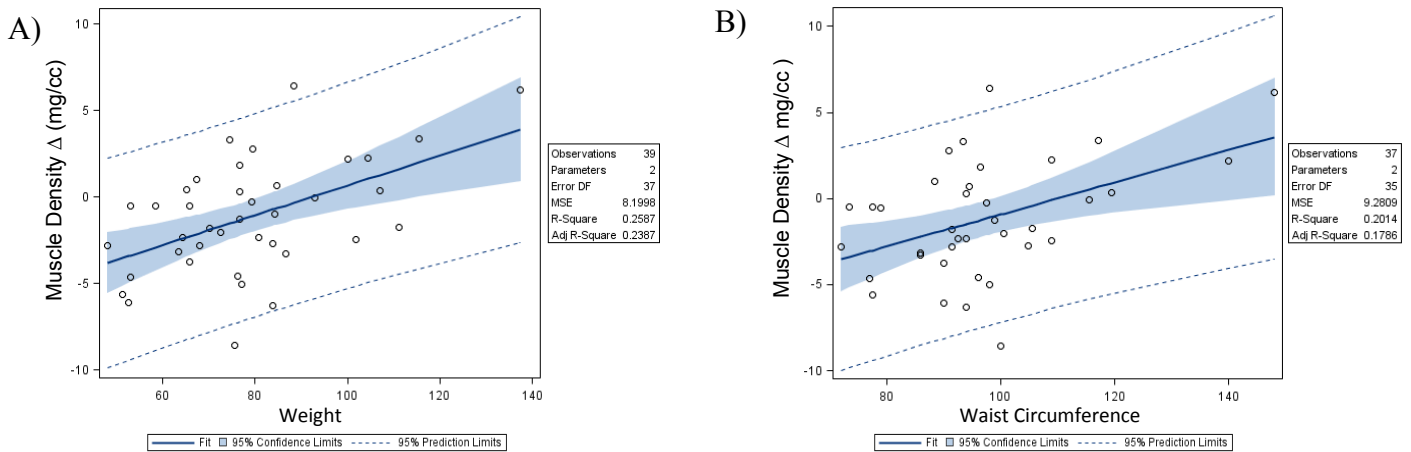


Figure 8 Relationship between Two-year change in Muscle Density and A) Weight and B) Waist Circumference



In a subsample of those with complete SCI, muscle density change ranged from a reduction of 11.3 mg/cc to a gain of 4.3 mg/cc. A mean reduction of 0.9 ± 3.2 mg/cc over one year was observed. Over a two-year period, a muscle density change ranging from a reduction of 6.3 mg/cc to a gain of 6.2 mg/cc, with a mean reduction of 1.0 ± 3.3 mg/cc observed (Table 14b).

The results of the rANOVA showed no significant change in muscle density between the three study visits for those with complete SCI (Table 15b, $p = 0.186$).

Individuals with incomplete injuries had a muscle density change ranging from a reduction of 5.2 mg/cc to a gain of 3.5 mg/cc over one year. A mean one-year reduction of 1.3 ± 2.2 mg/cc observed. Over two years, muscle density ranged from a reduction of 8.6 mg/cc to a gain of 6.4 mg/cc, with a mean reduction of 1.3 ± 3.3 mg/cc observed (Table 14c). The difference between the three visits did not reach significance ($p = 0.051$); however, a significant change was detected between baseline and year-1 (Table 15c, $p < 0.05$).

Weight ($R^2 = 0.127$, $p < 0.10$), waist circumference ($R^2 = 0.243$, $p < 0.05$), cLEMS ($R^2 = 0.094$, $p < 0.2$), moderate intensity physical activity ($R^2 = 0.095$, $p = 0.20$), total physical activity level ($R^2 = 0.113$, $p < 0.20$) were associated with a one-year change in muscle density in the subsample of participants with incomplete SCI. Weight and total minutes of daily physical activity were excluded as possible covariates in the multivariate model due to their association with waist circumference and daily minutes of moderate physical activity, respectively. After examining all possible variable combinations, only waist circumference was included in the best-fitting model for one-year change in muscle density for individuals with incomplete injuries ($R^2 = 0.243$, $p < 0.05$), such that a decreased waist circumference was associated with a reduction in muscle density (Figure 9). When one outlying data point representing a participant with a large waist circumference was removed from the model, the association between waist circumference and muscle density change did not reach statistical significance ($p=0.167$).

Figure 9 Relationship between one-year change in muscle density and waist circumference for participants with incomplete SCI

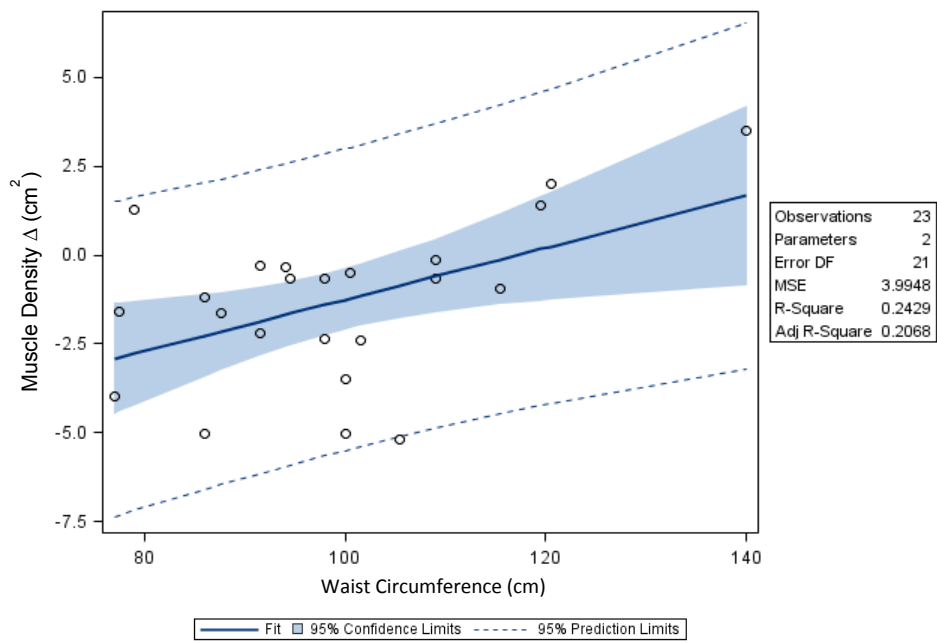


Table 14 One- and two-year muscle density change scores

a. Full Cohort		
	One-year Δ (mg/cc)	Two-year Δ (mg/cc)
N	49	39
Mean	-1.09	-1.16
SD	2.75	3.28
Maximum Reduction	-11.29	-8.57
Maximum Increase	4.26	6.39

b. Complete SCI		
	One-year Δ (mg/cc)	Two-year Δ (mg/cc)
N	26	21
Mean	-0.90	-1.08
SD	3.16	3.34
Maximum Reduction	-11.29	-6.3
Maximum Increase	4.26	6.17

c. Incomplete SCI		
	One-year Δ (mg/cc)	Two-year Δ (mg/cc)
N	23	18
Mean	-1.31	-1.26
SD	2.24	3.30
Maximum Reduction	-5.18	-8.57
Maximum Increase	3.49	6.39

Table 15 rANOVA results for between-visit change in muscle density

a. Full Cohort SCI, n=65				
Time period	Adjusted mean difference (mg/cc)	Standard Error (mg/cc)	Df	p
Baseline - Year-1	-0.97	0.42	86	0.023
Baseline - Year-2	-1.22	0.45	86	0.009
Overall Effect of Visit			(2, 86)	0.015

b. Motor-complete SCI, n = 31				
Time period	Adjusted mean difference (mg/cc)	Standard Error (mg/cc)	Df	p
Baseline - Year-1	-0.68	0.62	45	0.077
Baseline - Year-2	-1.21	0.67	45	0.275
Overall Effect of Visit			(2, 45)	0.186

c. Motor-SCI, n= 23				
Time period	Adjusted mean difference (mg/cc)	Standard Error (mg/cc)	Df	p
Baseline - Year-1	-1.31	0.57	39	0.028
Baseline - Year-2	-1.27	0.63	39	0.050
Overall Effect of Visit			(2, 39)	0.051

CHAPATER 6: DISCUSSION

6.1 Summary

This is the first study to prospectively measure lower-extremity muscle CSA and muscle density in a diverse cohort of men and women with chronic complete and incomplete SCI. Previous studies characterising lower-extremity muscle have focused on the acute and sub-acute stage of injury, whereas we explored muscle status years, if not decades, post-SCI. Individuals with complete-SCI had reductions in muscle CSA and muscle density values of 44.5% and 31.7% relative to their able-bodied peers, and individuals with incomplete-SCI had reductions in muscle CSA and muscle density values of 16.3% and 14.3% of their able-bodied peers, respectively. Furthermore, a decreased height, waist circumference, lower-extremity motor score and wheelchair use were associated with a decreased muscle CSA. Additionally, in those with complete SCI, a younger age at injury and decreased spasm frequency and severity were associated with decreased muscle CSA. In individuals with incomplete SCI, paraplegia was associated with a decreased muscle CSA. An older age, a decreased lower-extremity motor score, reduced spasm frequency and severity, less minutes of daily vigorous physical activity, and wheelchair use were associated with decreased muscle density. Longitudinally, we observed that individuals with complete and incomplete injures experienced a reduction in muscle CSA and muscle density over a two-year period. A greater waist circumference at baseline was associated with a reduction in muscle CSA, and lower weight and waist circumference at baseline were associated with a reduction in muscle density over two years.

6.2 Magnitude of Muscle CSA and Density Reduction after Chronic-SCI

6.2.1 Muscle CSA

Consistent with published literature (2,4,7) individuals with SCI had greater reductions in muscle CSA compared to age, gender, and height matched able-bodied controls and individuals with complete SCI had greater reductions in muscle CSA compared to those with incomplete SCI. Individuals with complete SCI experienced 45% reductions in muscle CSA compared to able-bodied controls. This reduction is comparable to reductions reported in previous studies examining lower-extremity MRI scans taken in the acute stage of complete-SCI, where decreases in gastrocnemius and soleus muscle CSAs were 54% and 68% relative to able-bodied controls (2). A 16% reduction in muscle CSA was observed in those with incomplete SCI. These reductions are less than that previously reported from in MRI scans of the partially paralyzed lower-extremity muscles of individuals with acute incomplete-SCI; which ranged from 76% and 67% of controls (4,11). It is possible that the increased muscle CSA values we observed are due to functional improvements or locomotor recovery in the chronic-stage of injury; however, it is more likely that the differences in CSA are due to the variation of preserved motor function in the incomplete-SCI population, or due to methodological differences between the two technologies (pQCT vs. MRI). Whereas MRI can examine individual muscles and segment muscle from inter- and intramuscular fat, pQCT examines the total CSA of all muscle groups and adipose tissue combined, and therefore it is possible that pQCT may overestimate muscle CSA values compared to MRI.

In summary, our results stress the prevalence of muscle atrophy in the chronic-SCI population. Muscle atrophy after SCI is closely associated with a decreased metabolic rate and increased adipose tissue storage if energy intake is not adjusted in accordance with energy

expenditure (37,41). Secondly, muscle atrophy results in reduced capacity for glucose uptake contributing to the development of type-II diabetes and other manifestations of metabolic disease (124,125). Evidence suggests that evoking skeletal muscle hypertrophy through therapy modalities like electrical stimulation may improve metabolic profile and reduce the risk of developing glucose intolerance, insulin resistance and dyslipidemia following chronic-SCI (46,269). Continued research investigating the effects of therapy modalities intended to preserve or improve muscle quantity, and their impact on an individual's metabolic profile is warranted.

6.2.2 Muscle Density

Reductions in muscle density of 32% and 14% were observed for those with complete and incomplete injuries, respectively. Although no other study has reported calf-muscle density values post-SCI, our results are consistent with an increased inter- and intramuscular fat content observed in MRI images of the thigh muscles following acute and chronic-SCI. After complete-SCI, Elder et al. (9) observed a fourfold increase in percentage intramuscular adipose tissue compared to matched controls. After incomplete-SCI Gorgey et al. (4) and Shah et al. (11) observed similar a threefold increase in relative intramuscular adipose tissue content. The observed increase in muscle density in those with incomplete-SCI compared to complete-SCI is likely due to the preservation of voluntary muscle contraction; however, regardless of injury completeness, individuals with SCI had muscle density values significantly less than their able-bodied peers.

