Clinical assessment of body composition after spinal cord injury. An observational study.

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: Persons who sustain a spinal cord injury (SCI) experience a dramatic loss of muscle and bone, and a dramatic increase in adipose tissue. It has been suggested that the muscle atrophy, obesity, and sublesional osteoporosis (SLOP) that occurs after SCI is due in part to the loss of voluntary control of the skeletal muscles in the lower extremities, impaired energy metabolism below the level of the lesion, and cessation of sufficient mechanical strain on bone. The prevalence of obesity and SLOP after SCI leads to increased cardiovascular disease and fracture risk, respectively. Current body composition screening procedures for the general population fail to identify individuals with SCI who are obese or have SLOP.

Muscle contractions provide physiological loads on bone; thereby a muscle-bone relationship is proposed with proportional declines in muscle and bone after SCI. In addition, both positive and negative relationships have been proposed between adipose tissue and bone; increased skeletal load bearing from excess adipose tissue mass may account for the positive associations reported to date. Due to a lack of load bearing activity after SCI, there should be a negative association between adipose tissue and bone.

Objectives: The primary objective is to characterize body composition among adults with chronic SCI using valid, reliable, and interpretable measures, and to suggest screening procedures for the detection of obesity and SLOP in this population. The secondary objectives are to explore the associations between: 1) muscle and bone, and 2) adipose tissue and bone.

Design and Setting: Cross sectional observational.

Population: A sample of 16 individuals (13 men, 3 women) with chronic SCI participated in this study. The neurological level of lesion ranged from C3-T12, with 9 motor complete and 7 incomplete SCI. Average±standard deviation for age was 51.12±12.37 years, and duration of injury 16.5±7.87 years. An additional 29 individuals with chronic SCI were included when exploring the relationship between muscle and bone. Forty-one individuals (31 men, 9 women) were included in this analysis; the neurological level of lesion ranged from C2-T12, with 13 motor complete and 28 incomplete SCI. Average±standard deviation for age was 48.7±13.36 years, and duration of injury 114.22±10.4 years.

Methods: Lean tissue, adipose tissue, and bone tissue were measured via surrogates of body adiposity, as well as two different scanning technologies. Lean tissue was assessed via muscle cross sectional area (CSA) (mm^2) and muscle density (mg/cm^3), and measured using peripheral quantitative computed tomography (pQCT). Adipose tissue was assessed via body mass index (BMI) (kg/m^2), waist circumference (WC) (cm), and % body fat, and measured using a floor scale, tape measure, and dual energy x-ray absorptiometry (DXA), respectively. Bone tissue was assessed via hip, distal femur, and proximal tibia areal bone mineral density (aBMD) (g/cm^2) using DXA, as well as cortical thickness (mm) and total volumetric bone mineral density (vBMD) (mg/cm^3) at the 1/3 proximal tibia, and trabecular vBMD (mg/cm^3) and total vBMD (mg/cm^3) at the distal tibia using pQCT. The relationships between muscle and bone, and adipose tissue and bone, were determined by correlating muscle CSA with indices of bone strength, and indices of obesity with indices of SLOP, respectively.

Results: The majority of participants had lean tissue values below able-bodied norms (67-100%). When using the able-bodied definition of BMI >30 kg/m^2, 19% of individuals were obese, whereas 63% and 81% were obese when using SCI-specific definitions of BMI >25 kg/m^2 or >22 kg/m^2, respectively. One hundred percent of individuals had SLOP using distal femur Z-score, and over 50% were at risk of fracture using distal femur fracture threshold of <0.78 g/cm^2. Weak (r=0.42) to moderate (r=0.57) correlations were found between muscle CSA and indices of bone strength, supporting the theory of a muscle-bone unit. No correlations were found between adipose tissue and bone.

Conclusions: Based on the cohort data, we propose that individuals with ≥2 risk factors (female, ≥60 years of age, duration of injury (DOI) ≥10, tetraplegia, motor complete) should be screened for obesity using % body fat from DXA as well as a combination of carefully interpreted SCI-specific BMI and WC. In addition, these same individuals should be screened for SLOP using a distal femur Z-score and fracture threshold from DXA. It is clear that due to the prevalence of obesity and SLOP in this population, intervention for prevention or treatment is essential. The presence of a muscle-bone unit indicates that muscle atrophy contributes to a reduction in bone strength; this is clinically important, as muscle strength is potentially amenable to rehabilitation intervention. No correlation was found between adipose tissue and bone. Future work should continue to explore these relationships using appropriate technology.

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GLOSSARY OF ACROYNMS

aBMD	Areal Bone Mineral Density
AIS	American Spinal Injury Association Impairment Scale
ApoB	Apolipoprotein B
ASIA	American Spinal Injury Association
BIA	Bioelectric Impedance Analysis
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index (kg/m^2)
CRP	C-Reactive Protein
CSA	Cross-Sectional Area (mm^2)
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
DOI	Duration of Injury
DXA	Dual-energy X-ray Absorptiometry
GDPH	α-Glycerophosphase Dehydrogenase
HDL-c	High-Density Lipoprotein Cholesterol
HU	Hounsfield Units
IL-6	Interleukin-6
IMAT	Intermuscular Adipose Tissue
IMCAT	Intramyocellular Adipose Tissue
ISCD	International Society for Clinical Densitometry
LDL-c	Low-Density-Lipoprotein Cholesterol
MHC	Myosin Heavy Chain
MRI	Magnetic Resonance Imaging
NIH	National Institute of Health
NLI	Neurological Level of Injury
NOF	National Osteoporosis Foundation
PAI-1	Plasminogen Activator Inhibitor
PPAR-γ	Peroxisome Proliferators Activated Receptor-γ
pQCT	Peripheral Quantitative Computed Tomography
PRP	Postural Re-Training Program
SAT	Subcutaneous Adipose Tissue
SCI	Spinal Cord Injury
SDH	Succinic Dehydrogenase
SLOP	Sublesional Osteoporosis
TAFI	Thrombin-Activatiable Fibrinolysis Inhibitor
TNF-α	Tumor-Necrosis Factor-Alpha
VAT	Visceral Adipose Tissue
vBMD	Volumetric Bone Mineral Density
VLDL-c	Very-Low-Density Lipoprotein Cholesterol
WBV	Whole Body Vibration
WC	Waist Circumference
WHO	World Health Organization

PREAMBLE

The present study is an observational investigation of body composition after spinal cord injury (SCI), embedded in two larger cross-sectional studies entitled, "Bone Quality in Individuals with Chronic Spinal Cord Injury" (CIHR-86521) and "Intermittent Whole Body Vibration and Passive Standing for Treatment of Sublesional Osteoporosis after Spinal Cord Injury Pilot Study Phase II: Safety and Efficacy Assessment" (ONF-SCI-2006-WAVE-44). A portion of the data from the two larger studies was obtained and utilized for the present study. In addition, data on bone health among individuals with SCI from several previous studies out of Lyndhurst Center, Toronto Rehabilitation Institute, will be utilized to assess one of the secondary aims: the relationship between muscle and bone. The main focus of this study is to report body composition (lean tissue, adipose tissue, and bone tissue) among a representative sample of adults with chronic SCI (injury for >2 years) including both sexes and diverse levels of impairment. In addition, to identify individuals who are obese, who have sublesional osteoporosis (SLOP), and/or who are at risk of fracture using SCI-specific and able-bodied definitions. Finally, screening procedures for detecting individuals at risk of obesity and SLOP based on the cohort data will be suggested. Secondary aims will explore potential associations between: a) muscle and bone, and b) adipose tissue and bone.

Outcome measures will include: 1) lean tissue body composition by way of muscle density (mg/cm^3) and muscle cross sectional area (CSA) (mm^2) at the 1/3 proximal tibia; 2) adipose tissue body composition by way of body mass index (BMI) (kg/m^2), waist circumference (WC) (cm), and whole body % fat; and 3) bone tissue body composition by way of hip, distal femur, and proximal tibia areal bone mineral density (aBMD) (g/cm^2), cortical thickness (mm) and total volumetric bone mineral density (vBMD) (mg/cm^3) at the 1/3 proximal tibia, and trabecular vBMD (mg/cm^3) and total vBMD (mg/cm^3) at the distal tibia.

The study may benefit the participants by providing them with information regarding their current body composition. The study will benefit the rehabilitation community by furthering our understanding of the body composition changes that occur after SCI, and recognizing the utility of present screening procedures for chronic disease in this population. In addition, the results from this study will broaden our understanding of the associations between muscle and bone, and adipose tissue and bone, after SCI.

1: INTRODUCTION

1.1: Rationale

Substantial muscle atrophy occurs after spinal cord injury (SCI). A decrease in muscle quantity (muscle cross sectional area [CSA]) and a decrease in muscle quality (change in fiber type, change in contractile proteins, decrease in muscle density) both contribute to muscle atrophy associated with SCI. It has been reported that a 45% to 80% reduction in muscle CSA (1, 2) occurs post-injury, which may impact the protective effect muscle contractions have on bone strength (3). A shift towards type II muscle fiber type (4-7) in addition to a shift towards myosin heavy chain (MHC) type II contractile proteins (2, 8, 9) have also been reported post-injury, resulting in highly fatigable muscle that may be difficult to activate for future functional use. The loss of muscle CSA and the decrease in muscle density results in less muscle available for glucose uptake, and therefore insulin resistance and obesity-related complications occur. Thus, muscle atrophy may cause reduced bone strength as well as augmented obesity-related complications after SCI.

In the able-bodied population, obesity is reaching epidemic proportions in both developed and developing countries; over the last 20 years obesity has become the most prevalent nutritional problem in the world (10). Obesity can be defined as an excess of whole body adipose tissue that frequently results in a significant impairment of health. Obesity is a chronic disease itself and creates an internal atherogenic milieu, and is a major risk factor for many subsequent chronic and non-communicable diseases. Obesity is considered to be a dominant factor in the development of cardiovascular disease (CVD), the leading cause of death in the United States and a major cause of disability. In 2004, 36% of all deaths in the United States were due to CVD (11) such as stroke, peripheral vascular disease, and type II diabetes mellitus (DM). In 2008 it was reported that the direct medical care expenditure for type II DM and obesity was more than $150 billion (US) (12). A subpopulation at an increased risk of becoming obese are those with SCI due to the dramatic lean tissue loss and adipose tissue gain post-injury (13, 14). Individuals with SCI may be obese without the physical presence of obesity due to the drastic body composition changes. The percentage of body weight as adipose tissue mass is 8-18% higher in persons with SCI versus age-, height-, and/or weight-matched able-bodied control subjects (15).

The decrease in lean tissue, loss of voluntary control of skeletal muscles, and reduction of weight-bearing activity following SCI results in insufficient mechanical strain on the bones of the lower extremities. Sublesional osteoporosis (SLOP) is a complication that can occur with paralysis; excessive bone resporption paired with little or no bone formation results in dramatic decreases in bone mineral density (BMD) of the hip and knee region following injury. Individuals with SCI experience a 3% to 4% per month decline in areal BMD (aBMD) of the hip and knee region for 12-18 months post-injury (16, 17). This reduction in aBMD results in an increased propensity for lower extremity fragility fracture; fragility fractures develop in 25-46% of persons living with chronic SCI and SLOP (18). There is a reported 2.8 times increased relative risk of fracture for each one standard deviation decrement in aBMD T-score at the femoral neck in men with SCI (19). Fragility fractures result in increased morbidity, increased attendant care and healthcare costs, and in extreme cases lower extremity amputation (20-22).

Several factors affect the severity of muscle atrophy, obesity, and SLOP among individuals with SCI, including sex, age, duration of injury (DOI), neurological level of injury (NLI), and American Spinal Injury Association Impairment Scale (AIS) classification. AIS classification differentiates between an individual with a motor complete injury (AIS A-B) and one with an incomplete injury (AIS C-D). This has important implications for lower extremity body composition and functional ability; much of the literature makes a distinction among individuals with SCI based on the completeness of their injury. In addition to demographic and impairment characteristics, physical inactivity has been suggested as a factor affecting body composition. Studies have reported that individuals who participate in physical activity are less likely to be obese in the able-bodied population (23-26) as well as after SCI (27, 28). Further, studies have reported that physical activity should be advocated for the prevention of osteoporosis, and implemented to reduce the likelihood of falling and its associated morbidity and mortality, in the able-bodied population (29); no published literature shows that physical activity prevents or treats SLOP after SCI.

Mechanostat theory suggests that bone strength is adapted to meet mechanical needs (30). Muscle contractions provide large physiological loads on bone, and therefore a relationship between muscle size and bone strength has been proposed (3). Pronounced muscle atrophy occurs following SCI, and so this muscle-bone relationship may help explain the high incidence

of fracture after SCI. If this relationship exists, it may be important clinically to test muscle size or strength as a predicting factor of osteoporosis and/or fracture risk.

Increasing biological and epidemiological evidence suggests a possible relationship between adipose tissue and bone. Excess adipose tissue may lead to obesity, while diminished bone tissue may lead to osteoporosis; both obesity and osteoporosis are both complex chronic diseases that share a pathophysiologic linkage (31). This may help explain the high incidence of both chronic diseases among individuals with SCI. Exploring the relationship between obesity and osteoporosis may expand our understanding of both chronic diseases independently, as well as the physiological basis of the association between them. In addition, determining a relationship between adipose tissue and bone may be clinically important since adipose tissue mass is a potentially preventable risk factor for fracture after chronic SCI.

1.2: Gaps in the Literature

The literature to date provides a good overview of body composition changes after SCI. However, the measurement sites and measurement techniques used to observe or quantify the changes to lean tissue, adipose tissue, and bone tissue vary across studies, making it difficult to compare or generalize findings. This observational study will characterize body composition among adults with chronic SCI using valid, reliable, and interpretable measures; care has been taken to ensure the validity and reproducibility of novel measures.