A growing body of evidence indicates that elevated fatty-infiltration is associated with diminished insulin sensitivity in skeletal muscle (49,64,167,285). Lipid accumulation is the result of enhanced fatty acid uptake into the muscle coupled with diminished mitochondrial lipid

oxidation (134). The excess fatty acids are stored or metabolized to various molecules that may interfere with normal insulin-mediated signal transduction; impairing cellular and whole-body glucose metabolism (135). An association between increased fat content in skeletal muscle and impaired glucose tolerance has been observed in the acute and chronic-SCI populations (4,9,11); however, given the different modalities (pQCT vs. MRI), and muscles examined (calf vs. thigh) it is difficult to directly compare muscle density with previous investigations of fatty-infiltration post-SCI. Calf-muscle density however, has been reported for able-bodied individuals with and without diabetes. Miljkovic-Gacic et al. reported that individuals with diabetes had a mean muscle density of 69.5 ± 6.5 mg/cc, which was significantly lower than those without diabetes (74.3 ± 4.3 mg/cc) (70). The mean muscle density values we observed for individuals with complete (48.8 ± 14.1 mg/cc) and incomplete (60.2 ± 9.2 mg/cc) SCI were lower than the density values reported for able-bodied individuals with diabetes. Therefore, increased fatty infiltration as demonstrated by decreased muscle density may be related to the increased prevalence of diabetes and dyslipidemia in the SCI community.

6.3 Determinants of Muscle CSA and Density

6.3.1 Determinants of Muscle CSA

This is the first investigation to examine the characteristics associated with calf-muscle CSA in a diverse sample of individuals with chronic-SCI. We observed that a decreased height, waist circumference, reduced cLEMS, and wheelchair use were associated with decreased muscle CSA for the entire cohort. In a sub-sample of participants with complete injuries, a decreased waist circumference, age at injury, and SFSS were associated with a decreased muscle CSA, and in a sub-sample of participants with incomplete injuries, paraplegia was associated

with a decreased muscle CSA. These characteristics were able to explain between 55% and 66% of CSA variation in our sample.

These results provide novel insight into who experiences the greatest muscle loss in the chronic phase of injury after varying degrees of paralysis. In the able-bodied population, characteristics such as age, gender, and physical activity are important determinants of muscle mass (286-288); however, the loss of muscle following SCI is distinct from muscle atrophy observed in the aged population (140,289), or in other disuse conditions (59,60,115,117,119) with respect to the rate of onset and severity of decline. Therefore, our examination of the demographic characteristics associated with muscle mass after SCI may aid in understanding who loses the most muscle, and who may be at greatest risk for the associated metabolic consequences.

Decreased height and waist circumference are associated with reduced muscle CSA. It is likely that this relationship is based on anthropometrics; in that smaller individuals have less muscle mass and a decreased waist circumference and height. Previously, it has been observed that obese individuals have a greater lower-extremity muscle CSA (290). Our results indicate that a positive relationship between body size and muscle mass remains despite chronic paralysis.

pQCT-derived muscle CSA was defined as the total CSA within the epimysium. Therefore increased inter- and intramuscular lipid deposits, often enlarged in those with increased central adiposity, may result in an overestimation of muscle CSA values; underwriting the relationship between waist circumference and muscle CSA. Studies using MRI technology have demonstrated that not accounting for enlarged inter- and intramuscular adipose stores may inflate thigh-muscle CSA values by 6% (4). As pQCT does not have the resolution to separate

lean tissue from adipose tissue, it is recommended that future studies use MRI technology to confirm the relationship between waist circumference and calf-muscle CSA.

For all SCI cases cLEMS was a determinant of muscle CSA. An association between the degree of voluntary muscle contraction and muscle mass was expected given the principles of muscle plasticity. It is possible that the significant association was driven by the difference in muscle CSA between those with complete and incomplete injuries (i.e., cLEMS 0 vs. >0) as cLEMS and muscle CSA were not associated in the sub-sample of those with incomplete SCI. The lack of statistical significance in the incomplete sub-sample suggests that variation in motor-score has a minimal effect on muscle CSA. It is possible that even though individuals with incomplete injuries have different amounts of voluntary function, they may experience similar loading profiles and consequently display minimal variation in muscle mass. Also, the sample of participants with incomplete SCI was 25 participants, and therefore may not have been of sufficient size to detect an association between cLEMS and muscle CSA. Future investigations examining a larger sample of individuals with incomplete injuries may add insight into the effect of motor-score variation on muscle CSA in those with incomplete injuries.

Wheelchair use was significantly associated with reduced muscle CSA. This association was expected as wheelchair use removes the loading associated with weight bearing and upright gait (291). Notably, the association between wheelchair use and muscle CSA remained significant after controlling for cLEMS; suggesting that loading patterns associated with upright ambulation promote increased muscle mass regardless of the degree of preserved voluntary muscle activation. Previously, Shah et al. observed reductions in muscle CSA values in both wheelchair dependant (range, 21%–39%) and ambulatory (range, 24%–38%) individuals relative to matched able-bodied controls approximately one-year after incomplete-SCI (7). However,

wheelchair users exhibited significantly greater plantarflexor muscle atrophy compared with the dorsiflexors, and a greater degree of atrophy in the medial gastrocnemius muscle compared to non-wheelchair users (7). By examining muscle CSA of a diverse group of individuals with chronic-SCI, we were able to expand on the relationship between wheelchair use and lower-extremity muscle mass by controlling for a variety of factors related to muscle status. Our results indicate a negative association between wheelchair use and lower-extremity muscle mass. This association stresses the importance of pursuing the restoration of upright posture after incomplete-SCI.

SFSS was significantly associated with muscle CSA in those with complete injuries. This finding concurs with our hypothesis that spasticity may maintain or improve skeletal muscle size, body composition, and metabolic profile (101,238-242). One study reported that six weeks post-incomplete SCI, thigh-muscle spasticity accounted for 54% of the variability in muscle CSA (238). In another study, spasticity was found to be related to pQCT-derived muscle mass in the thigh but not calf of 54 individuals motor-complete SCI five to 50 years post-injury; with Spearman correlation coefficients between spasticity and muscle CSA of 0.3 ($p=0.03$) for the thigh and 0.22 ($p=0.08$) for the calf (292). Our study differs from previous work in that we used self-reported spasm frequency and severity and not a clinical assessment, such as the Modified Ashworth Scale. Other have reported that SSFS is only moderately correlated with routine clinical examination, however, it adds the patient's perspective, and provides standardization of spasticity over time (195). This data indicates that lower-extremity spasticity has a positive effect on calf-muscle CSA in chronic stage of SCI. Future research should investigate the association between of spasticity score and metabolic health in the chronic-SCI population.

We observed that a younger age at injury was associated with a reduced muscle CSA in a sub-set of individuals with complete-SCI. This association was contrary to our hypothesis as we expected those injured at an older age to have a decreased muscle CSA as a result of age related functional declines prior to injury. The average age at injury for our cohort was 30 years, which likely explains why this was not the case. It is plausible that those injured at a younger age may not have reached their peak muscle mass, and therefore had a lower muscle CSA preceding injury. It was surprising that age at injury, and not age, or injury duration was associated with muscle CSA. In a post hoc analysis, we observed that age at injury was negatively correlated with injury duration ($r = -0.33$, $p < 0.05$), and therefore the association between a younger age at injury and decreased muscle CSA may be partially due to those injured at younger age being injured for longer. Our results indicate that age at injury, and possibly duration of injury, may be better than age at explaining a reduction in muscle CSA.

We observed that paraplegia was associated with a decreased muscle CSA in participants with incomplete-SCI. This was contrary to our hypothesis in that we expected higher level injuries to be related to impaired mobility, body composition, and autonomic function (124,229,230) ; resulting in individuals with tetraplegia having decreased muscle mass. One possible explanation for the contradictory findings is that individuals with tetraplegia may have had larger inter- and intramuscular fat deposits within the epimysium which caused an overestimation of muscle CSA. However waist circumference, which is a measure of central adiposity, was controlled for in the model. A post hoc analysis revealed that in the sub-sample of persons with incomplete SCI, tetraplegia trended towards having a higher cLEMS compared to participants with paraplegia. Motor-score differences could account for the association between level of injury and muscle CSA in the incomplete sub-sample; and explain why the

association between level of injury and muscle CSA did not reach statistical significance after controlling for cLEMS in the model for the entire cohort. Further investigation is needed to confirm the relationship between paraplegia and reduced muscle CSA.

Contrary to our hypothesis, muscle CSA was not associated with serum vitamin D level or physical activity level. It is possible that vitamin D level may moderate muscle CSA at concentrations lower than those observed in our cohort. . A 25(OH)D concentration of 60 nmol/l has been proposed as a minimum threshold for improved muscle health, such that impaired muscle function is observed in persons with a concentrations below 60 nmol/l as opposed to above 60 nmol/l (192). Our cohort had a mean 25(OH)D concentration of 86.49 ± 35.83 nmol/l, with only nine participant having a concentration below 60nmol/l. Therefore, it is possible that our sample was not able to demonstrate the effect of vitamin D insufficiency. In addition, it is possible that vitamin D may moderate muscle health through mechanisms not reflected in muscle CSA. Secondly, it was hypothesized that physical activity level would relate to muscle CSA by increasing lower-extremity muscle loading in those with incomplete injuries, and by eliciting a systemic response to improve glucose tolerance and low-level inflammation. It is possible that we did not observe an association between muscle CSA and physical activity in this study because the predominance of physical activity was performed with the upper body. Our results indicate that upper-body exercise is insufficient to induce an effect on lower-extremity muscle mass.

It was proposed that a combination of SCI-specific and traditional risk factors would determine an individual's muscle status post-SCI. The results of our study show that in those with chronic-SCI, 66% of the variation in calf-muscle CSA was explained by height, waist circumference, cLEMS, and wheelchair use. In those with complete-SCI, waist circumference

and age at injury, and spasm frequency and severity explained 55% of the variability in muscle CSA. In those with incomplete-SCI, waist circumference and level of injury explained 63% of the variation in muscle CSA. Our results have provided novel information as to who in the chronic-SCI population may experience the greatest degree of muscle atrophy and consequently may be at most risk for metabolic disease. Future work should focus on addressing the remaining unexplained variance in muscle CSA to possibly identify modifiable risk factors and therapy modalities to prevent or treat reductions in muscle mass.

6.3.2 Determinants of Muscle Density

Decreased muscle density is associated with impaired glucose tolerance due to increased fatty-infiltration of skeletal muscle (64). Therefore, understanding the factors associated with a reduced muscle density may identify those at most risk for diabetes and other forms of metabolic disease. Our results demonstrated that increased age, reduced cLEMS, reduced spasm frequency and severity, less minutes of daily vigorous physical activity and wheelchair use were associated with decreased muscle density in those with chronic-SCI. The models for the entire sample, and sub-samples of participants with complete and incomplete SCI explained 25-37% of the variation in muscle density, suggesting that other factors besides the variables we identified *a priori* may be involved in determining muscle density. However, incorporating the factors identified in this study into screening procedures may increase the sensitivity of metabolic disease prevention and detection practices in those with chronic-SCI.

Reduced muscle density is prevalent in the aged able-bodied population, and is associated with onset of metabolic morbidities (190,293) and functional declines (294); therefore, it is not surprising that age is a determinant of muscle density following SCI. However, the SCI-cohort

was much younger than the age typically associated with reduced muscle density in the able-bodied population. This agrees with evidence of premature aging observed previously in the SCI population (220,222); and suggests that metabolic disease screening and prevention should occur at an earlier age in those with SCI.

Calf-muscle lower-extremity motor score and wheelchair use trended towards a positive association with muscle density in the best-fitting model. These two variables are likely related, and when wheelchair use was removed from the model, cLEMS attained statistical significance. However, removing wheelchair use from the model decreased the R^2 -value by 4%; suggesting that wheelchair use has an effect on muscle density independent of motor-score. We observed that cLEMS was positively associated with muscle density in the entire sample, and participants with complete SCI had reduced muscle density values compared to participants with incomplete SCI. The association between motor-score and muscle density is congruent with previous studies showing a greater amount of intramuscular fat in the thigh muscles of those with complete injuries compared to those with incomplete injuries (4,9,11).

Wheelchair use, and not cLEMS, was included in the best-fitting model of muscle density in the sub-sample of participants with incomplete injuries. It is possible that the variability in muscle activity associated with the array of motor-scores observed in the incomplete-SCI population may be insufficient to evoke muscle density variability, or are undermined by the effect of loading pattern differences associated with upright versus wheelchair ambulation. Those who use a wheelchair for ambulation may be less active, have decreased daily caloric demands, and experience decreased loading of the lower-extremity muscles; and consequently, these individuals may be predisposed to metabolic dysregulation (185,235). Based on the muscle density values we observed, individuals with complete and incomplete injuries are at risk

for metabolic morbidities, and the degree of susceptibility is likely greater in those with complete SCI and in those who use a wheelchair for ambulation.

SFSS was positively associated with muscle density in the entire sample, and the association trended towards significance in the sub-sample of participants with complete injuries. This agrees with our hypothesis, and with previous literature showing that spasticity maintains or improves body composition and metabolic profile (100, 234-238). The association between spasticity and muscle density in those with chronic-SCI is a novel finding, as studies in the acute population did not observe a relationship between intramuscular fat and spasticity scores as assessed by the Modified Ashworth scale (238). However, mechanisms have been previously proposed that indirectly link spasticity to the prevention of intramuscular adipose tissue accumulation post-SCI (238). For example, there is evidence that spasticity contributes to the predominance of slow fibers in individuals with SCI. Slow fibers have a greater mitochondrial density compared to fast fibers, and consequently may facilitate increased oxidation of intramuscular fat (103,245). Spasticity was not related to muscle density in the sub-sample of participants with incomplete injuries; indicating the effects of spasticity on muscle density may have been eliminated by the presence of voluntary motor function. Given the association between spasticity and muscle quality, it is possible that spasticity may influence or mitigate metabolic health, especially in those with complete-SCI.

The regression model for muscle density indicated that increased daily minutes of vigorous physical activity trended towards an association with an increased muscle density. There is wide-spread evidence that physical activity can improve metabolic health (185,262,263). However, our results are novel in that upper-body physical activity was associated with decreased lipid storage in lower-body skeletal muscle. It is possible that the

systemic effect of vigorous exercise is due to increased skeletal muscle insulin sensitivity and the attenuation of inflammatory responses that are implicated in the increased storage of lipids in skeletal muscle (135,137,266). For example, a previous study has shown that arm cranking exercise improves low-grade systemic inflammation by decreasing plasma levels of inflammatory cytokines in adults with chronic-SCI (295). Our results suggest that the benefits of regular physical activity at a vigorous intensity extend to the reduction of fatty infiltration of lower-extremity skeletal muscle. To expand on our findings, it is recommended that the relationship between muscle density, metabolic health, and physical activity be explored with randomized, controlled exercise intervention studies.

Contrary to our hypothesis, gender, vitamin D level, waist circumference, and level of injury were not significantly associated with muscle density in the best-fitting models. It has been observed that females have a lower calf-muscle density compared to males in the able-population (70); however, our results indicate that gender differences are eliminated by complete and incomplete paralysis. Secondly, it was surprising that waist circumference, which has been proposed a measure of central adiposity and metabolic health in the SCI population (296-298), was not included in the best-fitting model for muscle density. Our results indicate that waist circumference may not be sensitive enough to detect fatty-infiltration of skeletal muscle in individuals with SCI, and therefore continued efforts to verify the validity of waist circumference as a measure of metabolic health in the SCI-population may be needed. Thirdly, one study previously observed a possible link between serum 25(OH)D insufficiency and increased fat infiltration of thigh-muscle (257); however, our results do not support a relationship between vitamin D and muscle density after chronic-SCI. Lastly, higher level injuries are characteristic of autonomic dysfunction, increased adipose tissue mass, and reduced activity levels which

predispose individuals with tetraplegia to poor metabolic health (232,233,299). It is possible that those with upper and lower level injuries experience similar reductions in muscle quality, or that the differences in metabolic health associated with level of injury are accounted for by other variables such as mobility and activity level.

It was hypothesized that a combination of SCI-specific and traditional risk factors would determine an individual's muscle status post-SCI. The results of our study show that in those with chronic-SCI, 37% of the variation in calf-muscle density was explained by age, motor score, spasm frequency and severity, vigorous physical activity level, and wheelchair use. In those with complete SCI, age and spasm frequency and severity explained 25% of the variability in muscle density. In those with incomplete SCI, age and wheelchair use explained 34% of the variation in muscle density. Our results provide novel information indicating who in the chronic-SCI population may experience the greatest degree of fat infiltration of lower-extremity skeletal muscle and consequently, who may be at most risk for metabolic disease. Future work should focus on addressing the remaining unexplained variance in muscle density to possibly identify modifiable risk-factors and therapy modalities to prevent or treat reductions in muscle quality.

6.4 Determinants of Muscle CSA and Density Change

6.4.1 Muscle CSA Change

The variability in individuals' one- and two-year muscle CSA change indicated that lower-extremity muscle mass can fluctuate after chronic-SCI. Precision analyses revealed a least significant change of 0.61cm^2 for the watershed image analysis technique used in this study. Based on this precision error, 23 participants exhibited a reduction and 13 participants exhibited an increase in muscle CSA. Notably, the frequency and magnitude of change for those who

experienced a reduction in muscle CSA was greater than for those who experienced an increase. The rANOVA indicated a significant reduction in muscle CSA over a two-year period in the cohort of individuals with complete and incomplete SCI. While the mean two-year reduction in muscle CSA was relatively small ($<2 \text{ cm}^2$), continued reductions of this magnitude extrapolated over a lifespan could have a major impact on metabolic function. The overall reduction and degree of individual variability in muscle CSA change was contrary to our hypothesis that proposed there would be no change in muscle CSA over a two-year period in those with chronic-SCI.

The majority of previous studies have examined muscle mass change with small sample sizes and relatively soon after SCI. While the general consensus in the literature is that a new steady-state of muscle atrophy is reached after an initial rapid loss of muscle mass in the acute stage of injury (10), a small number of studies have indicated the possibility of continued muscle mass reduction in those with chronic-SCI. One study observed that three individuals with SCI experienced reductions in thigh and lower-limb muscle CSA, ranging from -2.3% to -16.8% in one year (73). In another study, a control group of three men with chronic complete injuries experienced a 10% reduction in gracilis CSA after a twelve-week period; however, no change was observed in total thigh-muscle CSA (268). The evidence suggesting reductions in muscle mass following chronic-SCI is largely anecdotal as observations are from randomized controlled trials or case-series designs with small sample sizes underpowered to detect significant changes in muscle mass. This study was the first to prospectively measure lower-extremity muscle status in a diverse group of males and females years, if not decades, after SCI. These results are novel in that we not only report a statistically significant decrease in mean muscle CSA over two-years,

but that we observed some individuals with chronic-SCI experience muscle mass gains and reductions.

The factors associated with muscle CSA change remain unclear. A greater waist circumference at baseline explained a small proportion of muscle CSA variation in the regression model; however, the association was not statistically significant after outlying data points from two individuals with large waist circumferences were removed. There is evidence that obesity has a detrimental effect on skeletal muscle such that elevated toxic lipid metabolites, increased proinflammatory cytokines, and insulin and leptin resistance may contribute to decreased muscle regeneration and maintenance capacity (267). Therefore, it is possible that excessive lipid accumulation in those who are overweight or obese may result in lower-extremity muscle loss. The mechanisms responsible for decreased muscle regeneration in obesity remain largely unknown, and therefore continued research is needed to investigate this association, and underlying physiological pathways.

It must be noted that pQCT-derived muscle CSA values reflect the total soft-tissue area within the epimysium, and therefore it is possible that the decrease in muscle CSA we observed actually indicates a decrease in the size of inter- and intramuscular adipose tissue deposits. This hypothesis is supported in the observed concurrent improvement in muscle density over the two-year study duration, and by the relationship between a greater baseline waist circumference and improved muscle density. It is possible that individuals who are overweight or obese may have the greatest potential to experience improved fatty-acid oxidation in skeletal muscle, and consequently decreased fatty-infiltration (300). MRI technology, which is capable of segmenting lean from adipose tissue, could be used in future studies to confirm the relationship between a large waist circumference and a reduction in calf-muscle CSA.

The overall two-year reduction in muscle CSA indicates that individuals may experience continued muscle atrophy through the chronic stage of SCI. Declines in muscle mass are a component of natural ageing (140,301). It is possible that muscle atrophy after SCI is compounded by age-related reductions. In the older able-bodied population, men lose approximately 1% of their thigh muscle CSA per year, and women lose approximately 0.65% of their thigh muscle CSA per year (150). Age-related reductions in muscle mass may have an increasing impact as individuals live longer with SCI.

Muscle-targeting therapy interventions have shown the capability to increase lower-extremity muscle size following chronic-SCI. For example, ten weeks of electrically stimulated cycling resulted in a 4% increase in lean mass assessed by DXA in persons with complete and incomplete chronic-SCI (45). Six months of electrically stimulated bodyweight-supported treadmill walking improved quadriceps CSA by 15% in participants with complete tetraplegia, whereas manually facilitated bodyweight-supported treadmill walking showed no change (302). After incomplete SCI, bodyweight-supported treadmill walking without electrical stimulation has been observed to improve muscle CSA in observational case series (73,303) and in a randomized controlled trial, four months of electrically stimulated bodyweight-supported treadmill walking better preserved muscle CSA compared to traditional resistance and aerobic training exercise (118,304).

While electrically stimulated cycling and treadmill training have shown to elicit improvements in muscle CSA, the greatest hypertrophy has been observed after resistance training regimes (268,305,306). Twelve weeks of progressive electrically-stimulated knee extensor resistance training, in combination with a healthy diet, increased the muscle CSA of the whole thigh (28%), knee extensor (35%), and flexor (16%) muscle groups (305). Muscle

hypertrophy was associated with improved regional body composition, indices of carbohydrate metabolism, insulin resistance, and lipid profiles (269). Although evidence suggests that resistance training may be the best method to improve lower-extremity muscle size, small homogeneous samples denote that further research is necessary to confirm the external validity and determine the most effective therapy regimes following chronic SCI.

Precision analyses of pQCT-derived muscle CSA values from the watershed analysis technique indicated a least significant change of 0.61 mg/cc or 1.01%, which is similar to the reproducibility values reported Wong et al. (RMS-SD: 0.52cm², RMS-CV 1.4%). Therefore, changes greater than approximately 1.5% should be considered real change. However, real change does not necessarily indicate a clinically significant change. Further research incorporating physiologic and metabolic outcomes (e.g., muscle strength, glucose tolerance, lipid profile, etc.) are needed to determine the threshold for a clinically significant change in pQCT derived calf-muscle CSA that could be used to evaluate the success of therapy interventions. Additionally, the overall reduction and individual variability in muscle CSA highlights the need to re-consider the existence of a homeostatic “steady-state” after chronic-SCI. Our results stress that researchers must account for fluctuations in muscle mass when evaluating therapy efficacy; as it is possible that an intervention successful at preventing muscle loss may be disregarded, or that an intervention may be incorrectly deemed successful when in fact muscle gains were attributable to other physiological factors.

Continued research is needed to identify inherent and modifiable factors associated with muscle CSA losses and gains in the chronic-SCI population. Controlling for factors related to fluctuations in muscle mass may aid in our understanding of metabolic disease risk and improve the assessment of muscle-preserving therapy interventions. In this investigation, we only

examined the association between baseline characteristics and muscle CSA change.

Investigating factors that co-vary with muscle CSA should be the topic of future research.

Identifying stimuli or characteristics that co-vary with muscle gains and reductions may improve our understanding of the mechanisms responsible for the variability in muscle CSA change we observed.

6.4.2 Muscle Density Change

The range of one- and two-year muscle density change scores indicated that the degree of fat infiltration of lower-extremity muscle can fluctuate after chronic-SCI. Twenty one participants exhibited reductions, and nine participants experienced gains in muscle density greater than test-retest precision error. In addition, the rANOVA indicated a statistically significant reduction in muscle density over a one- and two- year period for the entire cohort, and a trend towards a statistically significant reduction over a one- and two-year period for the subsample of participants with incomplete injuries. While mean two-year reductions in muscle density were relatively small (<2 mg/cc), continued reductions of this magnitude extrapolated over a lifespan could have a major impact on metabolic health. Understanding the factors related to a change in muscle quality may help explain, and possibly reduce, the increased prevalence of metabolic disease in the chronic-SCI community.

Participants with complete and incomplete-SCI who experienced the largest reductions in muscle density were between two and four years post- injury. This indicates that the rapid increase in fatty-infiltration previously documented (4,9,11) continues beyond the acute and sub-acute stage, and into the early stage of chronic injury. However, we also observed that muscle density decreased up to 15% in participants greater than ten years post-SCI. These reductions

indicate that the degree of fatty-infiltration of skeletal muscle is not solely dependent on the duration of paralysis.

Many studies have proposed that fatty infiltration of muscle is an unavoidable consequence of normal aging (150,163,307,308). In a group of 1600 older adults between the ages of 70 and 79, a decrease in thigh muscle density was observed over a five-year period after accounting for race, weight changes, health status, and activity levels (309). In another study, Goodpaster et al. observed an 18% gain in thigh-muscle intramuscular adipose tissue over a one year period in a group of men and women between 70 and 89 years of age (310). After SCI, it is possible that fatty-infiltration of muscle does not follow a linear trajectory. For example, there may be an initial increase immediately after injury, followed by a gradual age-related increase that possibly accelerates as individuals reach older adulthood. The effect of aging on muscle quality warrants further investigation, especially as individuals are living longer with SCI.