The SCI-specific definitions for obesity (13, 32, 33) and SLOP or fracture risk (34, 35) are recent and continue to be open to discussion, and therefore not yet widely accepted. Consequently, limited studies identify individuals with SCI as being obese or having SLOP using tools specific to the SCI population. Current body composition screening procedures for the able-bodied population fail to identify individuals with SCI who are obese or have SLOP; however, some able-bodied definitions may be used among the SCI population if carefully and cautiously interpreted. As mentioned above, the completeness of injury has important implications for lower extremity body composition and functional ability. This observational study will determine the number of individuals with chronic SCI who are obese or have SLOP, taking into account the completeness of injury (motor complete [AIS A-B] vs. incomplete [AIS C-D]), based on SCI-specific and able-bodied definitions. Further, based on the cohort data, this study will suggest screening procedures for detection of obesity and SLOP after SCI.

A positive relationship between lean tissue and BMD has been proposed among the able-bodied population (3, 33, 36) as well as among individuals with SCI (33, 37). The studies among individuals with SCI utilized dual energy x-ray absorptiometry (DXA) technology, which provides a 2-dimensional view of bone and a composite of BMD and bone geometry. Due to the unique patterns of bone loss following SCI, it may be interesting to look at the relationship between lean tissue and different indices of bone strength using a technology that can provide values for trabecular bone and cortical bone separately. Peripheral quantitative computed tomography (pQCT) provides 3-dimentional images that can measure size, shape, and mineral density of bone, and was shown to predict failure load at the radius more accurately than DXA (38, 39). In addition, pQCT allows for the analysis of muscle CSA, which is considered an acceptable surrogate of muscle strength (3, 40). This study will explore the association between muscle and bone among individuals with chronic SCI using pQCT technology.

Both positive (41-43) and negative (44) relationships between adipose tissue mass and BMD have been proposed among the able-bodied population; few studies have looked at the relationship among individuals with SCI. Due to the increase in whole body and regional adipose tissue post-SCI and consequent increase in insulin and estrogen production, both hormones that contribute to increased bone mass, there may be a positive relationship between adipose tissue and BMD (45-51). However, it is recognized that a central contributor to bone strength is the gravitational and mechanical loading effect of weight bearing or ambulation. Due to the decreases in muscle and bone in parallel with increases in adiposity, as well as the lack of weight bearing or ambulation among individuals with SCI, there may be a negative relationship between adipose tissue and BMD. This study will explore the association between adipose tissue and bone among adults with chronic SCI.

The findings from this study will increase our understanding of the body composition changes that take place after SCI, provide preliminary screening suggestions for detection of obesity and SLOP in this population, and improve our understanding of the link between muscle and bone, as well as adipose tissue and bone.

1.3: Research Objectives

1.3.1: Primary Objectives

O1a: To characterize body composition among individuals with chronic SCI using the following outcomes: 1) lean tissue: muscle density (mg/cm^3) and muscle CSA (mm^2) at the 1/3 proximal tibia; 2) adipose tissue: body mass index (BMI) (kg/m^2), waist circumference (WC) (cm), whole body % fat; and 2) bone tissue: hip, distal femur, and proximal tibia aBMD (g/cm^2), cortical thickness (mm) and total volumetric BMD (vBMD) (mg/cm^3) at the 1/3 proximal tibia, and trabecular and total vBMD (mg/cm^3) at the distal tibia.

O1b: To determine the number of individuals with chronic SCI who are above and below able-bodied normative values for muscle CSA and muscle density. In addition, to determine the number of individuals with chronic SCI who are obese, who have SLOP, and/or who are at risk of fracture using SCI-specific and able-bodied definitions.

O1c: To suggest screening procedures for detection of obesity and SLOP based on cohort data describing the prevalence of obesity and SLOP after SCI.

1.3.2: Secondary Objectives

O2: To explore the association between muscle CSA and indices of bone strength (cortical bone CSA, cortical thickness, total BMC, total vBMD; trabecular vBMD) among a representative sample of adults with chronic SCI.

O3: To explore the association between indices of obesity (WC, BMI, % body fat) and indices of SLOP (distal femur aBMD, distal tibia trabecular vBMD) among a representative sample of adults with chronic SCI.

1.4: Research Hypotheses

1.4.1: Primary Hypotheses

H1b: Significant body composition changes occur following SCI. A dramatic reduction in lean tissue and increase in adipose tissue mass (13, 33, 52-54), a significantly higher % body fat compared to BMI-matched controls (52, 54-57), a greater WC (53), and a rapid decline in aBMD

(58-64) and vBMD (65, 66) in the lower extremities have all been reported following SCI. Individuals with motor complete (AIS A-B) SCI experience greater lean tissue losses, adipose tissue gains, and bone tissue losses when compared to persons with incomplete (AIS C-D) SCI (67-71). **It is hypothesized that a high proportion of individuals with SCI will have low muscle CSA, low muscle density, be obese, have SLOP, and be at risk of fracture. More specifically, it is hypothesized that individuals with motor complete (AIS A-B) SCI will have more adverse body composition measures than those with incomplete (AIS C-D) SCI. Further, it is hypothesized that individuals with SCI will not appear to be at risk for obesity when using the able-bodied BMI definition of >30 kg/m^2, but will be characterized as obese when using SCI-specific cutoffs of >22 kg/m^2 or >25 kg/m^2.**

1.4.2: Secondary Hypotheses

H2: Muscle CSA is a factor that may be used to characterize muscle atrophy. Indices of bone strength at the 1/3 proximal tibia and distal tibia may represent SLOP after SCI. **It is hypothesized that there will be a positive correlation between muscle CSA and indices of bone strength (cortical bone CSA, cortical thickness, total BMC, total vBMD, trabecular vBMD) among a representative population of individuals with chronic SCI.**

H3: WC, BMI, and % body fat are all factors that are used to define obesity. The distal femur and proximal tibia are the primary sites of fracture after SCI (22, 72, 73), and the distal femur is a more precise and reliable measure of aBMD than the proximal tibia when using DXA (74). In addition, it has been reported that fracture risk after SCI can be predicted from a fracture threshold at the distal tibia (35). Therefore, these indices of obesity (WC, BMI, % body fat) will be individually correlated with indices of SLOP (aBMD at the distal femur, trabecular vBMD at the distal tibia). It has been reported that excess adipose tissue may have deleterious effects on bone (44, 75-79) when the mechanical loading effect is statistically removed (44). Due to the body composition changes in combination with the lack of weight bearing or ambulation, **it is hypothesized that there will be an inverse relationship between indices of obesity (WC, BMI, % body fat) and indices of SLOP (distal femur aBMD, distal tibia trabecular vBMD) among a representative population of individuals with chronic SCI.**

2: BACKGROUND

2.1: Overview of Background

Individuals with SCI are perhaps at greater risk than any other segment of the population for muscle loss, adipose tissue gain, and bone loss. These dramatic body composition changes as a consequence of the neurological injury and physical inactivity may predispose individuals with SCI to obesity and sublesional osteoporosis (SLOP) post-injury (13, 80). The severity of body composition changes post-SCI is further affected by the completeness of injury (AIS A-B vs. AIS C-D) (67-71).

This chapter defines SCI, describes the body composition changes that take place post-injury, discusses measurement of body composition after SCI, and identifies SCI-specific and able-bodied definitions for obesity, SLOP, and fracture risk. Subsequently, the association between muscle and bone, as well as between adipose tissue and bone, will be explored.

2.2: Spinal Cord Injury

The American Spinal Injury Association (ASIA) defines SCI as "any injury within the neural canal below the level of the foramen magnum up to and including the cauda equina" (81). The spinal cord is situated within the spine, both of which are made up of segmental levels but do not necessarily correspond with one another. An individual can break their spine without sustaining a spinal cord injury, if only the vertebrae are damaged.

Causes of spinal cord damage can be either trauma (vehicular accidents, falls, gun shot wound, etc.) or disease (polio, spinal bifida, etc.). According to the Canadian Paraplegic Association, an estimated 900 Canadians sustain a traumatic SCI each year (82). The estimated total number of persons living with traumatic SCI in Canada is 36,000 (83), resulting in an estimated Canadian SCI prevalence of 1/10,000. Vehicular collision accounts for 35% of these injuries, while falls or industrial accidents account for 21.8% (82). Traumatic SCI predominately affects young adults between the ages 18-47, with a 4:1 ratio of men to women. A SCI is described by the NLI (cervical, thoracic, lumbar) and by the AIS (AIS A-D). The AIS is a clinical impairment scale to grade the severity of neurological loss (Table 1).

Table 1: American Spinal Injury Association Impairment Scale

Letter	Complete/Incomplete	Definition
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	Normal	Motor and sensory function are normal.

A simple means of interpreting the AIS with regards to upper motor neuron lesions is as follows: individuals with AIS A and B injuries (motor complete) with no voluntary movement of the legs are wheelchair bound about their home and community; individuals with AIS C injuries (incomplete) may achieve ambulation in their home, but may use a wheelchair for mobility in the community; and most individuals with AIS D injuries (incomplete) are able to achieve ambulation in their home and community (84).

Diverse sensory, motor, and autonomic impairments result from various levels and severities of injury. Damage or loss of function to the cervical segments of the spinal cord results in tetraplegia, or impaired function in the arms, legs, trunk, and pelvic organs. Damage or loss of function to the thoracic, lumbar, or sacral segments of the spinal cord results in paraplegia, or impaired function in the legs, trunk, and/or pelvic organs. A complete SCI (AIS A) refers to the absence of any neurological function (motor and sensory) below the level of the lesion, including the fourth and fifth sacral segments. An incomplete SCI (AIS B-D) refers to any preservation of motor and sensory function below the level of the lesion, including sacral sparing (85).

Some individuals with SCI can walk to a degree, while others are dependent on wheelchairs or other supportive devices. Further to sensory and motor losses, impotence and various degrees of urinary and fecal incontinence are common; catheters and/or a bowel management program are utilized to address these problems. Other autonomic effects of lesions above T6 may include the inability to regulate blood pressure effectively such as orthostatic hypotension, impaired thermoregulation, inability to sweat below the level of the lesion, and chronic pain. The majority

of individuals with SCI are fairly continent with involuntary urinary or bowel evacuation prevented with medication, catheters, and/or bowel management programs.

2.3: Lean Tissue after Spinal Cord Injury

2.3.1: Lean Tissue Changes after Spinal Cord Injury

Dramatic losses in the quantity and quality of skeletal muscle occur following SCI. A 45-80% muscle loss of the lower extremities has been reported in the acute phase after complete (AIS A) SCI, and a 24-31% lower muscle CSA of the lower extremities was reported in the chronic phase among individuals with incomplete (AIS C-D) SCI when compared to able-bodied controls. Skeletal muscle atrophy has been shown to be related to decreased muscle density, or the accumulation of intramyocellular adipose tissue (IMCAT) among individuals with complete and incomplete SCI (86, 87). The proposed rationales for a decrease in quantity and quality of skeletal muscle mass are multi-factorial, including: 1) psychosocial factors such as depression or isolation (15); 2) prolonged inactivity; and 3) skeletal muscle denervation of the lower extremities.

The decrease in quantity and quality of skeletal muscle mass contributes to the prevalence of both obesity and SLOP. The reduction in muscle can result in decreased metabolic rate and increased adipose tissue storage if energy intake is not adjusted relative to energy expenditure (88). In addition, the predominant peripheral action of insulin and 85% of total glucose uptake occurs in skeletal muscle (89, 90). Therefore, decreased quantity and quality of skeletal muscle mass following SCI has been suggested to be the largest contributor to hyperglycemia, peripheral insulin resistance, and consequently: 1) facilitated glucose oxidation over fatty acid oxidation; 2) stimulated synthesis of very low density lipoprotein cholesterol (VLDL-c) in the liver; and 3) enhanced storage of triglycerides in adipose tissue (91). This atherogenic internal environment contributes to obesity and obesity-related complications. Further, decreased quality of lean tissue by means of increased IMCAT or decreased muscle density has been reported to be an independent risk factor for obesity-related diseases such as type II diabetes mellitus (DM) (92).

The decrease in both quantity and quality of lean tissue that occurs following SCI results in a decrease or cessation of physiological loading on bone. Based on the mechanostat theory, this results in decreased bone strength and osteoporosis (3).

Table 2 summarizes some of the current literature reviewing muscular changes following SCI.

Table 2: Changes in Skeletal Muscle Following Spinal Cord Injury

Study	Population: N(M/F); type	DOI	Methods	Findings
Scelsi et al., 1982 (93)	22 (M), complete para	1-17mo.	Biopsy of RF	• 1-4mo: ↓ type II muscle fiber diameter • 4-9mo: ↓ type I and type II muscle fiber diameter • 10-17mo: ↓ type I muscle fiber no., ↑ type II muscle fiber no.
Lotta et al., 1991 (8)	10 (M), age 16-54yrs, complete para C5-T1	1-10mo.	Biopsy of G, S	• 1-6mo: ↓ type IIa muscle fiber diameter • 8-10mo: ↓ type I and relative↑ type IIb muscle fiber no.; ↑ type II MHC
Martin et al., 1992 (5)	5 (3M, 2F), age btw 22-38yrs, complete C6-T4	2-11yrs.	Biopsy of TA	• ↓ CSA • ↓ type I muscle fiber no. • ↓ SDH (48-67%)
Round et al., 1993 (6)	7 (M), age 24-47yrs, para	11mo-9yrs.	Biopsy of VL	• 5 participants showed marked or predominance of type II muscle fiber • 2 participants with shortest DOI showed preserved type I muscle fiber
Burnham et al., 1997 (9)	12 (8M, 4F), mean age 22.4yrs, C6-T8	0.5 to 219mo.	Biopsy of VL	• ↑ type II MHC btw 4-6wks • predominance of type II MHC and stable at ~70mo.
Gerrits et al., 1999 (94)	7 (6M, 1F), age btw 22-46yrs, AIS class A-C, C5-T5	1-21yrs	Isometric quad contractions via ES	• ↑ type II muscle fiber characteristics (faster rates contraction and relaxation; ↑ fatigability) • ↓ force-generating capacity
Castro et al., 1999 (2)	15 (13M, 2F), age btw 18-45yrs, complete C6-L1 (median injury T1)	>6mo.	Biopsy of VL	• ↓ CSA (-33%); • ↓ type I, IIa, IIax+IIx muscle fiber diameter (-27-56%) • ↓ type IIa muscle fiber no. • ↑ type IIax+IIx muscle fiber no. • ↑SDH & GPDH
Castro et al., 1999 (1)	14 (12M, 2F), AIS class A (C6-T10) (median injury T4); 10 para, 4 tetra	>6mo.	MRI of leg and thigh	• ↓ avg CSA (-45-80%) • ↓ avg CSA of G (-24%), S (-12%); QF (-16%), hamstrings (-14%); adductor (-16%)
Talmadge et al., 2002 (95)	6 (M), age 18-45yrs, complete, AIS class A, C6-T10; 5 para, 1 tetra	>6mo.	Biopsy of VL	• Muscle fibers mismatched for SERCA and MHC • ↑ type IIx MHC (all SERCA1) • high proportion of type I MHC and type IIa with both SERCA1 and SERCA2
Ditor et al., 2004 (96)	6 (5M, 1F), avg age 32yrs, complete para T4-	1-19yrs.	Biopsy of VL and AD	• ↓ [Na+K+-ATPase] in VL compared to AD (-66%) • neg. correlation btw [Na+K+-ATPase] and DOI

	T10, AIS class A			• higher proportion of type I muscle fiber than expected
Modlesky et al., 2004 (56)	8(M), complete C6-L1	>2yrs	MRI and DXA of thigh	• ↓ muscle mass and FFST • ↓ muscle mass *in* FFST (-15%) • ↑ %fat

DOI = duration of injury, ASIA = American Spinal Injury Association Impairment Scale, CSA = cross sectional area, ES = electrical stimulation, para = paraplegic, tetra = tetraplegic, no. = number

MRI = magnetic resonance imaging, DXA = dual energy x-ray absorptiometry, FFST = fat free soft tissue

VL = vastus lateralis, G = gastrocnemius, S = soleus, TA = tibialis anterior, QF = quadriceps femoris, AD = anterior deltoid

SDH = succinic dehydrogenase, GPDH = alpha-glycerophosphate dehydrogenase, PFK = phosphofructokinase, MHC = myosin heavy chain

SERCA = sarco(endo) plasmic reticulum calcium-adenosine triphosphatase, [Na+K+-ATPase] = Sodium, potassium-adenosine triphosphatase concentration

Following motor complete acute SCI (~6 months post-injury), a rapid and drastic decline in the quantity and quality of the denervated lower extremity musculature occurs. Muscle cross sectional area (CSA), muscle fiber type, muscle density, contractile proteins, and metabolic enzyme levels are affected following acute SCI. As mentioned above, one study reported a 45% to 80% decrease in quadriceps muscle CSA within the first 6 months post-SCI (1). Another study reported a ~60% reduction of muscle CSA within the first 6 weeks (2). The differences in reported muscle atrophy between studies could be attributed to varied methodology of muscle biopsy (histochemical) (2) vs. imaging (metabolic) (1). While most studies do not show a histochemical muscle fiber type transfer from type I to type II within the first 6 months of SCI, a transformation within type II muscle fibers from type IIa to type IIax+IIx has been observed (2). Interestingly, atrophy of primarily type II muscle fiber within the first 4 months post-injury, atrophy in both type I and type II between 4 and 9 months post-injury, and atrophy of primarily type I muscle fiber >12 months post-injury has been observed (8, 93). Regarding muscle quality, individuals with incomplete SCI were reported to have greater IMCAT accumulation (decreased muscle density) when compared to able-bodied controls six weeks post-injury (86). In addition to the dramatic decline in muscle CSA and muscle density, there are changes in the contractile capacity of muscle in the acute state of SCI.

Several studies have observed a shift within 6 months post-injury towards type II myosin heavy chain (MHC) (2, 8, 9). Myosin is the molecular motor of skeletal muscle, and is comprised of two MHC; the faster type II MHC is central to speed, energy demand, and efficacy of contraction. The transformation to faster MHC suggests that the muscle would have faster contractile speed and become highly fatigable. Ultimately, the increase in faster fibers impacts the ability to activate the paralyzed muscle or participate in endurance activity, which affects future functional use of the muscle. Finally, a change in metabolic enzyme levels, most notably succinic dehydrogenase (SDH; a marker of aerobic-oxidative capacity) and α-glycerophosphase dehydrogenase (GPDH) (2, 97) occurs following acute SCI. Observations of muscle quantity and quality suggest that acute motor complete SCI results in rapid and significant decreases in muscle CSA and a loss of MHC contractile protein (1, 2).

In the chronic stages of SCI (>2 year post-injury), muscle CSA continues to deteriorate but at a slower rate (9). One study compared lower extremity muscle CSA using MRI among 17 individuals with incomplete SCI 13 years post-injury to 17 age-, weight-, sex-, and height-

matched controls, and found that the individuals with SCI had 24%-31% lower muscle CSA than controls. The muscle CSA differences were highest in the thigh muscles (~31% in the quadriceps femoris) compared with the lower leg muscles (~25% in the tibialis anterior) (98). While contractile proteins and metabolic enzyme levels continue to change, the predominant skeletal muscle modifications that occur late after injury are muscle fiber type shifts from type I to type II muscle fiber (4-7). Several studies have reported an almost complete absence of type I muscle fibers 2-11 years post-injury (5-7, 99). Just as with the shift towards faster MHC type II, the shift towards faster muscle fibers (type II) may affect the person's ability to activate the paralyzed muscle or participate in endurance activity, which in turn may reduce their functional abilities. In contrast to the studies showing a shift towards type II muscle fibers, one study looking at histochemical muscle fiber type change among 22 paraplegic SCI participants 1-17 months post-injury observed type I muscle fiber atrophy, but with a marked decrease in the relative percentage of type II muscle fibers (93). Perhaps a shift towards type I muscle fiber would be observed following a longer duration of injury. A higher proportion of type I muscle fibers has been reported (96), however the participants in this study experienced considerable muscle spasticity which may have facilitated the preservation of type I muscle fibers and explain the contrary findings (100).

The NLI and AIS have implications on the severity of skeletal muscle loss and related complications (reduced metabolic rate and energy expenditure, impaired muscle function, obesity, type II DM, etc.). Individuals with complete SCI (AIS A) have reduced energy expenditure when compared with controls, and individuals with a higher NLI (i.e. tetraplegia) have reduced basal metabolic rates as well as significantly lower total daily energy expenditure when compared to individuals with a lower NLI (i.e. paraplegic) (101, 102). In addition, individuals with complete tetraplegia are more susceptible to obesity-related diseases such as type II DM; a recent study reported that 73% of participants with complete tetraplegia had type II DM compared to 24-44% of those with incomplete lesions or with paraplegia (103).

Age and sex are further factors affecting muscle quantity and quality; as individuals age, lean mass decreases (sarcopenia) (104) and IMCAT increases (105). Therefore, older individuals have decreased muscle quantity and quality. Among individuals with SCI, advancing age has been associated with a lower percent lean mass (33). In addition, women with SCI tend to have a lower lean mass than men (106).

In summary, the dramatic decline in skeletal muscle quantity and quality following SCI contributes to or helps precipitate insulin resistance and reduces mechanical strain on bone, ultimately contributing to further complications including obesity and SLOP.

2.3.2: Measures of Lean Tissue after Spinal Cord Injury

2.3.2.1: Muscle Cross Sectional Area

Since the dramatic reduction in muscle CSA that occurs post-SCI may have implications for the development of both obesity and SLOP (3, 88), it may be useful to include a measure of muscle CSA when assessing body composition. Muscle CSA can be measured using pQCT. To analyze a pQCT scan for muscle CSA, various thresholds are used to separate the muscle/bone/skin pixels from adipose tissue pixels, to determine the pixels belonging to bone, and finally to determine pixels belonging to skin. The bone and skin areas are then subtracted from the muscle/bone/skin area to get total muscle CSA (mm^2). In an unpublished study, reproducibility of pQCT muscle CSA at the 1/3 proximal tibia was assessed in 10 able-bodied participants scanned twice with a 2-week time interval between the first and second set of scans. The muscle area was determined with precision errors less than 3%[1]. This same study compared muscle CSA derived from a pQCT scan to muscle area derived from clinically used spiral CT among 18 able-bodied adults (9 men, 9 women), and reported that pQCT is just as reliable as a clinical CT scanner when determining muscle CSA.

2.3.2.2: Muscle Density

A decline in muscle density may contribute to the development of both obesity and SLOP after SCI (92, 107, 108). Therefore, including a measure of muscle density may be a valuable addition to the assessment of body composition post-SCI. Muscle density reflects the lipid content of skeletal muscle, and so a lower muscle density is associated with greater adipose tissue infiltration in skeletal muscle. Several studies have reported adipose tissue infiltration in skeletal muscle by measuring muscle density as a surrogate (109, 110). Muscle density is expressed in mg/cm^3 and has been shown to be a valid measure of IMCAT (111). Although one recent unpublished abstract reported that muscle density measured with pQCT Stratec XCT 2000

[1] CL Gordon, CE Webber, LF Beaumont. Accuracy and Precision Error of Muscle Cross-sectional Area Measured Using Peripheral Quantitative Computed Tomography in Adults. Abstract.

software from a pQCT scan is the most variable soft tissue to assess due to the difficulty in obtaining accurate segmentations and its physical nature within the muscle[2], some studies have published muscle density data via pQCT scan analyses among able-bodied persons (112). By obtaining a single calf-muscle slice using the present pQCT technology, a relatively small depot of skeletal muscle adipose tissue is obtained in comparison to other studies analyzing IMCAT via CT measures of the mid-thigh (111, 113, 114). However, a recent study has shown that CT muscle density of the mid-thigh is significantly correlated with muscle density of the calf (115).

The combination of a measure of muscle CSA and muscle density may provide a clinically meaningful approach of determining lean tissue body composition among individuals with SCI.

2.3.3: Normative Values for Lean Tissue Measures after Spinal Cord Injury

In this study, we have chosen muscle CSA and muscle density as measures of lean tissue among persons with SCI because of the dramatic loss of muscle quality and quantity documented to occur after SCI.

2.3.3.1: Muscle Cross Sectional Area

The unpublished study mentioned above that compared muscle CSA from pQCT to muscle area from a clinically used spiral CT reported an overall mean ± SD of muscle CSA among the 18 participants (9 men, 9 women) to be 7156.8 ± 1112.5 mm^2, using a voxel size of 0.4mm. Data from our lab using pQCT to assess muscle CSA at the 1/3 proximal tibia among 12 able-bodied persons of Caucasian descent (3 men, 9 women), average age 25.5±2.54, reported an overall mean ± SD of 7019.6±1331 mm^2. Female specific muscle CSA was 6918.4±933.53 mm^2, and male specific muscle CSA was 7323.0±2464.46 mm^2. There are a few limitations to using this data as normative values for the present study such as: it was a convenience sample and therefore not likely representative, the participants were not carefully screened for health issues that may compromise muscle, and it was a small sample of young participants (all under 30 years of age). However, it may be useful as preliminary normative data among a Caucasian able-bodied population, to compare with the muscle CSA from the SCI population in the present study. To

[2] F Caronzo, D Inglis, KA Beattie, C Gordon, JD Adachi. MRI vs. pQCT Imaging: Comparing the Variability Between Various Segmented Soft Tissue Areas. Abstract.

the author's knowledge, no published values of muscle CSA at the 1/3 proximal tibia using pQCT among individuals with SCI exist.

2.3.3.2: Muscle Density

One recent study looked at muscle density using pQCT among 471 individuals belonging to eight large multigenerational families of African ancestry. They reported a lower skeletal muscle density in women (72.4 mg/cm^3) than men (75.2 mg/cm^3) indicating a greater skeletal muscle adipose tissue infiltration among the female participants. In addition, they reported an age-effect on muscle density such that a 10% and 12% difference in muscle density among men and women, respectively, were found between the youngest group (18-29 years) and the oldest group (≥60 years) (110). Earlier studies reported similar findings of a lower skeletal muscle density among elderly Caucasian and African American women compared to men (116, 117), and lower skeletal muscle density in the elderly compared to the young (118). Studies have also shown a BMI-effect on muscle density such that a one-unit increase in BMI is associated with 1-5% decrease in muscle density (110). Although there is some literature looking at muscle density among populations of certain disease states (119-122), published muscle density values among individuals with SCI do not exist.

2.4: Adipose Tissue after Spinal Cord Injury

2.4.1: Adipose Tissue Changes after Spinal Cord Injury

Although it is well documented that an increase in adipose tissue mass occurs after SCI (13, 33), the determination of true adipose tissue gain following SCI is restricted as it is difficult to: 1) obtain baseline measurements within the first 2-4 weeks post-injury, and therefore true lean tissue losses and adipose tissue gains within the first year remain largely unmeasured; and 2) differentiate between an adipose tissue gain owing to paralysis rather than genetics or environmental factors (123). However, a study carried out among 8 pairs of male monozygotic twins showed a significant difference in total body adipose tissue mass and percent adipose tissue per unit BMI among the SCI twin compared to their able-bodied twin (37), suggesting a direct relationship between SCI and adipose tissue gain. Obesity is present in more than two thirds of those with SCI (13, 14), with adipose tissue mass reported as 8-18% higher among those with SCI when compared to able-bodied controls (15). Several studies have reported

significantly higher percent adipose tissue mass among adults with SCI when compared to BMI-matched controls using DXA (54-57) or deuterium dilution (52). Given that obesity can exist in the absence of weight gain or the physical appearance of obesity among individuals with SCI, there is a frequent failure to recognize obesity among the SCI population. This lack of recognition is due to the decrease in lean tissue mass and the increase in adipose tissue mass that occurs post-injury (13, 33, 52-54).