We observed that a greater bodyweight and waist circumference at baseline were associated with an increase in muscle density over two years. Increased weight and waist circumference may be representative of increased central adipose tissue storage. Overweight individuals may have the greatest capacity to improve fatty-acid oxidation in skeletal muscle, and consequently decrease fatty-infiltration (300). It is possible that “weight-loss” efforts to reduce adipose tissue storage may have resulted in improved metabolic health and consequently increased muscle density. It must be noted that weight and waist circumference were only able to explain 20-25% of the variation in muscle density change, and that the statistical significance of the association between waist circumference and muscle density was lost when outliers were removed. Continued investigation is needed to understand the association between regional body composition and muscle quality.

Precision analyses of pQCT-derived muscle density values from the watershed analysis technique indicated a least significant change of 0.57 mg/cc or 0.95%, which is similar to the reproducibility values reported Wong et al. (RMS-SD: 0.85mg/cc, RMS-CV:1.4%) . Therefore, changes greater than approximately 1.5% should be considered real. However, real change does not necessarily indicate a clinically significant change. Future studies incorporating metabolic outcomes (e.g., glucose tolerance, lipid profile, etc.) are needed to determine the threshold for a clinically significant change in pQCT derived calf-muscle density that could be used to evaluate the success of therapy interventions.

Numerous studies have observed that physical activity is capable of reducing or preventing age or obesity-related increases in fat infiltration of skeletal muscle (310-313). After SCI, electrical stimulation therapy has demonstrated the greatest potential to improve muscle quality(268,306,314). Gorgey et al., observed that electrical stimulation of the quadriceps muscles twice a week for twelve weeks combined with calorie restriction significantly decreased the amount of intramuscular fat in nine individuals with chronic complete-SCI, (269). The decrease in fat infiltration was relatively small (approximately 3%); however, the calorie-restricted control group experienced a 3% increase during this same period of time. Small homogeneous samples are a limitation to the studies investigating electrical stimulation therapy, however the results support previous speculation that increased muscle contraction improves the ability to use inter- and intramuscular fat as fuel, thus decreasing fat deposits within the muscle(315). The best electrical or functional electrical stimulation protocol (e.g., resistance training, cycling, walking, etc.) to maximize fatty-acid oxidation is not known and should be the topic of further investigation.

Our results suggest that a steady-state of inter- and intramuscular fat infiltration cannot be assumed following chronic-SCI. Therefore, considering inherent change in fat infiltration is imperative when muscle quality is used to evaluate the effect of exercise and lifestyle therapies. This is exemplified in the above study by Gorgey et al. (269), in that a 3% reduction in intramuscular fat was observed in the intervention group compared to a 3% increase in intramuscular fat in the control group. If the natural trajectory of muscle is towards increased fatty infiltration, then a therapy that demonstrates no change in fatty-infiltration may be successful in that it prevents a detrimental reduction from occurring.

In summary, high individual variation in the degree of muscle density change was observed in persons with both complete and incomplete paralyses. As muscle status is related to metabolic health, clinicians should recognize that metabolic disease risk may fluctuate over the duration of SCI. We were only able to explain a small proportion of the variability in muscle density change, and therefore continued investigation is needed to better understand the factors associated with fluctuations in muscle quality.

6.5 Limitations

This study provides important information to improve our understanding of muscle atrophy and fatty-infiltration of individuals with chronic-SCI; however, there are limitations to this investigation that must be addressed. First, there is the possibility that pQCT-derived muscle CSA values may be overestimated. Muscle CSA was defined as the soft-tissue area contained within the epimysium, and therefore includes the CSA of adipose and other non-contractile tissue. Studies using MRI technology have demonstrated that not accounting for inter- and intramuscular adipose stores may inflate thigh-muscle CSA values by up to 6% (4). Insufficient resolution to separate muscle from adipose tissue is a limitation of pQCT technology. We opted

to use a manual watershed-guided analysis technique in favour of an automated threshold-based technique in this study as we have observed that an automated method is unable to properly identify muscle in individuals with a high degree of fatty-infiltration. In addition, we observed that manual watershed-guided segmentation of muscle showed greater reproducibility and tighter retesting limits (71). Given the relatively small proportion of adipose tissue compared to lean tissue, watershed-guided segmentation of muscle is considered a valid method to measure muscle atrophy in the SCI population.

Individuals with SCI often experience lower-leg edema and venous pooling (98). It is unclear what effect this fluid shift has on pQCT-derived muscle CSA and density values. An increased fluid content in the lower-leg could overestimate muscle CSA and density. The literature base examining the effect of fluid pooling on muscle is limited. One study observed that the daily variation in CT derived lower-leg muscle volume was no different in one individual with lower-leg edema compared to those without edema (316). In another study, MRI derived muscle CSA increased $6.9 \pm 2.6\%$ after lower-body negative pressure (317); however, how lower-body negative pressure compares to SCI-related pathology is unknown. Until studies specifically isolate the effect of lower-leg fluid accumulation on pQCT outcomes, an effect cannot be ruled out.

The amount of missing data is a possible limitation of this study. Twenty-one percent, 26%, and 34% of participants had missing pQCT data at baseline, year-1 and year-2 time points, respectively. When possible, efforts were made to limit the effect of missing data. For example, scans and demographic data from year-1 or year-2 were used if baseline data was not available for the cross-sectional analysis. With this method, the scans of 65 participants (92%) were included in the cross-sectional analysis; however, some participants had missing independent

variables and therefore were excluded for the analysis. It is possible that missing data may have influenced the significance of independent variables in the regression models. Best-fitting models were selected based R^2 and $C(p)$ statistics with a sample-size equal to that of the independent variable with the least number of observations, and thus the data from all 65 individuals could not be used to select the best-fitting model. The reduced sample-size may have resulted in reduced power to detect a significant association between muscle status and related variables.

Variation in motor function characteristic of individuals with incomplete-SCI may interact with determinants of muscle status. It is possible that the sample of 25 individuals with incomplete-SCI was not large enough to detect significant associations between possible determinants of muscle status or changes in muscle status. For example, to avoid over-fitting regression models, we were limited to including only two independent variables in the multi-variable regression models. It is recommended future studies continue to explore muscle status in those with incomplete injuries by investigating larger samples of this population.

Periods of disuse and immobilization are associated with muscle atrophy. Individuals with SCI can experience events, such as lower-extremity fracture or extended illness that can limit their mobility for extended periods of time. For example, an immobilized lower-extremity or illness that confines a previously ambulatory individual to bed or wheelchair may be associated with reduced muscle status. In this investigation, we did not account for periods of extended immobility that may be related to the muscle status reductions we observed.

There is evidence that chronic inflammation is associated with decreased muscle quantity and quality (265-267,318). In the aged able-bodied population, chronic low-grade inflammation can affect muscle by: increasing the degree of oxidative stress, influencing muscle protein

balance, impeding muscle regeneration and repair, and triggering muscle cell apoptosis (318). Chronic inflammation is prevalent in the SCI population and characterized by increased serum concentrations of C-reactive protein, interleukin-6, and endothelin-1 (319,320). Muscle tissue is responsive to these catabolic cytokines that are unregulated during periods of inflammation, and therefore individuals who experience frequent or chronic periods of inflammation may experience detrimental changes in muscle. It is recommended that future cross-sectional and prospective studies include markers of inflammation to assess the association between chronic-inflammation, muscle status, and metabolic disease.

Nutrition is a key component of muscle size and quality. Although we accounted for serum vitamin D level, we did not account for the effect of other dietary factors on muscle status post-SCI. Inadequate protein intake for example, can be a determinant of muscle mass in other populations (321,322); however, the effect of protein intake on muscle after chronic-SCI is unknown. Secondly, inter- and intramuscular fat deposits can expand during periods of elevated lipid availability, and therefore high fat diets and diets that have excessive calories may contribute to increased fatty-infiltration of muscle (318,323,324). Therefore, future studies should examine the relationship between nutrient intake and muscle status.

We did not include a measure of glucose tolerance or dyslipidemia in this study. Uncontrolled blood sugar is associated with a greater age-related acceleration of muscle loss (325,326). In one study investigating thigh-muscle CSA of older adults, muscle CSA declined two-times faster in older women with diabetes than their non-diabetic counterparts (327). Given the high prevalence of diabetes in the SCI-population, glucose tolerance may be an important correlate of muscle mass, or change in muscle mass in those with SCI.

The greatest diagnostic yield from muscle density measurement may be in highlighting risk-factors related to dyslipidemia, glucose intolerance, and diabetes. Therefore, the most clinically relevant limitation on this study was that we were unable to report the direct association between muscle density and glucose tolerance or dyslipidemia after chronic-SCI. Although previous observational studies have examined the relationship between fatty-infiltration, pQCT-derived muscle density, and metabolic disease in other populations (70,212,216), the direct relationship between muscle density and glucose tolerance has not been reported in the SCI-community. It is possible that factors intrinsic to SCI (e.g., decreased lower-extremity muscle mass) may affect the relationship between muscle density, fatty-infiltration, and glucose tolerance derived from the able-bodied population. Therefore, studies are needed to confirm the validity of the relationship between muscle density and glucose tolerance post-SCI.

6.6 Conclusion

In summary, we found that individuals with motor-complete SCI had a 45% reduction in muscle CSA and a 32% reduction in muscle density relative to their able-bodied peers. Participants with motor-incomplete SCI had a 17% reduction in muscle CSA and a 14% reduction in muscle density relative to controls. With a set of 16 pre-selected variables, we were able to account for 55-66% of muscle CSA variation and 25-33% of muscle density variation in a diverse sample of individuals with chronic-SCI. A reduced height, waist circumference, calf-muscle lower-extremity motor score, wheelchair use, a younger age at injury, reduced spasm frequency and severity, and paraplegia were associated with a decreased muscle CSA. An older age, a decreased motor-score, reduced spasm frequency and severity, more minutes of daily vigorous physical activity, and wheelchair use were associated with a decreased muscle density.

Incorporating factors related to muscle status into metabolic disease risk assessment may increase the ability to identify those most at risk for metabolic disease.

Over a two-year period, we observed that individuals with chronic incomplete-SCI may experience continued reductions in muscle CSA and muscle density. Furthermore, we observed a high degree of individual variation in muscle CSA and density change in those with both complete and incomplete injuries. A greater waist circumference at baseline was associated with a reduction in muscle CSA, and a lower weight and waist circumference at baseline were associated with a reduction in muscle density over two years. The change in muscle status we observed suggests the need to re-define the definition of chronic-SCI when “chronic” implies the existence of a homeostatic “steady-state”. It can no longer be assumed that muscle does not change in the absence of intervention, and as muscle status is related to metabolic health, clinicians should recognize that metabolic disease risk may fluctuate over the duration of SCI. We were only able to explain a small proportion of the variability in muscle change, and therefore continued investigation to determine the mechanisms responsible for increases and decreases in muscle status is warranted.

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APPENDICES

Appendix A

1. Reliability of pQCT-derived Muscle Area and Density Measures on Water-Shed versus Threshold-Based Segmentation Methods
2. Accuracy and Precision Error of Muscle Cross-sectional Area Measured Using Peripheral Quantitative Computed Tomography in Adults
3. Influence of tester and timing on pQCT precision error
4. Bone Quality in Individuals with Chronic Spinal Cord Injury Participant Recruitment Flow Diagram
5. Standard Operating Procedure (SOP): pQCT image acquisition and analysis of 66%-site of the tibia
6. pQCT calibration curve for Statec XCT 2000 at Hamilton Health Sciences, Hamilton, ON

Reliability of pQCT-derived Muscle Area and Density Measures on Water-Shed versus Threshold-Based Segmentation Methods

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Objectives: To compare reliability of calf muscle measures obtained using peripheral quantitative computed tomography (pQCT) and analyzed with threshold-based versus watershed algorithms across a cohort with varying muscle area

Methods: Young adults (<30 years old), older adults (>60 years old) and individuals with spinal cord injury (SCI) were scanned twice on pQCT at the same visit with repositioning between acquisitions. A 2.3±0.5 mm thick slice was obtained from the 66% calf at 500 µm resolution. Images were randomized and blinded to the reader. Threshold-based and manual watershed segmentation of muscle from bone (threshold: 280, contour 1, peel 2) and subcutaneous fat (threshold: 40, contour 3, peel 1) was performed using Stratec V6.0 (Orthometrix) and sliceOmatic V4.3 (Tomovision), respectively. Tissue boundary identification was guided by the watershed tool and manually traced by a single reader. Muscle volumetric density (vMD) and cross-sectional area (MCSA) were computed in each analysis. Root mean square coefficients of variation (RMSCV) and standard deviations (RMSSD), and Bland-Altman limits of agreement (LOA) were determined for vMD and MCSA for both methods. A general linear model determined difference in vMD and MCSA between segmentation methods adjusting for participant subgroup.

Results: Most RMSCV and RMSSD values for threshold segmentation were larger than manual watershed segmentation (Table I) The LOA for vMD obtained using the watershed algorithm were -3.68 to 3.09 (N=85); versus on threshold-based segmentation, -4.75 to 4.75 (N=81). The LOA for MCSA was -132.80 to 137.50 (N=93) and -353.19 to 369.28 (N=81) for watershed versus threshold segmentation, respectively. Manual segmentation (70.2±9.2 mg/cc) provided larger (p<0.001) densities compared to threshold segmentation (67.4±10.3 mg/cc).

Conclusion: Watershed-guided segmentation of muscle from pQCT images showed greater reproducibility and tighter retesting limits. Although manual tracing may be less efficient, its higher reliability is favourable to longitudinal studies demanding greater analytical sensitivity.

Word count (298) + Figure (50) = 98.7% limit (350 words)

Table I. Comparing reliability of pQCT muscle measures obtained by water-shed versus threshold-based segmentation separated by participant subgroups. Young adult (age: 25.6±3.3,BMI:23.9±4.8), older adult (age:74.0±9.2, BMI:25.7±4.0), SCI (age:44.1±9.4,BMI:23.9±3.3). vMD = muscle volumetric density, MCSA = muscle cross-sectional area.

Reliability Data Variable & Method	RMSSD (units)			RMSCV (% error)		
	Young	Older	SCI	Young	Older	SCI
Water-Shed vMD (mg/cc)	0.89	1.43	0.85	1.18	2.01	1.42
Threshold-Based vMD (mg/cc)	1.73	1.22	2.43	2.36	1.77	4.06
Water-Shed MCSA (mm ²)	34.96	53.97	52.34	0.49	0.93	1.38
Threshold-Based MCSA (mm ²)	154.35	105.37	142.10	2.57	1.77	2.94

Accuracy and Precision Error of Muscle Cross-sectional Area Measured Using Peripheral Quantitative Computed Tomography in Adults

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(See PDF for high resolution image)

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Abstract

Purpose: Using muscle area from standard spiral Computed Tomography (CT) as the gold standard this study assessed the accuracy of muscle cross-sectional area derived from peripheral Quantitative Computed Tomography (pQCT) images acquired at 0.4 mm and 0.8 mm voxels. The precision error for muscle area derived at these two voxels was also determined.

Method: Twenty subjects participated in this study. Two pQCT images of the leg were obtained at 66% of the distance from the medial malleolus to the medial condyle of the tibia using a STRATEC XCT2000. Image #1 was acquired with standard scan parameters (voxel=0.8 mm, scan speed=30 mm/sec). For image #2 the voxel and speed were lowered to 0.4 mm and 20 mm/sec. The spiral CT images were acquired on a General Electric scanner with 0.4 mm voxels and 2 mm thick slices. pQCT muscle cross-sectional area was derived using standard analysis parameters. To reduce noise, median filtering was applied to the 0.4 mm voxel image before analysis. Custom software was used to extract muscle area from the spiral CT images. To assess precision, 10 subjects had the 0.8 mm and 0.4 mm voxel pQCT images acquired on three different days within three weeks. Subjects were not repositioned between the scans. Least squares analysis was used to compare pQCT and spiral CT muscle area. Precision error was assessed as percent coefficient of variation.

Results: There was good agreement between pQCT and spiral CT muscle cross-sectional area (R >0.9, p<0.001). As well, good agreement was found between muscle area derived from the 0.8 mm voxel pQCT image and the filtered 0.4 mm voxel image. The precision error for muscle cross-sectional area derived at either voxel setting was small (1-2%).

Conclusion: Muscle cross-sectional area derived from pQCT images is accurate when compared to muscle area from spiral CT. Second, filtering 0.4 mm voxel pQCT images to remove noise does not reduce the accuracy or precision of muscle measurements.

Introduction

pQCT is valuable for assessing the relationship between bone and muscle because it can simultaneously assess the cross-sectional area of bone and muscle in the limbs of adults and children. While assessment of muscle cross-sectional area has been widely used on clinical CT scanners the correlation between muscle area measured on a clinical CT scanner and a dedicated pQCT scanner has not been determined. **This work compares calf muscle cross-sectional area derived from a group of subjects scanned with pQCT and clinical CT.**

Subjects & methodology

Subjects: Comparison of muscle cross-sectional area from pQCT and spiral CT was done in 18 normal adult volunteers: 9 males, 9 females. Reproducibility of pQCT muscle area was assessed in 10 subjects scanned twice with a 2-week time interval between the first and second set of scans. Subjects were scanned at 0.4 mm and 0.8 mm voxel settings without repositioning.

Muscle assessment: Images of the left tibia were obtained at 66% of the distance from the medial malleolus to the medial condyle. Skin was marked with ink to ensure alignment of pQCT and spiral CT scan locations (pQCT and spiral CT done on the same day). Figure 1 details the scan parameters used in the study.

Image analysis: pQCT image data was analyzed using the manufacturer software and settings (Stratec XCT version 5.5). A median filter was applied to the 0.4 mm voxel pQCT images before muscle area segmentation. Spiral CT images were analyzed for muscle cross-sectional area using commercial software (BonAllyse Genie 1.3).

Results

Figures 2 and 3 show the relationship between muscle cross-sectional area derived from the spiral CT scanner and the pQCT scanner operating at 0.4 mm and 0.8 mm voxel settings. The results of a least squares fit to the data is shown on each plot.

Figure 2

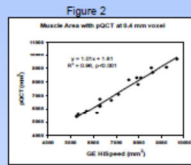
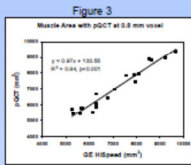


Figure 3



The table below gives the precision of muscle cross-sectional area derived for the pQCT scans acquired at two different voxel settings. The precision error terms (Root Mean Square Standard Deviation, RMSSD; and Coefficient of Variation, CV(%) were calculated according to the method of Gluer et al. (Osteo. Int. 5:262-270, 1995).

	0.4 mm Voxel	0.8 mm Voxel
Overall mean (mm ²) ± SD	7156.8 ± 1112.5	6995.4 ± 1138.5
RMSSD (mm ²)	181.1	208.4
CV(%)	2.5	2.9

Conclusions

Muscle cross-sectional area derived from a pQCT exam of the calf is accurate when compared to muscle area derived from spiral CT. Second, filtering 0.4 mm voxel pQCT images to reduce noise does not reduce the accuracy of muscle area determination. Third, muscle area is determined with precision errors less than 3%. We conclude that muscle cross-sectional area can be determined just as reliably on a pQCT unit as on a clinical CT scanner.

C. Gordon is a consultant for Orthometrix and holds shares in the company

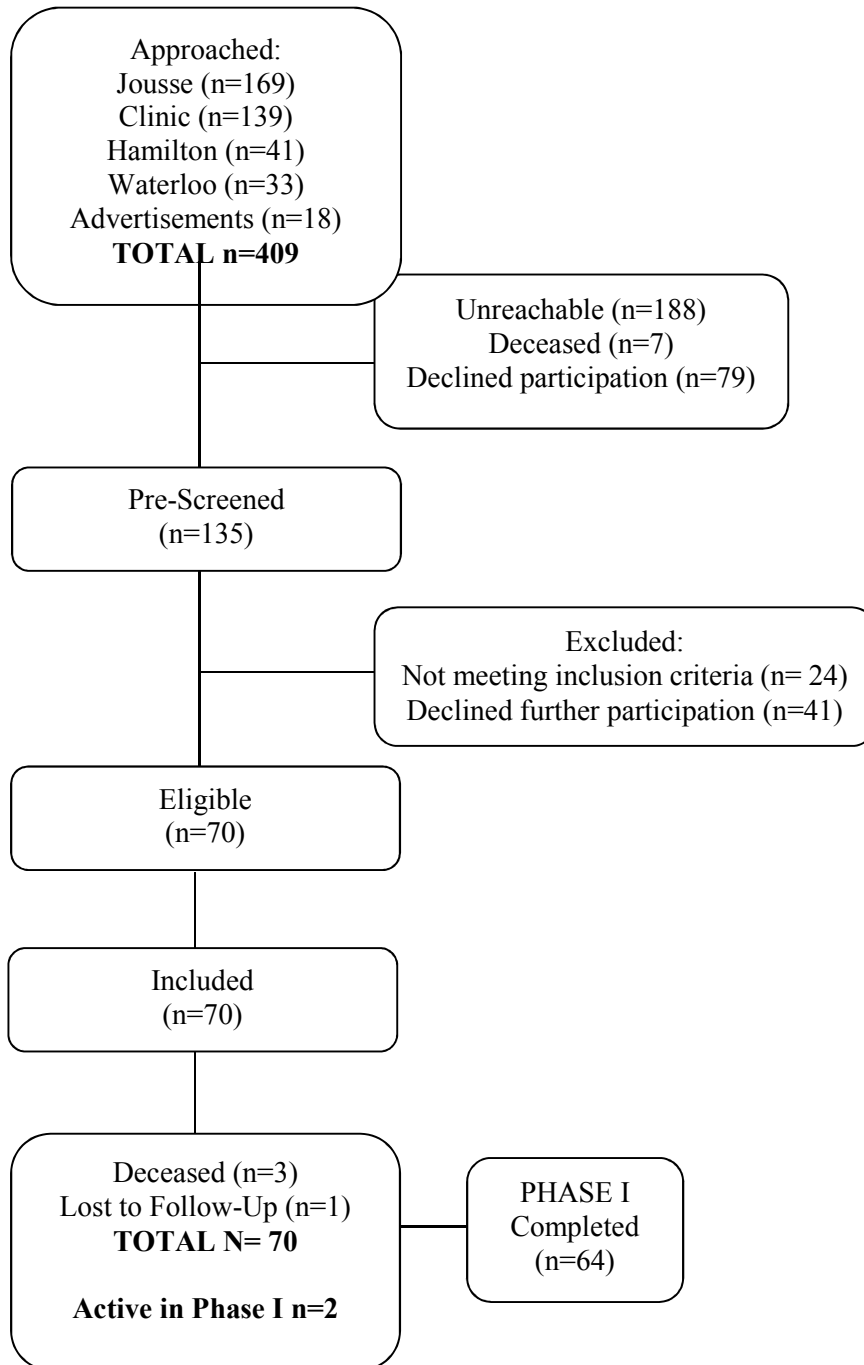
Influence of tester and timing on pQCT precision error (adapted from Swinford et al., 2010)

Data indicate individual absolute precision error measurements (\pm SD)

	Within-Day		Between-day		Two-way ANOVA results*		
	Within-tester	Between-tester	Within-tester	Between-tester	Tester	Timing	Interaction
Absolute muscle CSA (cm ²)	0.53 \pm 0.43†	0.42 \pm 0.31‡#	0.87 \pm 0.57‡	1.21 \pm 0.82†#	-a	-a	<0.05
Relative muscle CSA (%)	0.22 \pm 0.14	0.25 \pm 0.18	0.39 \pm 0.28	0.51 \pm 0.45	NS	<0.001	NS
Muscle Density (mg/cm ³)	0.31 \pm 0.35	0.23 \pm 0.15	0.40 \pm 0.38	0.33 \pm 0.28	NS	NS	NS

* - tester (within- vs. between-tester), timing (within- vs. between-day)
a - Tester and timing main effects were ignored in the presence of a significant tester x timing interaction
Symbols (†, ‡, #) indicate p<0.001 between groups with corresponding symbols, as determined by post-hoc pairwise unpaired t-tests

Bone Quality in Individuals with Chronic Spinal Cord Injury Participant Recruitment Flow Diagram
 (April 16, 2014)



Standard Operating Procedure (SOP): pQCT image acquisition and analysis of 66%-site of the tibia

Scan Acquisition:

Participant set-up

1. Greet participant and escort to pQCT scanner room
2. Remove shoe and roll up pant leg of leg to be scanned
3. Measure tibia length
 - Palpate and measure the distance between the tibiale mediale and sphyrion tibiale
 - Place a mark along the tibia that is 2/3 the distance measured distal to proximal
4. Remove wheelchair foot-plates to allow the participant to wheel as close as possible to the scanner
5. If possible, have participant slide their bum forward in their wheelchair
6. Position the participant's leg into the gantry and rest foot on the foot-plate. Provide extra padding for the foot and knee as needed
7. Tighten clamp around the knee to hold leg stationary

Scanner set-up and scan acquisition

1. Select the appropriate mask for the 66%-site measurement
 - Settings:
 - Voxel size: 0.5mm
 - Slice thickness: 2.2mm
 - Scan speed: 20 mm/second
2. Manually move scanner to the 66% site
3. Initiate scan
4. Check scan quality and repeat if needed