The increase in adipose tissue post-SCI can be attributed to the additive effects of poor nutritional habits and a reduction in energy expenditure (52, 101, 124, 125). The reduction of energy expenditure is due to several factors: 1) prolonged physical inactivity; 2) reduced basal metabolic rate due to loss of metabolically active skeletal muscle (13); and 3) impaired energy metabolism below the level of the lesion (124). Physical activity has been shown to suppress obesity among the able-bodied population (24-26), as well as after SCI (126-128). However, the physiological (diminished work capacity, neurogenic conditions, impaired thermoregulation, autonomic dysreflexia, impaired ventilatory capacity, spasticity, etc.), psychological (lack of motivation, interest, depression), and physical (cost or location of physical activity, accessibility of facility, knowledgeable instructors, etc.) barriers unfortunately result in low participation in physical activity among a large proportion of individuals with SCI.

Impairment characteristics are associated with energy expenditure, and consequently affect body adiposity. Twelve to 54% lower basal energy expenditure than controls, depending on NLI and AIS, have been reported due to the relative loss of metabolically active muscle tissue (88, 101, 102). With regards to the NLI, a person with a higher level of injury will experience greater reductions in energy metabolism, consequently increasing adipose tissue gain. For example, individuals with tetraplegia exhibit lower levels of serum high-density lipoprotein cholesterol (HDL-c) (signifying increased adiposity) when compared to those with paraplegia (67, 69, 103). With regards to the AIS, a greater decrease in energy expenditure is seen among individuals with motor complete SCI (AIS A-B) when compared to individuals with incomplete SCI (AIS C-D) because muscle activity is further limited in motor complete SCI. For example, individuals with motor complete injuries have lower levels of serum HDL-c when compared to those with motor incomplete injuries (67-69). With regards to DOI, during the acute stage of an SCI individuals tend to lose weight due to major trauma resulting in hypermetabolism and hypercatabolism (125,

129, 130). Following the acute phase and continuing into the chronic phase, decreased energy expenditure results in adipose tissue gain.

The distribution of adipose tissue has implications for the severity of obesity and frequency of obesity-related diseases (131-133). The risks associated with excess adiposity may in fact be more a function of where the adipose tissue is distributed rather than of the total amount of adipose tissue. Adipose tissue can be stored in several body compartments: directly beneath the skin (subcutaneous, SAT), within the abdomen bound by the parietal peritoneum (visceral, VAT), or within the muscle (inter-muscular adiposity [IMAT] directly beneath the *fascia lata*; and intramyocellular adipose tissue [IMCAT] within the muscle itself (120, 122, 134)). Both VAT and SAT contribute to abdominal obesity; however, excess VAT is more strongly associated with obesity-related disorders such as insulin resistance than other adipose tissue compartments in the able-bodied population (114, 135-139). Recent studies have shown both IMAT (92, 114, 140) and IMCAT (141-143) to be strongly correlated with complications of obesity, in particular insulin resistance. A strong relation between increased IMCAT and insulin resistance was found to be independent from central and overall adiposity among able-bodied persons (141, 144, 145). In addition, the reduction of lean tissue associated with an increase in IMCAT decreases the capacity for glucose uptake. One study reported that IMCAT may be a contributing factor to impaired glucose tolerance and type II DM after SCI (87).

Race (146-149), age (150, 151), sex (152), as well as SCI (13, 53) are all known to affect adipose tissue and adipose tissue distribution. To elucidate, obesity disproportionately affects populations of African origin (148, 149). As individuals age, adipose tissue mass accrues, and therefore older individuals have a higher percentage adipose tissue mass in the absence of weight gain (153). For a given BMI, men are reported to have more lean mass, and women to have higher adipose tissue mass. In addition, men have been found to have more VAT, while women carry more SAT (152).

Individuals with SCI have a unique distribution of adipose tissue (13, 53), including increased waist circumference and increased VAT (53), as well as increased IMAT (87) when compared to able-bodied controls. A recent study reported individuals with SCI to have 58% greater mean VAT than matched able-bodied controls after differences in weight were accounted for (53). The effect of excess adipose tissue, particularly VAT and IMCAT, on the internal environment directly contributes to an atherosclerotic milieu and an increased risk of obesity-

related disorders. Understanding the distribution of adipose tissue may therefore contribute to a more insightful and comprehensive assessment of body composition among persons with SCI.

2.4.1.1: Adipose Tissue and the Internal Environment

Adipose tissue is an active endocrine organ. Excess adipose tissue has recently been associated with pro-thrombosis, a state in which there is a risk of inappropriate blood coagulation. Adipose tissue directly secretes plasminogen activator inhibitor (PAI-1), and circulating lipids stimulate hepatic secretion of thrombin-activatable fibrinolysis inhibitor (TAFI). Both are pro-thrombotic agents that inhibit fibrinolysis, and both are directly associated with adipose tissue mass (154-156). Consequently, an increase in adiposity increases the risk of a pro-thrombotic state.

Adipose tissue also produces and secretes hormones including leptin, adiponectin, and resistin. These adipokines are not only associated with adipose tissue mass, predominantly VAT, but also interact with other tissues and cells in the body [including bone cells]. A disruption in the secretion, function, and balance of adipokines occurs in the course of obesity resulting in changes in metabolic processes and accelerated atherosclerosis. Research looking at fasting levels of adipokines among individuals with SCI is limited; 3 studies have reported higher levels of serum leptin among men with SCI when compared to able-bodied controls (157-159), and one study showed a tendency for higher levels of serum adiponectin among men with SCI (157).

Abdominal adipose tissue, in particular VAT, is an independent predictor of obesity-related diseases such as CVD (160, 161). In both men and women, VAT deposits of >130 cm^2 are associated with disturbances of glucose-insulin homeostasis as well as pro-atherogenic changes in the plasma lipoprotein-lipid profile (162). VAT secretes large amounts of circulating pro-inflammatory cytokines including interleukin-6 (IL-6) and tumor-necrosis factor-alpha (TNF-α) (163). Both IL-6 and TNF-α ultimately cause low-grade vascular inflammation and the synthesis of C-reactive protein (CRP) (164, 165), which has been linked to increased risk of type II DM and CVD in able-bodied and SCI (166, 167). Due to the associations between: 1) VAT and CRP, and 2) CRP and CVD, some scientific literature has reported CRP levels as an indicator of increased VAT (168, 169). However, given that CRP levels may be elevated for several reasons after SCI other than excess VAT including bladder infection or pressure sores, these associations may be inaccurate. In addition, the hypertriglyceridemia of abdominal obesity leads to low

density lipoprotein cholesterol (LDL-c) production, increased apolipoprotein B (ApoB) levels, and reduced HDL-c levels. The metabolic environment created by VAT contributes to obesity and obesity-related complications (170).

VAT and SAT may not completely explain the atherogenic internal environment resulting from obesity. There has been a recent increase in interest in IMCAT due to its association with type II DM (142, 171-174) and impaired muscle function (116, 150). There appears to be an age- (110, 118), sex- (110, 116, 117) and race-effect (109, 175-177) on IMCAT. The underlying mechanism for increased accumulation of adipose tissue within the muscle is unknown, but some studies have suggested decreased fat oxidation (178), decreased lipolysis (179), and/or increased fatty-acid uptake and higher expressions of fatty acid transport proteins (180) as possible rationales. Obesity alone may explain and perpetuate the harmful internal environment causing and resulting from IMCAT among able-bodied persons. Among individuals with SCI, however, this detrimental internal environment occurs due to a combination of obesity, physical inactivity, and impaired energy metabolism below the level of the lesion.

2.4.2: Measures of Adipose Tissue after Spinal Cord Injury

Understanding the methods for measuring body composition and their underlying assumptions and limitations is key to interpreting body composition data after SCI. Individuals with SCI are perhaps at greater risk of obesity than any other segment of the population. The literature regarding the prevalence of obesity among persons with SCI is poorly substantiated (13), and there are inadequate established guidelines for accurate classification of obesity in this population. Due to the differences in body topography between individuals with SCI and able-bodied persons, techniques developed for the able-bodied population to assess and monitor body composition cannot be used to accurately quantify body composition in the SCI population. The following sections describe several measures of body composition, and discuss their utility in the SCI population.

2.4.2.1: Hydrostatic Weighing, Air Displacement Plethysmography, Bioelectrical Impedance Analysis, Skin-Fold Measures

Hydrostatic weighing holds assumptions that are violated among those with SCI including: 1) the components of fat-free mass (water, protein, mineral) are proportionally constant to a

reference non-SCI cadaver; and 2) residual lung volumes are larger in the able-bodied and therefore inaccurately measured for persons with SCI (181).

Air displacement plethysmography and bioelectrical impedance analysis (BIA) both rely on body density predicted from hydrostatic weighing and violates these same assumptions. In addition, BIA assumes constant hydration and therefore does not take into account any fluid shifts that may occur among the SCI population such as lower-extremity edema or venous pooling (13). Further, a study looking at different body positions during BIA among the SCI population found that a seated position deviated the most from an accurate body composition prediction (182). Finally, BIA devices tend to lose accuracy in severely obese persons. Anthropometric equations developed for skin-fold measurements are also based on hydrostatic weighing assumptions, and are population-specific. A similar equation has not been developed or validated for the SCI population. Due to considerable alterations in fluid states and substantial changes in muscle mass and bone density post-SCI, the validity of these aforementioned measures is questionable (183-187).

2.4.2.2: Body Mass Index

BMI (weight [kg] divided by height [m] squared) is often used among the able-bodied population as a screening tool for diagnosing obesity-related disorders (188); there is a link between BMI and chronic disease among the able-bodied population (189). In fact, BMI is often used to define obesity among the general population. The main assumption when using BMI guidelines is that body mass, adjusted for height squared, is closely related to body adiposity and therefore morbidity and mortality (188). However, the relationship between BMI and body adipose tissue content varies with age, sex, and race; therefore, cut-off points could be lower or higher than the WHO recommended figures.

When the WHO criteria are used among individuals with SCI, obesity may not be accurately categorized (33, 54, 190); variable relationships have been found between BMI and chronic disease in people with SCI (191-194). Individuals with SCI are frequently said to be normal or overweight instead of obese since the mass of adipose tissue is less than that of lean tissue; therefore, this inaccurate categorization contributes to an underestimation of obesity.

A further cause for inaccurate categorization of BMI among individuals with SCI is measurement error during weight and height calculation. To obtain a BMI measurement, a

wheelchair scale is typically used for attaining a weight measurement, but obtaining a height measurement is more difficult. Many studies have used subject height recall (33, 192, 193), however this is not recommended as recalled height and measured length have been found to be inconsistent among those with SCI regardless of age or DOI (195). Measured length can be used as an alternative, as it has been shown to be closely associated with height (196).

2.4.2.3: Waist Circumference

BMI does not provide information regarding the distribution of adipose tissue, which is an important factor when assessing health risks resulting from obesity. Therefore, in combination with BMI, it is important to consider the location of adipose tissue when determining body composition. Adipose tissue in the abdominal region, particularly VAT, is strongly correlated with risk factors for CVD. WC is positively correlated with abdominal adipose tissue content, and is an accurate, reliable, and reproducible surrogate measure of VAT.

In large epidemiological studies, WC has been shown to be strongly, significantly, and independently correlated with several obesity-related complications. In addition, the able-bodied literature (197-200) as well as the third National Health and Nutrition Examination Survey (201) found that WC was more strongly correlated with three obesity-related risk factors than BMI. The location of a WC measurement is controversial. WC is measured among able-bodied persons in a standing position with a measuring tape placed around the abdomen in a horizontal plane after normal expiration. According to the World Health Organization (WHO), the location of the measurement is the midpoint between the lower border of the rib cage and the iliac crest. According to the National Institute of Health (NIH), the location of the measurement is at the superior border of the iliac crest. A prior study reported equally high reproducibility with WC values measured at four different sites of immediately below the lowest rib, at the narrowest waist, midpoint between the lowest rib and the iliac crest, and immediately above the iliac crest (202). The narrowest waist (found to be at the lowest rib) was reported to be the most frequently recommended, as the site of the lowest rib is easy to identify, even in obese persons (202).

Among those with SCI, WC measured below lowest rib after normal expiration in a supine position showed high reproducibility, and appeared to be a simple means of obtaining the WC measurement (53). This method of WC measurement is highly correlated with VAT among a cohort of individuals with complete, incomplete, paraplegic and tetraplegic SCI (53). Research

continues to support a relationship between WC and chronic disease risk such as obesity and CVD in the SCI population (15, 203).

2.4.2.4: Percent Body Fat

BMI and WC are both surrogates for assessing body adiposity due to the practical and cost effective nature of obtaining these measures. However, more accurate means of assessing body adiposity exist, such as magnetic resonance imaging (MRI) or DXA. MRI has the capability to maximize the contrast between different tissues (i.e. muscle, bone, cartilage, etc.), allowing them to be analyzed separately. However, MRI is expensive, associated with long weight times, not often readily available, and not always possible for use among individuals with SCI due to metal implants.

DXA has been used to assess body composition among several different populations including SCI (55, 158, 204-208); DXA can measure total as well as regional lean tissue mass, adipose tissue mass, and % body fat (209-211). Appropriate software must be used to determine the lean and adipose tissue mass from DXA scans. The principle of DXA is such that x-ray beams of two peak energies are produced (low and high-energy photons), and are attenuated differently in bone and soft tissue. When the x-ray or photon source is placed on one side of the person [or object], the intensity and energy of the beam on the other side of the person [or object] is related to its thickness, density, and chemical composition. The differences in attenuation through bone, lean tissue, and adipose tissue reflect the different chemical composition of each component. The energies used are selected to optimize separation of the mineralized and soft tissue components of the area analyzed. With increasing photon energy, the difference in attenuation properties for each tissue decreases. Based on theoretical and experimental studies, it has been found that if the low-energy photon is 40 keV and the high-energy photon is in the range of 70-100 keV, estimates of the bone mass and overlaying soft tissue mass can be calculated (212-214).