Post-scan

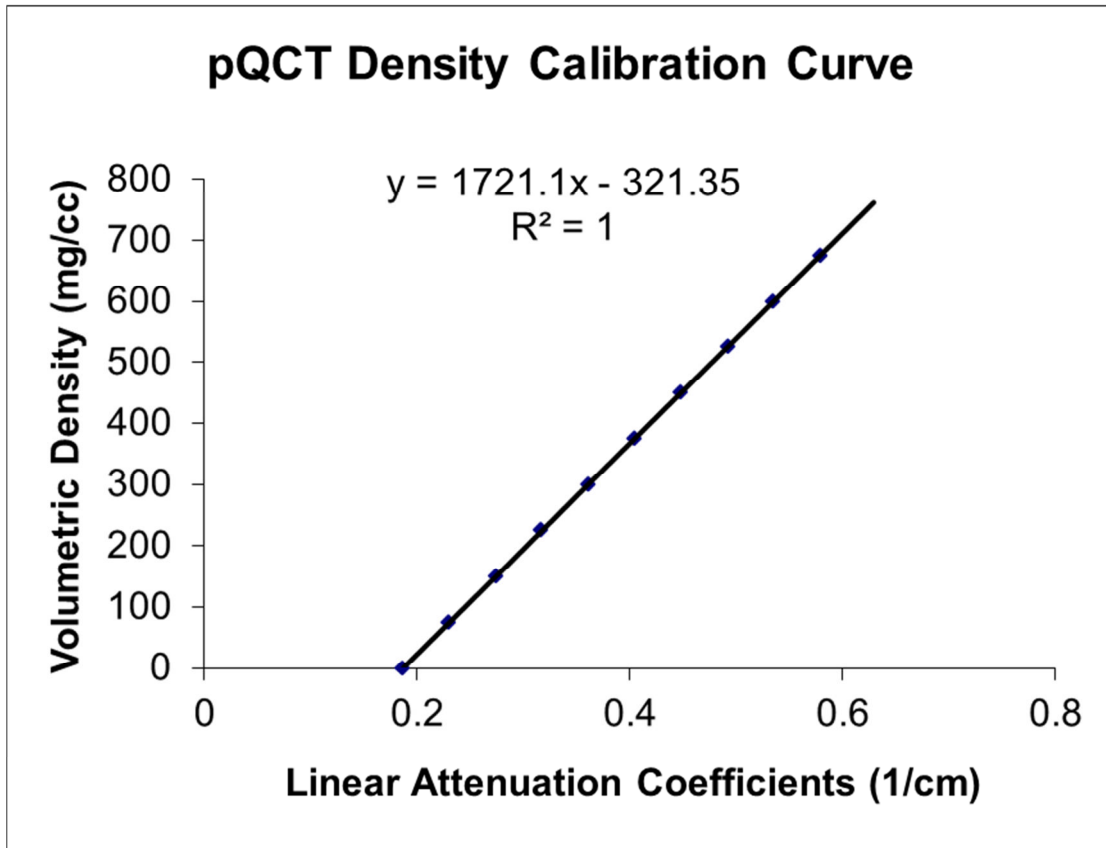
1. Remove participant's leg from gantry
2. Re-assemble wheelchair
3. Assist with putting on shoes and adjusting clothing items

Image analysis:

1. Open SliceOmatic software
2. Load scanner specific calibration file
3. Open pQCT image
4. Perform Gamma correction
5. Select "morpho" mode
6. Select "watershed" analysis
 - Settings:
 - a. Merge level: 1
 - b. Line Thickness: Off
 - c. Hue Slider: Yellow
 - d. Param values: default
7. Tag muscle
8. Enter "edit" mode and manually correct for watershed spillover
9. Tag subcutaneous fat

10. Enter “edit” mode and manually correct for watershed spillover
11. Tag bone
12. Enter “edit” mode and manually correct for watershed spillover
13. Export CSA data to Excel
14. Export density data to Excel

pQCT calibration curve for Statec XCT 2000 at Hamilton Health Sciences, Hamilton, ON



Linear Attenuation coefficient (1/cm)	Density (mg/cm ³)
0.187	0
0.23	75
0.274	150
0.317	225
0.361	300
0.405	375
0.448	450
0.492	525
0.535	600
0.579	675

Appendix B

1. Letter of Invitation
2. Physician Referral Form
3. Telephone Screening Form
4. Information and Consent
5. Past Medical History
6. Concurrent Medications
7. Health Demographics
8. Current Health Status
9. Spasm Frequency and Severity Scale
10. Physical Activity Recall Assessment for People with Spinal Cord Injury
11. pQCT

Primary Investigators:
Dr. Lora Giangregorio
Dr. Catharine B. Craven

Bone quality in individuals with chronic spinal cord injury

Lyndhurst Centre 520 Sutherland Drive Toronto, Ontario M4G3V9

Co-investigators:
Dr. A. Papaioannou
Dr. M. Popovic
Dr. L. Thabane
Dr. N. McCartney
Dr. J.D. Adachi



Medicine
UNIVERSITY OF TORONTO



<Date>

<Address>

RE: Research Study

Dear <Name>:

You are being asked to take part in a research study called “Bone quality in individuals with chronic SCI”. Myself and other researchers at McMaster University, the University of Waterloo, University of Toronto and the Toronto Rehabilitation Institute are conducting the study. The Canadian Institutes of Health Research are funding this study (www.cihr-irsc.gc.ca). The purpose of the study is to examine the bone health of men and women with chronic spinal cord injury.

If you agree to take part in the study, you will be asked to have your bone density measured once a year for 2 years. You will also be asked to report your past and current medical history and medications, followed by a brief examination of your sensation and muscle activity. You will participate in two types of bone density scans in the study; one at Lyndhurst and one at McMaster University. Transportation to McMaster University will be provided. The overall time commitment for the study is 10-15 hours over the 2 year period. This includes three visits to Lyndhurst (2-3 hours each) as well as three visits to McMaster (30 minutes each) and five telephone follow-up calls (30 minutes each). All participants will receive a \$40 honorarium at the 0 (start), 1 year and 2 year time points.

At some point in the next two weeks you will receive a telephone call from a research assistant. The assistant will ask you if you are interested in participating in this study. If you are not interested, you can tell the assistant at this time. If you would prefer not to have the assistant call you at all, please call (416) 597-3422, extension 6301. Leave a message with our research coordinator, Lindsay Robertson, saying that you would prefer not to be contacted. Alternatively, you can also e-mail robertson.lindsie@torontorehab.on.ca.

It is important for us to know if people who participate in the study are very different from people who choose not to participate. If you choose not to participate, the research assistant will ask you if you mind answering a few brief questions, such as your age or

whether you have ever broken a bone before. Your name will not be stored with this information. You can choose not to answer these questions if you wish.

If you decide to participate in the study, all information you provide will be confidential. Your name will not appear on any forms. You can stop participating at any time without having to give a reason. A decision not to volunteer or to withdraw from the study after you have enrolled will not have any impact on the care you receive at Lyndhurst. If you have any questions about the study you can contact Lindsie Robertson at the number listed above or Dr. Cathy Craven at (416) 597-3422 extension 6122.

Your contribution to this research will help us better understand who is at risk for bone loss and broken bones. We eventually want to understand better ways to diagnose and prevent broken bones among people with spinal cord injury. Thank you for your consideration.

Sincerely,

insert physician name here

This study has been reviewed and received ethics clearance through the Office of Research Ethics at the University of Waterloo, the Research Ethics Board at the Toronto Rehabilitation Institute and the Research Ethics Board of Hamilton Health Sciences/McMaster University Faculty of Health Sciences.. If you have any questions regarding your rights as a research participant, you may contact: Dr. Gaetan Tardif, Research Ethics Board at (416) 597-3422 x 3730 or Dr. Susan Sykes University of Waterloo Research Ethics Board at 519-888-4567, x 36005, ssykes@uwaterloo.ca or Office of the Chair of Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board at (905) 521- 2100 x42013.



Lyndhurst Centre
520 Sutherland Drive
Toronto, Ontario M4G 3V9
Tel: 416-597-3422
www.torontorehab.com

Insert patient label here

RESEARCH: Bone Quality in Individuals with Chronic Spinal Cord Injury

Background: A cohort of 80 adult men and women, with traumatic SCI, two years post-injury, will be established. Data collected will include: medical history; bone density (BMD) and body composition; tibia volumetric BMD, bone geometry, muscle area and trabecular structure; and x-ray reports to verify fractures (if any). Data will be collected at 6 month intervals over a 24 month period. This research will form the basis for studies of bone quality and fractures in the SCI population.

Patient has verbally consented to the above personal health information being forwarded to a research team member and being approached with more information about the study

- YES
- NO

If no, is patient agreeable to completing a refusal questionnaire by phone?

- YES
- NO

Please forward to Lindsie Blencowe (x6301, room 206-D)

Thanks!

Date Signature of Physician

Assessors Initials: _____

Bone Quality in Individuals with Chronic Spinal Cord Injury

Participant ID

Telephone Screening Form

Date of Assessment / /

Y Y Y Y M M D D

Gender: M F

	Inclusion Criteria	Yes	No	Comments
1.	Participant is ≥18 years of age	<input type="checkbox"/>	<input type="checkbox"/>	
2.	Participant is able to understand instructions in English.	<input type="checkbox"/>	<input type="checkbox"/>	
3.	“What is the level of your spinal cord injury?” Potential participant has a level of injury at or between C2 and T12	<input type="checkbox"/>	<input type="checkbox"/>	<i>Insert Level of Injury</i> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/>
4.	“What was the cause of your spinal cord injury?” Potential participant has a neurological impairment secondary to a spinal cord injury of sudden onset (<24 hours onset).	<input type="checkbox"/>	<input type="checkbox"/>	<i>Insert Cause of Injury</i>
5.	“When did you have your spinal cord injury?” Potential participant’s spinal cord injury occurred at least 24 months prior to screening.	<input type="checkbox"/>	<input type="checkbox"/>	<i>Date of Injury:</i> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> / <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> / <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> Y Y Y Y M M D D
6.	“Do you know if you have or have had any conditions that might affect your bones, such as cancer or liver disease?” Potential participant has no secondary causes of osteoporosis.	<input type="checkbox"/>	<input type="checkbox"/>	
7. 0	“Are you willing to attend three visits to Lyndhurst and three visits to McMaster University over the course of two years?” Potential participant is willing to attend 3 visits to Lyndhurst & McMaster.	<input type="checkbox"/>	<input type="checkbox"/>	

If potential participant is eligible for the study, arrange for a visit to Lyndhurst to complete information and consent form and first testing visit (if consent is provided).

Primary Investigators:
Dr. Lora Giangregorio
Dr. Catharine B. Craven

Bone quality in individuals with chronic spinal cord injury

Lyndhurst Centre 520 Sutherland Drive Toronto, Ontario M4G3V9

Co-investigators:
Dr. A. Papaioannou
Dr. M. Popovic
Dr. L. Thabane
Dr. N. McCartney
Dr. J.D. Adachi



Medicine

UNIVERSITY OF TORONTO



Participant Information Sheet and Consent Form

Title of Study: Bone Quality in Individuals with Chronic Spinal Cord Injury

Primary Investigators: Dr. Lora Giangregorio and Dr. Catharine B. Craven

Co-investigators: Dr. Papaioannou, Dr. Popovic, Dr. Thabane, Dr. McCartney, and Dr. Adachi

Student Investigators: Kayla Hummel, Deena Lala, and Julia Totosy de Zepetnek, Dept. of Kinesiology, University of Waterloo

Sponsor: Canadian Institutes of Health Research, Ontario Neurotrauma Foundation, and SCI Solutions Network

You are being invited to participate in a research study. To decide whether or not you want to be a part of this research study, you should understand what is involved and the potential risks and benefits. This form gives detailed information about the research study, which will be discussed with you. Once you understand the study, you will be asked to sign the form at the end of this information letter if you wish to participate. If you are not able to sign the form but are able to provide verbal consent, it will be documented by the person obtaining consent. Please take your time to make your decision. Feel free to discuss it with your friends and family, or your family physician.

WHY IS THIS RESEARCH BEING DONE?

Individuals with spinal cord injury (SCI) often experience bone loss. Bone loss can cause a person to be more likely to break a bone in the future. We are conducting this study to examine in more detail the bone loss that occurs after SCI.

WHAT WILL I BE ASKED TO DO IF I DECIDE TO TAKE PART IN THE STUDY?

This study will require 10-15 hours of your time over a 2 year period.

This study is being conducted at multiple sites. You may participate at Lyndhurst Hospital (Toronto) OR Chedoke Hospital (Hamilton) – whichever is most convenient for you.