From a whole-body DXA scan, bone-containing pixels make up 40-45%, and the remaining pixels are used to estimate the body's adipose tissue-to-lean tissue ratio. Concern has been expressed that the relative adipose tissue-to-lean tissue ratio is thus based on sampling only one-half of the whole body, as well as hydration status of the persons lean tissues (215). However, DXA-derived values for lean and adipose tissue mass have been compared with multi-

compartment models with good agreement (216). Estimates for the bone, lean, or adipose tissue mass have since been theoretically shown to be unaffected by a person's hydration status (217-220). Radiation doses from DXA depend on the site measured (i.e. whole body vs. lumbar spine), but are typically ~30 μSv, which is less than doses received annually from background radiation (2500 μSv). The radiation dose of ~30μSv is roughly equal to the dose of radiation received over 3 day by every Canadian from natural sources of radiation in the environment. DXA is costly and time consuming, and requires trained personnel to administer the scan. In addition, for accurate comparison within person, the same DXA equipment, acquisition, and analysis protocols should be used.

2.4.3: Defining Obesity after Spinal Cord Injury

In this study, we have chosen BMI, WC, and body fat % via DXA as measures of adiposity among persons with SCI. Rationale for using BMI includes its widely accepted use and simplicity of attaining the measure, as well as recently suggested SCI-specific BMI cut-offs for defining obesity. Rationale for using WC includes the simplicity of attaining the measure, as well as the importance of characterizing adipose tissue location for obesity and obesity-related diseases. Rationale for using % body fat via DXA includes its widely accepted use and reliability in determining body composition.

2.4.3.1: Body Mass Index

BMI is widely used due to its simplicity and correlation with WHO criteria of underweight (BMI <18.5), normal weight (BMI 18.5-24.9), overweight (BMI 25-29.9), obese class I (BMI 30-34.9), obese class II (BMI 35-39.9), and obese class III (BMI >40) classification among able-bodied persons (221).

In studies of persons with chronic SCI, mean BMI values range from 20 to 27 kg/m^2 (33, 54, 191, 192, 222-224). A study comparing BMI to four-compartment modeling [reported to accurately assess body composition among individuals with SCI (225)] showed that 77% of those with paraplegia had a mean BMI in the normal range (BMI 18.5-24.9 kg/m^2), but a body fat % in the obese range (≥26% for men, ≥39% for women) (181). Other SCI studies reporting both BMI and body fat % showed a mean BMI in the low 20s and a mean body fat % in the obese range (33, 37, 52, 54-56, 181, 222, 226, 227). Seventy-seven adults with chronic SCI

underwent anthropometric measures (body fat % via BIA, and BMI), and reported a BMI cutoff of 30 kg/m^2 failed to identify 73.9% of obese participants. A recent study has identified lowered BMI cutoffs to better identify obese persons with SCI; it was concluded that a BMI cutoff of >22 kg/m^2 is appropriate for identifying individuals with SCI who are at high risk of obesity and obesity-related chronic diseases (32). Further, two groups of experts in the field of body composition after SCI, independent of each other, suggested a BMI cutoff of >25 kg/m^2 (13, 33)[3,4] to identify individuals with SCI who are obese.

2.4.3.2: Waist Circumference

It has been reported that a WC of >95 cm is a good surrogate for a visceral adipose depot of >130 cm^2 among able-bodied men and women (228). The NIH states that the WC at which there is an increased relative risk of CVD is defined as >102 cm (>40 in) for men, and >88 cm (>35 in) for women, and should be used in conjunction with BMI among able-bodied persons with a BMI between 25 and 29.9 kg/m^2 (229). There are no SCI-specific values for WC measurement.

2.4.3.3: Percent Body Fat

As DXA measures are often expensive and not readily available, there are no universally accepted % body fat ranges to define overweight and obesity. However, several researchers have suggested % body fat ranges for normal, overweight, and obesity; one study reported a working approach to developing age-, race- and sex-specific % body fat ranges that correspond to published BMI guidelines for underweight (<18.5), overweight (≥25) and obesity (≥30) (230). Body fat % was measured via DXA, and BMI was calculated via height and weight among 1626 men and women of three groups (Caucasian, African American, and Asian) and three age categories (20-39, 40-59, and 60-79 years). The authors developed an equation to convert DXA to a 4-compartment % body fat, and for Caucasian men aged 20-39 they reported a % body fat range of 8-20% for normal weight (BMI 18.5-24.9 kg/m2), 21-25% for overweight (25-29.9 kg/m2), and ≥26% for obese (BMI ≥30 kg/m2). For Caucasian women aged 20-39 they reported a % body fat of 21-32% for normal weight, 33-38% for overweight, and ≥39% for obese (230).

[3] Personal Communication: Dr. David Gater; 2009 Congress on Spinal Cord Medicine and Rehabilitation, September 22-26, Dallas, TX.
[4] Personal Communication: Dr. Ann Spungen; 2009 Congress on Spinal Cord Medicine and Rehabilitation, September 22-26, Dallas, TX

2.5: Bone Tissue after Spinal Cord Injury

2.5.1: Bone Tissue Changes after Spinal Cord Injury

The WHO has defined osteoporosis as a skeletal disease characterized by "low bone density and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture." (231). It has been well accepted that the most powerful and measurable determinant of fracture risk is the amount of bone in the skeleton measured by bone mineral density (BMD) or bone mineral content (BMC) (232, 233). The prevalence and relative risk of fracture increases dramatically with decreased BMD (234).

A subpopulation that is at an increased risk of developing osteoporosis and subsequent increased fracture risk are those with SCI. The sites where fractures most commonly occur following SCI are the distal femur and tibia, the proximal tibia, the femoral and tibia shaft, and less commonly the femoral neck and bones of the foot (235, 236). Over 90% of the reported fractures occur in the distal femur or proximal tibia (22, 72, 73). The majority of fragility fractures occur due to minor trauma events such as a transfer from a wheelchair or rolling over in bed (237). Fractures after SCI can lead to increased morbidity, decreased functional mobility, and increased attendant care and healthcare costs.

Regional changes in areal and volumetric BMD, changes in the shape and structure of the long bones of the legs, and alterations in bone CSA have been reported following SCI (61, 238), all of which predispose individuals with SCI to SLOP. SLOP is distinct from postmenopausal osteoporosis in its rate of onset, rate and severity of decline in BMD, etiology and associated regional fracture risk, micro-architecture of bone, and skeletal distribution (239-242). It is characteristic of persons with traumatic SCI to experience a 3%-4% per month decline in aBMD of the hip and knee region within 12-18 months post-injury (243, 244). Several studies have reported that the rapid decline in aBMD following injury amounts to the hip, distal femur, and proximal tibia being 28%, 37-43%, and 36-50% below that of age-matched able-bodied individuals (58-64).

The decline in vBMD of the hip and knee region is predominantly peri-articular, with relative preservation of cortical bone and reduced trabecular volume (65, 66). In agreement with these findings, a recent study measured vBMD using pQCT among persons at least 5-7 years post-injury, and found a 54% and 73% loss in trabecular vBMD of the distal femur and distal tibia,

respectively (245). Further, a study using MRI to examine bone health among individuals with chronic motor complete SCI reported reduced bone volume, reduced trabeculae number, and increased spacing between bone trabeculae at the distal femur and proximal tibia compared to controls (246, 247). These findings suggest that trabecular bone is more affected than cortical bone following SCI. One study looked at vBMD of the tibia among 6 individuals with acute tetraplegia (12 months post-injury) and reported a decrease of 15% and 7% of trabecular and cortical vBMD, respectively (238). Another study looking at vBMD of the tibia among 8 individuals with chronic SCI (>2 years post-injury) found a decrease of 35.3% and 12.9% in the trabecular and cortical compartments, respectively (248). Further, one study reported exponential decay in cortical wall thickness of the tibial and femoral shafts, but not cortical vBMD following SCI (245).

Some studies suggest that aBMD stabilizes by 1-2 years after SCI, at 25-50% below that of able-bodied peers, in the hip and knee regions (244, 249). A more recent study reported that a new steady-state was reached at 50% of the mean value of a reference group after 3 years in the femur, and at 40% after 5 years in the tibia distal epiphysis. This same study reported a decrease in cortical wall thickness but not cortical vBMD, reaching a steady state after 5 years at 65% and 7 years at 70% below that of reference values in the femoral and tibial shafts, respectively (245). Contrary to these studies, recent investigations support a continual decline in aBMD with time post injury of 3% per year, and that a steady-state of lower extremity bone mineral homeostasis is not reached (64, 65, 240, 250).

Contributing factors to changes in bone mass/BMD may include: decreases in lean tissue, loss of voluntary control of skeletal muscles, reduction of weight-bearing activity, increased renal calcium excretion and reduced intestinal absorption of calcium, hormonal and metabolic changes, alterations in blood flow, and alterations in the immune system (251-255). The relative importance of each of these factors has not been clearly established. Physical activity has been shown to have potential in modifying fracture risk among the able-bodied population (256), but no rehabilitation intervention to date has documented a sustained increase in hip or knee region BMD among individuals with SCI (257-261), or demonstrated ability to prevent fractures. It is plausible that in the small sample sizes examined to date, the short duration of treatment interventions and insufficient mechanical stresses failed to induce osteoblast activity or decrease osteoclast activity, resulting in the lack of treatment effects.

Five factors that should be taken into consideration when determining an individuals' risk of developing SLOP and subsequent fracture risk following SCI: age (≥60 years), sex (female), DOI (≥10 years), NLI (tetraplegia), AIS (motor complete), and muscle CSA (234, 262-265). The incidence of fracture has been reported to be 2-6% per year, and increases with the duration of SCI (18, 266); one study out of the United States reported a 14% incidence of fractures among those injured 5 years, 28% incidence among those injured 10 years, and a 39% incidence among those injured 15 years (18). Although individuals with complete paraplegia and complete tetraplegia will experience similar bone loss of the lower extremities, individuals with paraplegia have been reported to have a higher incidence of lower extremity fracture when compared to those with tetraplegia (22), perhaps due to the higher use of manual wheelchairs and higher occurrence of independent transfers, and therefore a greater chance of falls. Individuals with complete SCI tend to lose more bone than those with incomplete SCI (70, 71), and therefore fractures are more common among individuals with complete injuries (72).

Accurate assessments of these changes are costly and difficult, but are important when assessing SLOP and risk of fracture.

2.5.2: Bone Tissue Measures after Spinal Cord Injury

2.5.2.1: Areal Bone Mineral Density and Fracture Threshold/Breakpoint

DXA can be used to measure total and regional BMD. In fact, DXA is the most widely applied method of measuring bone density. DXA measurements allow for the mass of bone mineral to be calculated in the whole body as well as regionally, and expressed as an areal bone density (aBMD) in grams per square centimeter (g/cm^2). DXA is most commonly used to scan the lumbar spine, femoral neck, and whole body; the areas of the lumbar spine and femoral neck are the sites of common fracture among able-bodied individuals. Due to the fact that cortical bone is greater when looking at a whole body scan, BMD and therefore fracture risk may not be accurately represented at specific sites.

To interpret aBMD results from a DXA scan, appropriate race- and sex-matched aBMD reference ranges are required (267, 268). The participant's result can then be expressed as a T-score, which has been validated for whole body aBMD. A T-score compares actual bone density to sex-matched peak bone density of young adults, reported as a number of standard deviations below the average, with one digit (e.g. -2.3). The WHO defines a T-score of >-1 as normal, a T-

score of -1 to -2.5 as low bone density, a T-score of <-2.5 as osteoporosis, and a T- score of ≤-2.5 in conjunction with at least one or more fragility fractures as severe osteoporosis. These criterion, as well as those from the National Osteoporosis Foundation (NOF) of the United States for osteoporosis is intended to diagnose postmenopausal women only (269, 270).

The International Society for Clinical Densitometry (ISCD) provides expanded guidelines to include postmenopausal women, premenopausal women, and men under the age of 50. According to ISCD, T-scores are preferred and the WHO densitometric classification is applicable for postmenopausal women and men aged 50 and older. For women prior to menopause and for men younger than age 50, however, Z-score are preferred. A Z-score compares actual bone density to the bone density of age-, weight-, sex-, and race-matched persons. ISCD classifies a Z-score of -2.0 or lower to define, "below the expected range for age", and a Z-score of above -2.0 to define, "within the expected range for age" (271).

Unfortunately, there is no specific protocol for assessing or interpreting aBMD among individuals with SCI. The distal femur and proximal tibia, skeletal sites most likely to be fractured after SCI (22, 72, 73), are not included in routine scanning protocols using DXA. To predict fracture risk of the paralyzed legs, it is important to measure aBMD at the sites in which most of the fractures occur. Therefore, it is important to have a protocol for scanning and criterion for interpreting the scan at the distal femur and proximal tibia sites. Barriers to BMD testing among individuals with SCI contribute to the lack of available protocols for assessing bone health in this population. Barriers may include scanner design, limited accessibility, increased typical scanning time, and increased staff necessary (272). However, one recently published article evaluated the precision of a DXA scanning protocol for measuring BMD at the knee in SCI, as well as specifically the distal femur vs. proximal tibia. It was reported that the determination of BMD at the knee was precise, and was free of many sources of variation common in scanning the spine (day-to-day changes in abdomen contents related to gut peristalsis and meals). In addition, it was reported that BMD assessed at the distal femur was more precise than at the proximal tibia among individuals with SCI (74).

An alternative means for determining osteoporotic risk and therefore fracture risk is to assess the fracture threshold, the aBMD at a specific site below which fractures begin to occur, or fracture breakpoint, the aBMD value at which the majority of fractures occur. Fracture threshold values using DXA have been established among postmenopausal able-bodied women at 0.97

g/cm^2, 0.95 g/cm^2, and 0.92 g/cm^2 (Z scores of -2.3, -2.4, and -2.2, respectively) at the lumbar spine, femoral neck, and intertrochanteric region of the femur, respectively (273).

Fractures are uncommon at the spine or hip among individuals with SCI, and so fracture thresholds at the lower extremity sites, specifically at the knee, would be more beneficial for this population. A recent study established a fracture threshold and a fracture breakpoint at the knee among a cohort of individuals with SCI. Data from 168 participants (141 had no lower extremity fractures, and 27 had sustained a lower extremity fracture post-injury) was reviewed, and aBMD at the knee was compared in the non-fracture group vs. the fracture group. It was reported that when the knee was used as a proxy for the entire lower extremity, the fracture threshold was 0.86 g/cm^2 and the fracture breakpoint was 0.49 g/cm^2. This article reported a fracture threshold at the knee of 0.78 g/cm^2, and fracture breakpoint of 0.49 g/cm^2. The article noted that a low aBMD at the knee cannot entirely predict who will fracture; risk factors important to consider, in this order: low BMD (<0.78 g/cm^2), complete paraplegia, sex (female), prior fracture, DOI, and age (34).

2.5.2.2: Volumetric Bone Mineral Density and Fracture Threshold

A tool for bone assessment that is beginning to receive more attention due to its ability to calculate volumetric densities (mg/cm^3) is pQCT. The cross-sectional approach of pQCT allows for the separation between cortical and trabecular bone compartments with calculation of separate vBMD, as well as assessment of various bone geometric properties such as trabecular spacing or cortical thickness. Individuals with chronic SCI experience a unique pattern of bone loss including substantially reduced trabecular vBMD, relatively preserved cortical vBMD, and reduced cortical thickness. Therefore, separate trabecular and cortical measures directly at the bone sites most prone to fractures may prove to be invaluable in predicting fracture risk in this population. Like DXA, pQCT is costly, time-consuming, and requires experienced personnel to administer the scan. In addition, the repositioning of an individual when obtaining a pQCT scan may affect the reproducibility of measures; however, a recent study reported a good precision of pQCT in measurement of the tibia (274).

Assessing fracture thresholds at sites of common fracture after SCI using pQCT technology would be beneficial for assessing fracture risk. Accelerated bone loss and fractures often manifest at skeletal sites with a higher proportion of trabecular bone, and trabecular vBMD has

been shown to be associated with fractures in cross-sectional studies. A recent study has suggested fracture thresholds of the femur and tibia among individuals with SCI (35). Bone measurements and fracture assessments were obtained from 99 individuals with motor complete SCI (para- and tetraplegic, AIS A and B), 27 of whom had sustained a fracture of the lower extremities. The participants with and without femur fractures had mean femur trabecular vBMDs of 84.8 ± 23.8 mg/cm^3 and 116.8 ± 26.0 mg/cm^3, respectively. The participants with and without tibia fractures had mean tibia trabecular vBMDs of 46.9 ± 21.8 mg/cm^3 and 68.4 ± 22.4 mg/cm^3, respectively. The data from this study implied a fracture threshold at approximately 110 mg/cm^3 at the distal femur and 70 mg/cm^3 at the distal tibia (35).

2.5.3: Defining Sublesional Osteoporosis after Spinal Cord Injury

In this study, we have chosen hip, distal femur, and proximal tibia aBMD Z-scores from a DXA scan to define SLOP. Rationale for using Z-scores at these sites includes the widely accepted use of Z-scores among the able-bodied population for defining osteoporosis, as well as the importance of assessing SLOP at sites most common to fracture after SCI. In addition, SCI-specific distal femur aBMD fracture threshold and distal femur aBMD fracture breakpoint from a DXA scan were used to determine fracture risk after SCI. Further, distal tibia trabecular vBMD fracture threshold from a pQCT scan was used to determine fracture risk after SCI. Rationale for using these measures includes the specificity to the present population, as well as the importance of characterizing fracture risk at sites most common to fracture after SCI.

2.5.3.1: Areal Bone Mineral Density and Fracture Threshold/Fracture Breakpoint

The ISCD guidelines, as mentioned above, are expanded to include premenopausal women and men <50 years, and therefore may be more useful than the WHO guidelines for defining osteoporosis among subpopulations such as SCI. From a DXA scan, a Z-score of <-2.0 can be used to define osteoporosis at the hip, distal femur, and proximal tibia among individuals with chronic SCI (271).

A DXA scan can also provide aBMD values at the hip, distal femur, and proximal tibia. As mentioned above, a recent study established a fracture threshold and fracture breakpoint at the knee among a cohort of individuals with chronic SCI. The fracture threshold at the distal femur

of 0.78 g/cm^2 and the fracture breakpoint at the distal femur of 0.49 g/cm^2 can be used to predict fracture risk among individuals with chronic SCI (34).

2.5.3.2: Volumetric Bone Mineral Density and Fracture Threshold

The distal tibia is one of the sites common to fracture after SCI (235, 236). As mentioned above, one study determined a fracture threshold at the distal tibia among a cohort of individuals with SCI. Using a pQCT scan, the fracture threshold of approximately 70 mg/cm^3 at the distal tibia can be used to predict fracture risk among individuals with chronic SCI (35).

2.6: Identifying Risk Factors for Obesity and Sublesional Osteoporosis

Several factors affect the severity of muscle atrophy, obesity, and SLOP among individuals with SCI: sex (female) (275, 276), age (≥60 years) (153, 277), DOI (≥10 years) (275), NLI (tetraplegia) (67, 69, 103), and AIS (motor complete) (67-69, 235). As mentioned previously, AIS classification differentiates between an individual with a motor complete injury (AIS A-B) and one with an incomplete injury (AIS C-D). This has important implications for lower extremity body composition and functional ability, and therefore may have a greater contribution to adverse body composition changes after SCI than the other risk factors. The relative risk of each of the abovementioned factors may be important when applying them as a means of identifying individuals at risk of obesity and SLOP. Further elements that may influence the prevalence of obesity and SLOP after SCI that should be taken into consideration when applying the 5 risk factors described above include: previous fracture, drug intake, dietary intake, level of physical activity, and/or socioeconomic status.

2.7: Muscle-Bone Unit after Spinal Cord Injury

Both the skeleton and musculature undergo harmonic and concordant physiological growth, and in aging these changes are reciprocal. In the able-bodied population, muscle loss is associated with increased fracture risk due to various mechanisms including increased bone remodelling and alterations in the sense of equilibrium, leading to greater predisposition towards falling (278). Both lean tissue loss and bone loss following SCI is common, and these changes combined increase the risk of fracture after SCI. Fragility fractures often occur due to minor trauma events such as a transfer from a wheelchair or rolling over in bed (237). Due to the

substantial human and economic costs of fractures, it is important to understand the mechanisms by which bone strength is developed and maintained.

The mechanostat theory suggests that bone strength is adapted to meet mechanical needs (30). It has been proposed that bone quality is made up of baseline bone strength at birth and typical peak voluntary mechanical loads. These typical peak voluntary mechanical loads are from activities of daily living or from purposeful training. Two types of loading determine the strength of bone: direct mechanical loading (walking, running, jumping) and indirect physiological loading (muscle contractions). Muscle contractions provide the largest physiological loads on bone, and therefore a linear relationship has been proposed between muscle size and bone strength (3). Studies have reported associations between an index of muscle strength (muscle cross sectional area [CSA]) and indices of bone strength (i.e. BMC, BMD) among the able bodied population (3, 33, 36, 279-281), supporting the concept that muscle strength is one of the main determinants of the robustness of bone. In the various phases of life, the ratio between muscle and bone remains almost constant (282, 283), such that muscle loss is one of the main determinants of bone fragility. If a muscle-bone relationship exists, in conditions of muscle atrophy such as after SCI, there should be a corresponding decline in bone strength.

Few studies have looked at the muscle-bone relationship among individuals with SCI. One study reported a strong association between muscle and aBMC in the legs among individuals with incomplete SCI (37), while another reported a strong association between muscle and aBMD in the arms among individuals with SCI, regardless of the NLI or AIS (33). These studies used DXA that provides aBMD, a 2-dimentional view of bone and a composite of BMD and bone geometry. The original muscle-bone unit theory was presented using pQCT, which provides a 3-dimentional image that can measure size, shape, and mineral density of bone, and was shown to predict failure load at the radius more accurately than DXA (38, 39). pQCT can also provide muscle CSA, which is considered an acceptable surrogate of muscle strength (3, 40). Given that individuals with SCI experience a unique pattern of bone loss including a predominant loss of trabecular vBMD and cortical thinning (245), it may be valuable to look at the separate components of bone, as well as muscle CSA, using pQCT technology.

Cortical bone CSA, cortical thickness, total vBMD, and total BMC at the 1/3 proximal tibia, and trabecular bone CSA, trabecular vBMD, total vBMD, and total BMC at the distal tibia may

be good indices of bone strength to associate with muscle CSA among individuals with chronic SCI.

2.8: Adipose Tissue and Bone after Spinal Cord Injury

Obesity and osteoporosis are both complex chronic diseases. Both diseases are affected by genetic and environmental factors, normal aging is associated with both diseases, and both adipocytes and osteoblasts are derived from a common precursor – the mesenchymal stem cell – in bone marrow (284). The activation of the peroxisome proliferators activated receptor-γ (PPAR-γ) pathway favours differentiation of mesenchymal stem cells into adipocytes over osteoblasts (285, 286), while the Wnt signaling pathway inhibits adipogenesis in preadipocyte cells (287, 288) and promotes osteogenesis (289-291). Whether a relationship exists between obesity and osteoporosis, and the basic mechanisms underlying the relationship are unclear, although several potential mechanisms have been proposed to support either a positive or negative relationship. Increased skeletal load bearing from excess adipose tissue mass (41-43), the association of adipose tissue mass with the secretion of bone active hormones (i.e. insulin, amylin, preptin) (46, 292), or the secretion of bone active hormones from adipocytes (i.e. estrogen) (47-51), may account for the positive associations reported to date. Absence of load bearing (44) may account for the negative associations reported to date.

A relationship between body weight and bone mass is well represented in the literature; it is recognized that a larger body mass (contributed to by both adipose tissue mass and lean mass) imposes a greater mechanical loading on bone, and that bone mass increases to accommodate the greater load. This relationship is plausible with increasing adipose tissue mass, as the extra weight increases the load that the skeleton is required to bear. Clinical observations have shown that obesity is associated with increased BMD (41). The reverse has also been shown in that a decrease in body weight leads to bone loss (293). Further, many studies have shown that adiposity and bone mass are directly correlated (42, 43). It has been reported that individuals who lose bone rapidly have significantly lower adipose tissue mass than individuals who lose bone slowly (294). One study explored the adipose tissue and bone mass relationship in a cohort of healthy postmenopausal women and found that aBMD was more closely related to weight, BMI, and adipose tissue mass, and less closely related to lean mass (295). Several other studies reported similar results (292, 293, 296). The relationship appears to be dependent on sex (weaker

in men) (297), menopausal status (stronger post-menopause) (298), and level of physical activity (stronger among sedentary persons) (299).

It is likely that if adipose tissue mass impacts bone mass, it would do so by modulating activity of bone cells. Obesity is associated with hyperinsulinemia, and insulin is a potential regulator of bone growth since osteoblasts have insulin receptors (45). In addition, insulin has been shown to directly stimulate osteoblast proliferation in vitro (46). It is thought that the direct effects of insulin on bone are reinforced by two other hormones, amylin and preptin, that are co-secreted with insulin. In humans, the high plasma insulin, amylin, and preptin levels may increase sex hormones (i.e. estrogen), increase osteoblast activity, and decrease osteoclast activity, all pathways that contribute to increased bone mass (292). Further, it has been hypothesized that in the able-bodied population, enhanced estrogen production due to adiposity may be related to BMD (47-51).

Contrary to a positive relationship between adipose tissue mass and bone mass, the reverse has been reported in the literature. If the mechanical loading effect of total body weight is statistically removed, a negative correlation between adipose tissue mass and bone mass is found, indicating that excess adipose tissue mass actually has a detrimental effect on bone (44). Further research has shown that excessive adipose tissue mass may not protect against decreases in bone mass (75-79), and that the risk of osteoporosis is higher for individuals with higher body adiposity, independent of body weight (77). One study reported that a higher proportion of adipose tissue mass was negatively associated with bone mass among 153 premenopausal women (78). Another study among late adolescent women reported that excess weight in the form of adipose tissue mass may have a negative effect on adolescent bone (300).

Persons with chronic SCI have an increase in whole body and regional adiposity (37). The increase in adipose tissue may result in hyperinsulinemia, as well as enhance estrogen production, thereby providing a protective effect on bone. On the other hand, individuals with chronic SCI experience decreases in muscle and bone in parallel with increases in adipose tissue, thus presenting a setting in which an inverse relationship between adipose tissue and bone may exist. Further, for those individuals with SCI who are unable to weight bear or ambulate, reduction of gravitational and mechanical forces may attenuate the association found between body weight and bone mass.

The current study will investigate the relationship between indices of obesity (BMI, WC, and % body fat) and lower limb bone density (distal femur aBMD, distal tibia trabecular vBMD). BMI, WC, and % body fat are all used as indices to describe or define obesity. Able-bodied men and women are considered overweight with a BMI of 25-29.9 kg/m^2 and obese with a BMI of ≥30 kg/m^2 (301). One potential problem with using BMI as an index of obesity is that BMI may not necessarily represent obesity *per se* as it is excessive adipose tissue mass, rather than total body weight, that defines obesity. In addition, characterization of overweight and obesity using BMI among individuals with SCI is unreliable. However, there is a link between BMI and chronic disease among the able-bodied population (189), and adjusted BMI values have been published for individuals with SCI such that individuals with chronic SCI and BMI values of >22 kg/m^2 (32) or >25 kg/m^2 (13, 33) are considered at high risk of obesity and obesity-related chronic diseases. Able-bodied men and women are considered at risk of obesity and obesity-related diseases (e.g. CVD) with a WC of >102 cm and >90 cm, respectively (301). WC is highly correlated with VAT among individuals with SCI (53), and therefore potentially contributes to chronic disease risk in this population (228). Able-bodied men and women <40 years of age are considered overweight with a % body fat of >20% and >33%, respectively, and are considered obese with a % body fat of >25% and >39%, respectively. Excess body adiposity is a widely accepted definition of obesity, and is associated with increased chronic disease risk (302).