If you decide to participate in the study, we will ask you to do the following things:

Visit to Lyndhurst or Chedoke

- ♦ Complete a medical history that asks questions about your injury characteristics as well as your past and current medical health, medications and lifestyle. You may be asked to have an ASIA exam, which tests your sense of touch and your sense of movement, if we do not have record of an exam for you. This will take approximately 45 minutes.
- ♦ On your first visit to Lyndhurst, you will be asked to provide a blood sample. Fasting conditions will be required. Participants will be asked to fast for at least 12 hours. For those participants unable to fast, a breakfast of toast and apple juice or orange juice will be allowed and blood will be drawn 4 hours after. The blood sample will be used to measure protein markers of bone metabolism, vitamin D, parathyroid hormone (PTH), and ionized calcium levels in your blood. The blood sample will be drawn by a trained phlebotomist. We will take about two tablespoons of blood by inserting a needle in a vein in your arm.
- ♦ Participate in 1 set of 6 bone density scans. Bone density scans are x-rays that measure how much bone mineral you have in certain bones. Individuals with low amounts of bone mineral may be at increased risk of fracture. The scans will be taken of your hips, above and below your knee, your spine and your whole body. During the scans you will be transferred to a scanning table. If you are not able to transfer yourself, we will use a special lift device. You will not feel anything when the scanner is on. The scanning will take approximately 60 minutes.
- ♦ Complete some questionnaires by phone three days after your visit. The questionnaires will gather information regarding your activity and diet. This telephone call will last approximately 30 minutes.

Visit to McMaster

- ♦ Participate in a second visit at McMaster University Medical Centre for a second type of bone density scan. The scanner is called a peripheral

quantitative computed tomography scanner and also uses x-rays to measure bone density. During this visit, you will be asked to participate in 1 set of 3 scans that measure the shape and structure of your bones. A researcher will take 3 scans, one at your ankle, the second at mid-calf and the third at the widest cross-section of your calf. During the scans the limb being measured will be placed in a positioning device. Please refer to the pictures we have provided. We will conduct the scans while you are seated in a chair or wheelchair. You will not feel anything when the scanner is on. This visit will take 45 minutes.

Yearly Follow-up for 2 years

- ◆ You will be asked to return annually for the next two years to repeat the medical history, bone density scans, and scans at McMaster. You will be called at 6 and 18 months during the two year study to monitor any changes in your health, medication and record if you have had any fractures. You will also be asked to report any broken bones to the study coordinator over the two-year period when they occur. These phone calls will take approximately 30 minutes or less.

If you have severe spasticity: During the scans at McMaster, it may be difficult for the technologist to position you if you have lower body muscle spasms. **Only if you have severe lower body muscle spasms**, you will be asked to take a small dose of Lorazepam (otherwise known as Ativan, dose is 0.5-1.0 mg below the tongue) to prevent spasms while the scan is taking place. **If you do not have severe spasticity, you will not need to take Lorazepam.** Lorazepam is a short acting muscle relaxant that reduces muscle spasms. Many people with SCI have taken Lorazepam early after their injury to help with sleeping while in hospital. Adverse reactions to Lorazepam, when they occur, are usually observed at the beginning of the dose and generally decrease in severity or disappear after 2-3 hours. If you become very drowsy with Lorazepam, you may not remember having the pQCT scan. If needed, the Lorazepam will be prescribed for you by Dr. Craven on the day of your scan. These precautions are taken mainly to reduce the chance of injury in the event that a spasm occurs when your leg is placed in the scanning device. **You do not have to agree to take Lorazepam if you do not wish to do so.** However, we may decide not to try to scan you if the spasticity limits our ability to position you safely. If you have metal implants in both lower legs, have broken your shinbones in the past, or have severe leg spasms and are allergic to Lorazepam, you will not be able to participate in the study. Also, women who may be pregnant or who plan on becoming pregnant

cannot participate. If you are a woman, a urine pregnancy test may be performed to ensure that it is safe for you to participate.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

The risks to participants are small. Bone Density scans involve exposure to small amounts of radiation. The level of exposure associated with the scans proposed in this study is ~30 μSv , which is less than doses received during a computed tomography (CT) scan of the chest (30-60 μSv) or annually from background radiation (2500 μSv). The radiation dose is roughly equal to the dose of radiation received over 3 days by every Canadian from natural sources of radiation in the environment. Repeated exposure to radiation has a cumulative risk over time but the radiation risk from participating in this study considered minimal.

If you are asked to take Lorazepam to reduce your leg spasms during scans in Hamilton, there is a risk of side effects. Amongst a study of 3500 people, the most common side effects were sedation (15.9%), dizziness (6.9%), weakness (4.2%) and unsteadiness walking (3.4%). Less frequent side effects include disorientation, depression, nausea, change in appetite, headache and agitation. Most side effects, if they occur, occur with the first dose of the drug. Lorazepam will only be given to you if necessary. If you need Lorazepam, it will provided to you at no cost. After taking Lorazepam, the study staff will monitor you for an hour or so, to make sure you have not had any side effects. A physician will be available for supervision. You should not drive or perform other tasks that require alertness immediately after taking Lorazepam. Also, you cannot take Lorazepam if you are currently taking the fungal medications ketoconazole (Nizoral or Xolegel) or itraconazole (Sporanox).

Women who may be pregnant or who plan on becoming pregnant cannot participate in the study as there are risks to exposing a fetus or unborn baby to ionizing radiation.

Fasting blood draws can also have side effects and discomforts. Fasting may cause hunger, headache, dizziness and/or weakness. As a result of the blood draw, there is a possibility that you may experience pain, bruising, bleeding or infection at the site of the needle puncture. Blood draws may also temporarily cause headache, nausea and lightheadedness.

HOW MANY PEOPLE WILL BE IN THIS STUDY?

80 individuals with SCI will be recruited to participate.

WHAT ARE THE POSSIBLE BENEFITS OF THE STUDY FOR ME AND/OR SOCIETY?

We cannot promise any personal benefits to you from your participation in the study. If you are interested in learning what your bone density is, we can send your bone density scan results to your physician. The study will help us understand bone loss in individuals with SCI, and determine risk factors related to bone loss in SCI.

CONFIDENTIALITY AND SECURITY OF DATA

Your data will not be shared with anyone except with your consent or as required by law. All personal information will be removed from the data and will be replaced with a number. A list linking the number with your name will be kept in a secure place, separate from your file. The data will be securely stored in a locked office. For the purposes of ensuring the proper monitoring of the research study, it is possible that a member of the Office of Research Ethics at the University of Waterloo, Hamilton Health Sciences Research Ethics Board or Toronto Rehab Research Ethics Board may consult your research data and medical records. However, no records that identify you by name or initials will be allowed to leave the hospital. By signing this consent form, you authorize such access. If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published without your specific consent to the disclosure. However, it is important to note that a copy of your signed consent form and the data that follows may be included in your health record. The data will be retained indefinitely.

CAN PARTICIPATION IN THE STUDY END EARLY?

If you volunteer to be in this study, you may withdraw at any time and this will in no way affect the quality of care you receive at this institution. You have the option of removing your data from the study. You may also refuse to answer any questions you don't want to answer and still remain in the study. The investigator may withdraw you from this research if circumstances arise which make it unsafe for you to continue participating and it is in your best interest to withdraw. You will also be informed in a timely manner of any new

information that arises during the course of the study that may influence your decision to participate.

WILL I BE PAID TO PARTICIPATE IN THIS STUDY?

You will receive a \$40 honorarium to participate in the study. We will provide transportation for the study visits and you are welcome to have someone accompany you on the trip. For those wishing to use their own transportation for travel, we will reimburse the costs of parking and mileage (\$0.50 per kilometer) associated with participating in the study.

WILL THERE BE ANY COSTS?

Your participation in this research project will not involve any additional costs to you or your health care insurer.

WHAT HAPPENS IF I HAVE A RESEARCH-RELATED INJURY?

If you are injured as a direct result of taking part in this study, all necessary medical treatment will be made available to you at no cost. Financial compensation for such things as lost wages, disability or discomfort due to this type of injury is not routinely available. However, if you sign this consent form it does not mean that you waive any legal rights you may have under the law, nor does it mean that you are releasing the investigator(s), institution(s) and/or sponsor(s) from their legal and professional responsibilities.

IF I HAVE ANY QUESTIONS OR PROBLEMS, WHOM CAN I CALL?

If you have any questions about the research now or later, if you wish to withdraw from the study at any time or if you think you have a research-related injury, please contact the research coordinator for the study, Lindsie Robertson at (416) 597-3422 x6301, pager (416) 644-6936 or one of the study investigators below:

Dr. Craven (416)597-3422 x6122

Dr. Lora Giangregorio (519) 888-4567 x36357

Kayla Hummel via e-mail, khummel@uwaterloo.ca

This study has been reviewed and received ethics clearance through the Office of Research Ethics (ORE) at the University of Waterloo, the Research Ethics Board at the Toronto Rehabilitation Institute and the Research Ethics Board of

Hamilton Health Sciences/McMaster University Faculty of Health Sciences. If you have any questions regarding your rights as a research participant, you may contact any/all of the offices listed below:

Office of Research Ethics (ORE) at the University of Waterloo (519) 888-4567 x6005

Dr. Gaetan Tardif - Chair, Toronto Rehab Research Ethics Board (416) 597-3422 x 3730

Office of the Chair of Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board (905) 521- 2100 x42013

IF I DO NOT WANT TO TAKE PART IN THE STUDY

It is important for you to know that you can choose not to participate in the study. Your doctor can do tests to look at your bone density even if you do not participate in this study. Choosing not to participate will in no way affect the regular therapy or health care that you receive.

If do not want to participate, it is important for us to know if there are significant differences between people who choose to participate in our study and people who don't. We ask if you would mind answering 7 brief questions that will be used to determine if the group of people who did not participate are different than those who did. You can also choose not to answer these questions, it is entirely your decision. If you do not want to be in the study but might want to answer the questions, we will review them with you and let you decide. Neither your name or any identifying information will be used with this information.

CONSENT STATEMENT
SIGNATURE OF PARTICIPANT/LEGALLY-AUTHORIZED
REPRESENTATIVE

I have read the preceding information thoroughly. I have had the opportunity to ask questions, and all of my questions have been answered to my satisfaction. I agree to participate in this study. I understand that I will receive a signed copy of this form.

Name of Participant

Signature of Participant

Date

If verbal consent is obtained in lieu of a signature, the person obtaining consent will initial here: _____

Consent form administered and explained in person by:

I confirm that I have explained the nature and purpose of the study to the participant name above. I have answered all questions. I believe the participant has the legal capacity to give informed consent to participate in this research study.

Name and title

Signature

Date

SIGNATURE OF PRINCIPAL INVESTIGATOR:

I have delegated the informed consent discussion to _____

Signature of Principal Investigator

Date

Access to Medical Charts

Title of Study: Bone Quality in Individuals with Chronic Spinal Cord Injury

Primary Investigators: Dr. Lora Giangregorio and Dr. Catharine B. Craven

Co-investigators: Dr. Papaioannou, Dr. Popovic, Dr. Thabane, Dr. McCartney and Dr. Adachi

Student Investigators: Kayla Hummel, Deena Lala, and Julia Totosy de Zepetnek, Dept. of Kinesiology, University of Waterloo

Sponsor: Canadian Institutes of Health Research, Ontario Neurotrauma Foundation, and SCI Solutions Network

We would like to access your medical chart to verify your medical history. We would like to confirm your ASIA classification to see if it has changed, check your surgical and medical history and see any bone density scans you have had. By signing below, you are giving your consent to allow the coordinator of the study and lead investigators to look at your chart. You have the right to choose not to have anyone look at your chart if that is your wish. The information collected from your chart will be used for research purposes only.

Consent to give access to chart at Toronto Rehab:

Name

Signature

Date

Assessors Initials: _____

Bone Quality in Individuals with Chronic Spinal Cord Injury

Participant ID	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Past Medical History
Date of Assessment	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> Y Y Y Y M M D D	Visit 01

Gender: <input type="checkbox"/> Male <input type="checkbox"/> Female	Date of Birth: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> Y Y Y Y M M D D
Date of injury/onset: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> Y Y Y Y M M D D	Time Post Injury: <input type="text"/> <input type="text"/> <input type="text"/> years
Level of Injury (e.g. T12, C06): <input type="text"/> <input type="text"/> <input type="checkbox"/> <input type="checkbox"/> N/A	Cause of injury : _____
ASIA Impairment (A-D): <input type="text"/>	ASIA Total Motor Score: <input type="text"/> <input type="text"/> <input type="text"/> ASIA LEMS: <input type="text"/> <input type="text"/> <input type="text"/> ASIA Sensory Score: <input type="text"/> <input type="text"/> <input type="text"/>

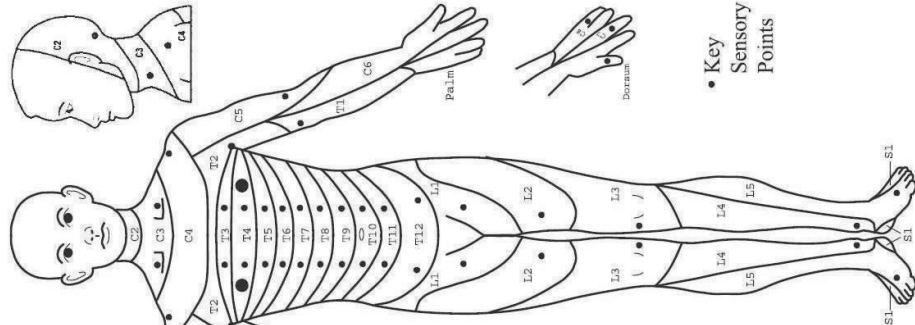
Bone Quality in Individuals with Chronic Spinal Cord Injury

Participant ID

Date of Assessment / /
 Y Y Y Y M M D D

Past Medical History

Visit **01**



ASIA AMERICAN SPINAL INJURY ASSOCIATION
STANDARD NEUROLOGICAL CLASSIFICATION OF SPINAL CORD INJURY
ISCS

KEY SENSORY POINTS

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

KEY MUSCLES
 (score on reverse side)

MOTOR

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

C5 Elbow flexors
 C6 Wrist extensors
 C7 Elbow extensors
 C8 Finger flexors (distal phalanx of middle finger)
 T1 Finger abductors (little finger)

L2 Hip flexors
 L3 Knee extensors
 L4 Ankle dorsiflexors
 L5 Long toe extensors
 S1 Ankle plantar flexors

Voluntary anal contraction (Yes/No)

Comments:

	LIGHT TOUCH		PIN PRICK	
	R	L	R	L
C2				
C3				
C4				
C5				
C6				
C7				
C8				
T1				
T2				
T3				
T4				
T5				
T6				
T7				
T8				
T9				
T10				
T11				
T12				
L1				
L2				
L3				
L4				
L5				
S1				
S2				
S3				
S4-5				

Any anal sensation (Yes/No)

PIN PRICK SCORE (max: 112)

LIGHT TOUCH SCORE (max: 112)

TOTALS (MAXIMUM) (56) (56) = (56)

NEUROLOGICAL LEVEL (The most caudal segment with normal function)

COMPLETE OR INCOMPLETE? COMPLETE INCOMPLETE

ASIA IMPAIRMENT SCALE

ZONE OF PARTIAL PRESERVATION (Caudal extent of partially preserved segments)

SENSORY MOTOR

This form may be copied freely but should not be altered without permission from the American Spinal Injury Association.

Bone Quality in Individuals with Chronic Spinal Cord Injury

Participant ID	<input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/>	Past Medical History
Date of Assessment	<input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> / <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> / <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/>	Visit 01
	Y Y Y Y M M D D	

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.
- Determine ASIA Impairment Scale (AIS) Grade:
Is injury Complete?
 NO → **Is injury motor incomplete?** If NO, AIS=B
 (Yes=voluntary anal contraction OR motor function more than three levels below the motor level on a given side.)
 YES → **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.

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

MUSCLE GRADING

- total paralysis
- palpable or visible contraction
- active movement, full range of motion, gravity eliminated
- active movement, full range of motion, against gravity
- active movement, full range of motion, against gravity and provides some resistance
- active movement, full range of motion, against gravity and provides normal resistance
- 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.

Assessors Initials: _____

Bone Quality in Individuals with Chronic Spinal Cord Injury

Participant ID	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Health Demographics
Date of Assessment	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> Y Y Y Y M M D D	Visit <input type="text"/> <input type="text"/>

HEIGHT: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> cm <input type="checkbox"/> Not Available	WEIGHT: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> kg <input type="checkbox"/> Not Available
WAIST CIRCUMFERENCE: <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> cm <input type="checkbox"/> Not Available (taken at lowest rib)	

FEMALES ONLY:

ARE YOU PRE-MENOPAUSAL, PERI-MENOPAUSAL OR POST-MENOPAUSAL?
If they are unsure, skip to next question.
 PRE PERI POST

If they are pre- or peri-menopausal, or unsure ask: **HOW LONG AGO WAS YOUR LAST PERIOD?** (do not count periods that occurred while taking hormones)
 LESS THAN ONE YEAR 1-3 YRS
 3-10 YRS MORE THAN 10 YEARS

If they are post-menopausal, ask: **WAS YOUR LAST PERIOD GREATER THAN 10 YEARS AGO?**
 NO YES

If NO, ask: **WAS YOUR LAST PERIOD LESS THAN 5 YEARS AGO?**
 NO YES

HAVE YOU EVER HAD A HYSTERECTOMY OR HAD BOTH YOUR OVARIES REMOVED OR RADIATED?
 NO YES: SPECIFY PROCEDURE, AND AGE WHEN PERFORMED

Assessors Initials: _____

Bone Quality in Individuals with Chronic Spinal Cord Injury

Participant ID

Health Demographics

Date of Assessment / /
Y Y Y Y M M D D

Visit

DO YOU CURRENTLY SMOKE? YES NO #/DAY

HAVE YOU EVER BEEN A SMOKER? YES NO

IF **YES TO ABOVE**, PLEASE WRITE DOWN WHEN THEY STARTED AND STOPPED SMOKING (YEAR). ALSO PLEASE INDICATE HOW MANY CIGARETTES PER DAY, ON AVERAGE. IF AMOUNT SMOKED VARIED OVER TIME, PLEASE DESCRIBE.

START STOP #/DAY
Y Y Y Y Y Y Y Y

DO YOU CURRENTLY DRINK ALCOHOL? YES NO #/DAY n/a

BEER (bottles per week)

WINE (glasses per week)

LIQUOR (oz. per week)

DO YOU HAVE A HISTORY OF ALCOHOL CONSUMPTION?

YES NO #YEARS n/a

BEER (bottles per week)

WINE (glasses per week)

LIQUOR (oz. per week)

CAGE

HAVE YOU EVER FELT YOU SHOULD CUT DOWN ON YOUR DRINKING?

YES NO

HAVE PEOPLE ANNOYED YOU BY CRITICISING YOUR DRINKING?

YES NO

HAVE YOUR EVERY FELT BAD OR GUILTY ABOUT YOUR DRINKING?

YES NO

HAVE YOUR EVER HAD A DRINK FIRST THING IN THE MORNING TO STEADY YOUR NERVES OR GET RID OF A HANGOVER (EYE-OPENER)?

YES NO

Assessors Initials: _____

Bone Quality in Individuals with Chronic Spinal Cord Injury

Participant ID	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Health Demographics
Date of Assessment	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> Y Y Y Y M M D D	Visit <input type="text"/> <input type="text"/>

COMPLICATIONS

PLEASE INQUIRE IF THE PARTICIPANT HAS EXPERIENCED ANY OF THESE COMPLICATIONS IN THE PAST 3 MONTHS (CHECK ALL THAT APPLY):

- AUTONOMIC DYSREFLEXIA BLADDER INFECTION
- PAIN DEEP VEIN THROMBOSIS
- PRESSURE SORE CONSTIPATION
- SPASTICITY HETEROTOPIC OSSIFICATION
- HEMORRHOIDS BLADDER/KIDNEY STONES
- INGROWN TOE NAIL DRUG ADDICTION
- GI BLEED NEUROLOGIC DETERIORATION
- LOW BLOOD PRESSURE GYNECOLOGICAL PROBLEMS
- SURGERY
- OTHER (SPECIFY) _____

DETAILS:

Bone Quality in Individuals with Chronic Spinal Cord Injury

Participant ID	<input type="text"/>	Spasm Frequency & Severity Scale
Date of Assessment	<input type="text"/> / <input type="text"/> / <input type="text"/>	Visit <input type="text"/>
	Y Y Y Y M M D D	

SPASM FREQUENCY		
0 = No Spasm 1 = Spasm induced only by stimulation 2 = Infrequent spontaneous spasms occurring less than once per hour 3 = Spontaneous spasms occurring more than once per hour 4 = Spontaneous spasms occurring more than ten times per hour		
Right		Left
<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4	Arm	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4
<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4	Leg	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4
<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4	Trunk	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4
SPASM SEVERITY		
1 = Weak 2 = Moderate 3 = Strong		
Right		Left
<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	Arm	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3
<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	Leg	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3
<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	Trunk	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3

If severe and frequent lower extremity spasticity, complete following page prior to administration of Ativan and pQCT scan

PARA-SCI

Date: YYYY / MM / DD

Intensity: Mild=mild, Mod=moderate, Heavy=heavy, NAA=nothing at all **Duration:** in min **Type:** ADL or LTPA

Be sure to record the date!		Date:		Day: <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3			
		Activity	Intensity	Duration	Type		
Morning routine	Wake up time						
	Transfer						
	Bowel & Bladder Management						
	Bathing						
	Personal Hygiene						
	Dressing	Upper Body					
		Lower Body					
	Other						
Breakfast							
Morning							
Lunch							

PARA-SCI

Date: YYYY / MM / DD

		Activity	Intensity	Min	Type	
Afternoon						
Dinner						
Evening						
Evening routine	Bedtime					
	Transfer					
	Bowel & Bladder Management					
	Bathing					
	Personal Hygiene					
	Dressing	Upper Body				
		Lower Body				
Other						

Bone Quality in Individuals with Chronic Spinal Cord Injury

Participant ID <input style="width: 50px;" type="text"/>	pQCT
Date of Assessment <input style="width: 40px;" type="text"/> / <input style="width: 40px;" type="text"/> / <input style="width: 40px;" type="text"/>	Visit <input style="width: 40px;" type="text"/>
Y Y Y Y M M D D	

pQCT Participant #: <input style="width: 50px;" type="text"/>	Side: Right <input type="checkbox"/> Left <input type="checkbox"/>
Leg Length: <input style="width: 30px;" type="text"/> <input style="width: 30px;" type="text"/> <input style="width: 30px;" type="text"/> mm	Voxel Size: <input style="width: 30px;" type="text"/> <input style="width: 30px;" type="text"/> <input style="width: 30px;" type="text"/>
Name of ROI: _____	
Comments: _____	

4% CT ID: <input style="width: 40px;" type="text"/>	CONTMODE: <input style="width: 40px;" type="text"/>	PEELMODE: <input style="width: 40px;" type="text"/>	
Threshold 1 : <input style="width: 40px;" type="text"/>	Threshold 2 : <input style="width: 40px;" type="text"/>	Threshold 3 : <input style="width: 40px;" type="text"/>	
Total	BMC / 1mm slice: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 30px;" type="text"/> mg/mm	BMD: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 30px;" type="text"/> mg/cm ³	Area: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 30px;" type="text"/> mm ²
Trabecular	BMC / 1mm slice: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 30px;" type="text"/> mg/mm	BMD: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 30px;" type="text"/> mg/cm ³	Area: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 30px;" type="text"/> mm ²
Cortical Thickness: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 30px;" type="text"/> mm	Mean Hole Size: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 30px;" type="text"/> mm	Max. Hole Size: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 30px;" type="text"/> mm	
Connectivity Index: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>		# Nodes: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	

Scans completed by (initials)

Bone Quality in Individuals with Chronic Spinal Cord Injury

Participant ID <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	pQCT
Date of Assessment <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> / <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> / <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	Visit <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>
Y Y Y Y M M D D	

38% CT ID: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	CONTMODE: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	PEELMODE: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	
Threshold 1 : <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	Threshold 2 : <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	Threshold 3 : <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	
Total	BMC / 1mm slice: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mg/mm	BMD: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mg/cm ³	Area: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mm ²
Cortical & Sub-cortical	BMC / 1mm slice: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mg/mm	BMD: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mg/cm ³	Area: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mm ²
Cortical Thickness: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mm		Polar x-sectional MOI: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mm ⁴	
Connectivity Index: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>		# Nodes: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	

66% CT ID: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	CONTMODE: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	PEELMODE: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	
Threshold 1 : <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	Threshold 2 : <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	Threshold 3 : <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	
Total	BMC / 1mm slice: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mg/mm	BMD: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mg/cm ³	Area: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mm ²
Cortical & Sub-cortical	BMC / 1mm slice: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mg/mm	BMD: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mg/cm ³	Area: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mm ²
Cortical Thickness: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mm		Polar x-sectional MOI: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> . <input style="width: 40px;" type="text"/> mm ⁴	
Connectivity Index: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>		# Nodes: <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/> <input style="width: 40px;" type="text"/>	

Scans completed by (initials)