Regarding indices of SLOP, over 90% of the reported fractures occur in the distal femur or proximal tibia (22, 72, 73), and the distal femur is a more precise and reliable measure of BMD than the proximal tibia when using DXA (74). In addition, it has been reported that fracture risk after SCI can be predicted from a fracture threshold at the distal tibia (35). Therefore, the aBMD at the distal femur site and the trabecular vBMD of the distal tibia are the most appropriate measures to correlate with indices of obesity.

2.9: Summary of Study Rationale and Background

Dramatic lean tissue losses, adipose tissue gains, and bone tissue losses occur after spinal cord injury, predisposing this population to obesity and SLOP. Obesity can lead to many secondary complications, most notably cardiovascular disease (CVD), the leading cause of death after SCI. Forty-six percent of deaths are due to CVD for individuals 30 years post-injury (303-305). SLOP can also lead to secondary complications, most notably fracture. The incidence of

fracture has been reported to be 2-6% per year, and increases with the duration of SCI (18, 266); one study reported a 39% incidence of fracture among those injured 15 years (18). Fragility fractures result in increased morbidity, increased attendant care and healthcare costs, and in extreme cases lower extremity amputation (20-22). Both obesity and SLOP are influenced by demographics (age and sex) and injury related characteristics (DOI, NLI, and AIS). Determining the lean tissue, adipose tissue, and bone tissue composition of individuals with SCI is therefore important to help understand, manage, and hopefully improve the chronic disease risk of obesity and SLOP in this population. Of note, most body composition assessments are developed for the able-bodied population, resulting in erroneous categorization among the SCI population. Body composition assessment may be achieved via DXA and pQCT, as well as surrogates of body adiposity (BMI and WC).

A relationship between lean mass and bone has been suggested, which may help explain the high incidence of SLOP and fracture after SCI. The proposed relationship is such that the reduction or cessation of physiological loading from muscle contractions on bone after SCI results in deceased bone strength and SLOP (3). Exploring the relationship between muscle and bone may expand our understanding of the mechanisms involved in bone loss and fracture after SCI.

A relationship between obesity and osteoporosis has been suggested, which may help explain the high incidence of both chronic diseases among individuals with SCI. Exploring the relationship between obesity and SLOP may expand our understanding of both chronic diseases independently, as well as the physiological basis of the association between them.

3: METHODS

3.1: Overview of Study Design

The present study was an observational study on body composition after SCI, embedded in two larger studies entitled, "Bone Quality in Individuals with Chronic Spinal Cord Injury" (Bone Quality Study) and "Intermittent Whole Body Vibration and Passive Standing for Treatment of Sublesional Osteoporosis after Spinal Cord Injury Pilot Study Phase II: Safety and Efficacy Assessment" (WBV Study). The main focus of the first larger study is to establish a pilot cohort of individuals with chronic SCI; the cohort can create the potential for future prospective longitudinal studies evaluating predictors of fracture in the SCI population. Eighty individuals with chronic SCI are to be recruited to participate in this larger study; the recruitment is on-going. Fourteen of these individuals (recruited prior to September 2009) were included in the body composition analysis for the present study. The main focus of the second larger study is to determine the safety and therapeutic potential of whole body vibration (WBV) on bone health after SCI. Ten adult men with chronic motor complete paraplegia are to be recruited to participate in this larger study; the recruitment is on-going. Two of these individuals (recruited prior to September 2009) were included in the body composition analysis for the present study. In addition, muscle CSA and indices of bone strength from a further 29 individuals were included when exploring the association between muscle and bone. These data were taken from two previous studies out of McMaster University and the University of Waterloo, entitled "Functional Electrical Stimulation-Assisted Walking: Reduction of Secondary Complications due to Spinal Cord Injury" and "Reproducibility of a New Bone Density Technique", respectively.

The main focus of the present study was to characterize body composition (lean tissue, adipose tissue, and bone tissue) among a representative sample of individuals with chronic SCI (injury for >2 years) including both sexes and diverse levels of impairment. Additional objectives included: a) determining the number of individuals with chronic SCI who were above and below able-bodied normative values for muscle CSA and muscle density; b) determining the number of individuals with chronic SCI who were obese, had SLOP, and/or were at risk of fracture using SCI-specific and able-bodied definitions; and c) suggesting screening procedures

for detection of obesity and SLOP after chronic SCI. Secondary aims were to explore potential associations between: a) muscle and bone, and 2) adipose tissue and bone.

A portion of the data from the larger studies was obtained and utilized for the present study. The study visits of consequence for the present observational study were as follows:

1. A visit to Lyndhurst Center consisting of a medical history questionnaire including injury etiology and impairment descriptors; a DXA scan that measured whole body % fat as well as hip, distal femur, and proximal tibia aBMD (g/cm^2); a WC measure (cm), and a height (m) and weight (kg) measure.
2. A visit to Hamilton Health Sciences' McMaster University consisting of a pQCT scan that measured muscle density (mg/cm^3), muscle CSA (mm^2), cortical bone CSA (mm^2), cortical thickness (mm), total vBMD (mg/cm^3), and total bone BMC (mg/mm) from the 1/3 proximal tibia site, and trabecular bone CSA (mm^2), trabecular vBMD (mg/cm^3), total vBMD (mg/cm^3), and total BMC (mg/mm) from the distal tibia site.

3.2: Recruitment and Screening

3.2.1: Recruitment

Potential participants for both larger studies were identified through a number of recruitment mechanisms including: 1) a poster campaign, 2) online publication on Canadian Paraplegic Association (CPA) Ontario website, 3) referral by a rehabilitation service provider, 4) at the individual's request, or 5) with the use of the SCI Long-Term Follow-Up Database. The SCI Long-Term Follow-Up Database contains a list of individuals interested in finding out more information about ongoing SCI research at Lyndhurst Centre. The Long-Term Follow-Up Database contains the contact and demographic information for individuals with SCI who have consented to be contacted for the purpose of receiving information about relevant research.

Prior to data collection, potential participants were required to meet inclusion criteria, as well as not meet exclusion criteria. These criteria were different for each larger study. NLI and AIS scores were confirmed via an AIS exam done by a physiatrist at Lyndhurst Center, Toronto Rehabilitation Institute. The following inclusion and exclusion criteria were required for participation in the first study, "Bone Quality in Individuals with Chronic Spinal Cord Injury":

Table 3: Participant Inclusion and Exclusion Criteria for Bone Quality Study

Inclusion Criteria	Exclusion Criteria
• Able to understand instructions in English or has an interpreter that is willing to accompany them (e.g. family member) • A traumatic spinal cord impairment (C2-T10 ASIA A-D) associated with a stable upper motor neuron • >1 yr post-injury • Ability to give informed consent • Age ≥ 18 years	• Current or prior known conditions other than paralysis that are known to influence bone metabolism including: metabolic disorders, oral glucocorticoid use for ≥3 months, malignancy, known liver or malabsorption condition • Weight > 270 lbs (limit for bone density machine) • Contraindications to pQCT testing (e.g. bilateral metal implants, severe spasticity and allergy to Ativan)

For the second study, "Intermittent whole body vibration and passive standing for treatment of sublesional osteoporosis after spinal cord injury pilot study phase II: safety and efficacy assessment", the inclusion and exclusion criteria were more rigorous. The inclusion criteria were intended to select a homogenous sample of adult men with chronic motor complete paraplegia. The exclusion criteria were intended to identify potential subjects for whom exposure to intermittent whole body vibration would be unsafe or whom passive standing would be unsafe. Inclusion and exclusion criteria are outlined below:

Table 4: Participant Inclusion and Exclusion Criteria for Whole Body Vibration Study

Inclusion	Exclusion
• Men • Motor complete paraplegia (T2 to T10, AIS A & B) • 20-60 yrs of age • Traumatic SCI • Chronic (>2 yrs) SCI	• Failed PRP • >250lbs (113kg) and >6ft (183cm) • Unable to complete PRP in 5 sessions • History or development of: uncontrolled autonomic dysreflexia, untreated orthostatic hypotension, seizure disorder, migraine headaches, rheumatoid arthritis, kidney stones, arrhythmias, valvular heart disease, non-union fragility fracture, dislocated hip, cochlear implants, deep vein thrombosis, spondylolisthesis, joint implant, diabetes, gallstones, pacemaker, cancer, lower extremity pressure ulcer • Conditions of: bilateral heterotopic ossification of the hip or knee region, plantar flexor contractures of >.20°, combined hip and knee

	flexion contracture >30° • Use of oral bisphosphonate • Concurrent participation in another intervention study or program which would confound interpretation of the study results • Cancer or radiotherapy

3.2.2: Screening

The screening protocol for the bone quality study consisted of determining the participants' eligibility and providing them a detailed description of the study.

Due to the potential safety hazard of the WBV intervention, this study had a rigorous screening protocol. The screening visit included collecting written informed consent, a medical history form, a physical examination, serum Vitamin D and hemoglobin screening, an ultrasound of the kidneys and bladder to ensure the participant did not have kidney stones or hydronephrosis, an x-ray of the spine to ensure the participant did not have loose or broken hardware, and [if necessary] a postural retraining program (PRP). A physician who was part of the research team evaluated the participants' medical history to determine if there was any reason they should not participate.

Individuals with SCI experience autonomic nervous system impairments following injury, including difficulty with blood pressure regulation. Orthostatic hypotension can result when changing body positions, such as from sitting to standing. Orthostatic hypotension is defined as a drop in systolic blood pressure below 70mmHg, diastolic blood pressure below 40mmHg, or heart rate below 50 beats per minute. Therefore, participants with SCI who did not stand on a regular basis were asked to complete a PRP prior to commencement of the study to ensure safe standing during the study. Postural retraining is a means of using a tilt table to trigger baroreceptors to accommodate for changes in the participant's position (sit to stand). This tool has been previously used to identify participants who were unsafe to engage in passive standing, and successful completion of PRP was predictive of safe standing (306).

The PRP was deemed complete when the participant could stand in a tilt table for 30 minutes at a near erect posture without symptoms of orthostatic hypotension or a significant drop in heart rate or blood pressure from their seated posture. To date, the side effects from previous studies on standing in the SCI population at Lyndhurst include rare occurrences of orthostatic hypotension, three cases of syncope, one case of deep vein thrombosis, ten cases of pressure

sores, and five reports of pain. The PRP helps the participants body get used to being upright before using the standing device. Up to five training sessions over a two-week period were dedicated to getting the participant used to standing again. If a participant was not able to become accustomed to standing after the two-week period, the participant was excluded from further participation so as to minimize risks associated with standing during the pilot intervention period. In previous standing studies conducted at Lyndhurst Center, only 3 of 60 participants failed the postural re-training program.

3.2.3: Participants

A sample of 16 individuals (13 men, 3 women) with chronic SCI participated in this study, and the data were obtained at Lyndhurst Center, Toronto Rehabilitation Institute as well as at McMaster University. Informed consent was obtained from each of the participants within their respective studies (Appendix A and Appendix B). When exploring the relationship between muscle and bone, 41 individuals (32 men, 9 women) with chronic SCI participated, and the data were obtained at McMaster University. Informed consent was obtained prior to participation.

3.3: Methodology

3.3.1: Primary Outcome Measures

Lean tissue, adipose tissue, and bone tissue composition was measured via DXA and pQCT, in addition to surrogates of body adiposity (BMI and WC). Lean tissue was measured via muscle CSA (mm^2) and muscle density (mg/cm^3) using pQCT. Adipose tissue was measured via BMI (kg/m^2) and WC (cm) using a floor scale and tape measure, and whole body % fat using DXA. Bone tissue was measured via hip, distal femur, and proximal tibia aBMD (g/cm^2) using DXA; cortical thickness (mm), cortical bone CSA (mm^2), total vBMD (mg/cm^3), and total BMC (mm/mg) at the 1/3 proximal tibia using pQCT; and trabecular vBMD (mg/cm^3), total vBMD (mg/cm^3), and total BMC (mg/mm) at the distal tibia using pQCT.

3.3.1.1: Lean Tissue

Muscle CSA (mm^2) and muscle density (mg/cm^3) were obtained from pQCT scans of the 1/3 proximal tibia. Images were acquired using a Stratec XCT 2000 scanner (Stratec Medizintechnik, Germany) (picture within Appendix B); a translate-rotate, small-bore computed tomography

scanner that acquires a transaxial image from 145 projection scans. Bony landmarks at the knee joint and medial malleolus were palpated and a measuring tape was used to measure the distance between them. The 1/3 proximal tibia site was 66% of tibia length, measuring from distal landmark. Sixty-six percent of the tibia length was chosen because in this region the muscle has the highest circumference and cross-sectional area (307, 308). The participants' lower leg was placed into the scanner. The scanner obtained slice widths of 2.2mm, and a voxel size of 0.5mm. A slice width of 2.2mm was chosen because it has been reported that accurate measures of vBMD obtained from pQCT scans can be obtained at slice widths of >2mm (309).

To analyze a pQCT scan for muscle CSA, the Stratec XCT 2000 software was used. Contour mode is used to find the edge using a contour detection algorithm. It will find pixels with similar values along the boundary of a tissue using the threshold that is set. When performing the first step in the muscle CSA analysis, the contour mode is used to detect the boundary between skin and air. Peel mode is used to separate between two types of tissues, using a threshold. Contour mode 1, peel mode 2, and -100/40mg/mm^3 thresholding was used to separate muscle/bone/skin pixels from pixels containing adipose tissue. Contour mode 1 and 710mg/mm^3 threshold was used to determine the pixels belonging to bone, and finally contour mode 4 and -100/2000mg/mm^3 was used to determine the pixels belonging to skin. The bone and skin areas were then subtracted from the muscle/bone/skin area to obtain total muscle CSA (mm^2).

To analyze a pQCT scan for muscle density, the Stratec XCT 2000 software was used. The first step was to remove the skin and SAT using a contour mode 3, peel mode 1, and threshold of 40mg/mm^3. Total muscle & bone area as well as total muscle & bone density were the outcomes; total muscle & bone mass was subsequently determined. The second step was to remove the skin, SAT, and muscle using a contour mode 1, peel mode 2, and threshold of 280mg/mm^3. Total bone area and total bone density were the outcomes; total bone mass was subsequently determined. Muscle mass and muscle area were then calculated by subtracting the total bone mass from the total muscle & bone mass, and the total bone area from the total muscle & bone area, respectively. Finally, muscle density was determined by dividing the muscle mass by the muscle area, and reported as mg/cm^3.

3.3.1.2: Adipose tissue

BMI (kg/m^2) and WC (cm) were measured as surrogates of body adiposity. For BMI,

participants were weighed using a scale (BMH Medical Inc., model 6059) that was attached to a ceiling lift. As the participant was being transferred from their wheelchair to the DXA table, their weight was recorded in pounds, and subsequently converted to the nearest 0.1 kg. If the participant did not use the ceiling lift, they were weighed in their wheelchair to the nearest 0.1 kg on a floor scale at Lyndhurst Center (Seiko Scale, Stathmos, type 513-417). Once they transferred from their wheelchair for other measurements, their wheelchair weight was measured and subtracted from the total weight to determine body weight. Length measurements were made using a flexible non-elastic Gulick II tape measure (Country Technology Inc, Gay Mills, WI) to the nearest 0.001 m. All measurements were taken on the right side of the body from the heel to the crown of the head while the participant lay in a supine position. The participant's feet were stretched into dorsiflexion where possible. Length measures were taken in segments from the heel to crown if participants had contractures that prevented the straightening of their legs. BMI was determined by dividing the participants body weight (kg) by their length (m) squared.

The measurement for WC was taken after normal expiration immediately below the lowest rib in a supine position (53, 202) with the same tape measure as used for participant length (Gulick II). For each WC measurement, the tape measure was placed directly on the skin with the participants' arms by their sides. Each measurement was taken to the nearest 0.1 cm.

Whole body % fat was measured with a Hologic DXA device (Hologic Inc., Hologic QDR-4500A; MA, USA) (picture within Appendix B), using standard protocols provided by the manufacturer. Whole body scans were analyzed using commercially available software from Hologic. The precision and accuracy of DXA for soft tissue has been reported to be 99% and <1% error (310). The participant was positioned supine on the scanning table, and scanning was performed in a rectilinear fashion, taking approximately 15 minutes. For the whole body scanning required for % body fat measurement, it was essential that all parts of the body (including the arms) were included in the scan field.

3.3.1.3: Bone Tissue

Areal BMD of the hip, distal femur, and proximal tibia were measured with DXA. The hip scan was obtained using a standard protocol provided by the manufacturer. Distal femur and proximal tibia scans were acquired and analyzed using a lower extremity positioning device and protocol whose reliability and accuracy have been previously determined (56). The participant

was positioned supine on the scanning table, and scanning was performed in a rectilinear fashion, taking approximately 15 minutes per site examined. Intra-class correlation coefficients for repeated distal femur and proximal tibia BMD measures are 0.99 and 0.97, respectively. The accuracy of DXA for aBMD measurement is 3%-8% (311-313), and the precision of DXA differs depending on the anatomical site measured. The precision of DXA is better among individuals with normal BMD than among osteoporotic persons. For the regional scanning of the hip, distal femur, and proximal tibia, it was important to position the body for the specific site being measured, as different leg positions can cause errors in BMD measurement. Trained technologists in the Bone Density Lab at Lyndhurst Centre performed the scans. The site is equipped with a lift for transferring patients onto the scan bed.

Cortical bone CSA (mm^2), cortical thickness (mm), total vBMD (mg/cm^3), and total BMC (mg/mm) at the 1/3 proximal tibia; and trabecular CSA (mm^2), trabecular vBMD (mg/cm^3), total vBMD (mg/cm^3), and total BMC (mg/mm) at the distal tibia were obtained from pQCT scans of the tibia. Just as for the lean tissue images from pQCT, the bone tissue images were acquired using a Stratec XCT 2000 scanner. Bony landmarks at the knee joint and medial malleolus were palpated and a measuring tape was used to measure the distance between them. The 1/3 proximal tibia site was 66% of tibia length, measuring from the distal landmark, and the distal tibia site was 4% of tibia length, measuring from the distal landmark. The participants' lower leg was placed through the scanner. The scanner obtained slice widths of 2.2mm at both the 1/3 proximal tibia and distal tibia sites, and a voxel size of 0.5mm and 0.2mm at the 1/3 proximal tibia and distal tibia, respectively.

To analyze the pQCT scans for bone parameters, the Stratec XCT 2000 software was used, and different contour modes, peel modes, and thresholds were used at the 1/3 proximal tibia and distal tibia sites. At the 1/3 proximal tibia site (66%), total and cortical parameters were analyzed using contour mode 1 and $280mg/mm^3$ threshold. Contour mode 1 is the default analysis mode; it is threshold driven and used to separate soft tissue from the outer edge of bone. Any voxel with a density below the set threshold is eliminated, and $280mg/mm^3$ is considered to be the threshold for soft tissue (adipose tissue and lean tissue). At the distal tibia site (4%), total and trabecular parameters were analyzed using contour mode 3, peel mode 2, and $130/400mg/mm^3$ outer threshold/inner threshold values. Contour mode 3 employs a contour detection algorithm to find the bone edge; it has user-defined thresholds and in the present study $130mg/mm^3$ was chosen.

Peel mode 2 uses inner thresholds to separate the total area into trabecular and subcortical bone, and provides information on trabecular bone parameters; it uses thresholds based on density and in the present study 400mg/mm^3 was used (38).

3.3.2: Summary of Primary Outcome Measures

Table 5 provides a summary of the lean tissue, adipose tissue, and bone tissue outcomes used in this study. In addition, lean tissue normative values (110) as well as adipose tissue (13, 32, 33, 221, 229, 230) and bone tissue (34, 35, 271) SCI-specific and able-bodied definitions for obesity, SLOP, and fracture risk are included.

Table 5: Summary of Normative Values and Definitions for Body Composition Assessment

<table>
<tr><th rowspan="2">Outcomes</th><th colspan="2">Able-Bodied Normative Values / Definitions of Obesity and Osteoporosis</th><th rowspan="2">Spinal Cord Injury-Specific Definitions</th></tr>
<tr><th>Men</th><th>Women</th></tr>
<tr><td>Muscle Density (mg/cm^3)
(pQCT)</td><td>Normative Value:
75.2 mg/cm^3 (110)</td><td>Normative Value:
72.4 mg/cm^3 (110)</td><td>N/A</td></tr>
<tr><td>Muscle CSA at Calf (mm^2)
(pQCT)</td><td colspan="2">Normative Value: 7019.6±1331 mm^2</td><td>N/A</td></tr>
<tr><td>Body Mass Index (kg/m^2)
(floor scale and tape measure)</td><td colspan="2">Underweight: <18.5
Normal: 18.5-24.9
Overweight: 25-29.9
Obese I: 30-34.9
Obese II: 35-39.9
Obese III: ≥40 (221)</td><td>High risk for obesity and obesity-related diseases:
>22 kg/m^2 (32)
>25 kg/m^2 (13, 33)
(No universally accepted value)</td></tr>
<tr><td>Waist Circumference (cm)
(tape measure)</td><td>Obese: >102 cm (>40 in) (229)</td><td>Obese: >88 cm (>35 in) (229)</td><td>N/A</td></tr>
<tr><td>Whole Body % Fat
(DXA)</td><td><table><tr><th>Age</th><th>BMI</th><th>%bf</th></tr><tr><td>20-39</td><td>18.5-24.9</td><td>8-19</td></tr><tr><td></td><td>25-29.9</td><td>20-24</td></tr><tr><td></td><td>≥30</td><td>≥25</td></tr><tr><td>40-59</td><td>18.5-24.9</td><td>11-21</td></tr><tr><td></td><td>25-29.9</td><td>22-27</td></tr><tr><td></td><td>≥30</td><td>≥28</td></tr><tr><td>60-79</td><td>18.5-24.9</td><td>13-24</td></tr><tr><td></td><td>25-29.9</td><td>25-29</td></tr><tr><td></td><td>≥30</td><td>≥30</td></tr></table>(230)</td><td><table><tr><th>Age</th><th>BMI</th><th>%bf</th></tr><tr><td>20-39</td><td>18.5-24.9</td><td>21-32</td></tr><tr><td></td><td>25-29.9</td><td>33-38</td></tr><tr><td></td><td>≥30</td><td>≥39</td></tr><tr><td>40-59</td><td>18.5-24.9</td><td>23-33</td></tr><tr><td></td><td>25-29.9</td><td>34-39</td></tr><tr><td></td><td>≥30</td><td>≥40</td></tr><tr><td>60-79</td><td>18.5-24.9</td><td>24-35</td></tr><tr><td></td><td>25-29.9</td><td>36-41</td></tr><tr><td></td><td>≥30</td><td>≥42</td></tr></table>(230)</td><td>N/A</td></tr>
<tr><td>aBMD (g/cm^2)
(DXA)</td><td colspan="2">Premenopausal women and men <50 yrs; can be used to define osteoporosis at the hip, distal femur, and proximal tibia (271):
Below expected range: Z-score: <-2.0
Within expected range: Z-score: >-2.0</td><td>Fracture threshold distal femur:
0.78g/cm^2 (34)
Fracture breakpoint distal femur:
0.49g/cm^2 (34)</td></tr>
<tr><td>Trabecular vBMD (mg/cm^3)
(pQCT)</td><td>N/A</td><td>N/A</td><td>Fracture threshold distal tibia:
70mg/cm^3 (35)</td></tr>
</table>

3.3.3: Secondary Outcome Measures

3.3.3.1: Muscle-Bone Unit

The relationship between muscle and bone among the present cohort of individuals with chronic SCI, as well as an additional 29 individuals from previous studies out of Lyndhurst Center, Toronto Rehabilitation Institute, was determined by correlating an index of muscle strength with indices of bone strength. Muscle CSA has been shown to be a good index of muscle strength (3, 40). It has been suggested that a reduction in all of cortical thickness, cortical bone CSA, and total BMC at the 1/3 proximal tibia (66%), and total vBMD, trabecular vBMD, and total BMC at the distal tibia (4%) occurs following SCI. All of the measures are valid and interpretable.

3.3.3.2: Adipose Tissue and Bone

The relationship between obesity and osteoporosis among the present cohort of individuals with chronic SCI was determined by correlating indices of obesity with indices of SLOP. The indices of obesity [WC, BMI, and whole body % fat] were chosen because they are all used to define obesity and identify people at high risk for obesity-related diseases (32, 53, 197-201, 221). The first index of SLOP [distal femur aBMD, g/cm^2] was chosen because it is the most frequent site of fracture after SCI (22, 72, 73). The distal femur is a more precise and reliable measure of aBMD when using DXA than the proximal tibia (74). The second index of SLOP [distal tibia trabecular vBMD] was chosen because accelerated bone loss and fractures often manifest at skeletal sites with a higher proportion of trabecular bone, and trabecular vBMD has been shown to be associated with fractures in cross-sectional studies (35). Each of the aforementioned measures is valid and interpretable.

3.4: Data Analysis

Descriptive statistics were used to describe the participant's demographic and impairment characteristics. Lean tissue, adipose tissue, and bone tissue composition were presented as average±standard deviation for continuous variable and count (%) for categorical variables. The number of individuals with chronic SCI that had values above and below able-bodied normative values for muscle CSA and muscle density listed in Table 5 were reported. In addition, the

number of individuals with chronic SCI who were obese, had SLOP, and/or who were at risk of fracture using SCI-specific and able-bodied definitions listed in Table 5 were reported.

Linear regression analysis was performed to determine the relationship between % body fat and BMI. The line of best fit was used to approximate the % body fat associated with a BMI of 22 kg/m^2 (32), 25 kg/m^2 (13, 33), and 30 kg/m^2 (221).

Participants were characterized based on 5 risk factors of sex (female), age (≥60 years), DOI (≥10 years), NLI (tetraplegia), and AIS (motor complete). A BMI >22 kg/m^2 defined the presence of obesity, and a distal femur Z-score of <-2.0 defined the presence of SLOP. The number of participants correctly and incorrectly identified to have obesity and SLOP based on the presence of ≥2 or ≥3 risk factors was determined.

Pearson Correlations were used to assess the associations between an index of muscle strength (muscle CSA) and indices of bone strength (cortical bone CSA, cortical thickness, total vBMD, and total BMC at the 1/3 proximal tibia [66%], and trabecular CSA, trabecular vBMD, total vBMD, and total BMC at the distal tibia [4%]). The strength of the relationship was determined based on the correlation values and interpretation in Table 6 (314). The data was evaluated for normality.

Pearson Correlations were used to assess the associations between indices of obesity (WC, BMI, and whole body % fat) and indices of SLOP (distal femur aBMD, and distal tibia volumetric vBMD). The strength of the relationship was determined based on the correlation values and interpretation in Table 6 (314). For all analyses, significance was accepted at the $p<0.05$ level. All statistical analyses were performed using SAS 9.1.3 software.

Table 6: Interpreting Pearson Correlation Values

Correlation Value	Interpretation
≤0.29	Very weak
0.30 to 0.49	Weak
0.50 to 0.69	Moderate
0.70 to 0.89	Strong
≥0.90	Very Strong

4: RESULTS

4.1: Participants

The cohort consisted of 16 participants, 13 men (81%) and 3 women (19%). Data from 14 participants (11 men, 3 women) was utilized from the Bone Quality Study, and data from 2 participants (2 men) was utilized from the WBV Study. One hundred and twenty-nine individuals with chronic SCI were contacted for the Bone Quality Study, 16 were screened, and 14 were enrolled. Twenty-one individuals were contacted for the WBV study, 4 were screened, and 2 were enrolled. A flow chart of the subjects contacted, screened, and enrolled can be found in Figure 1.

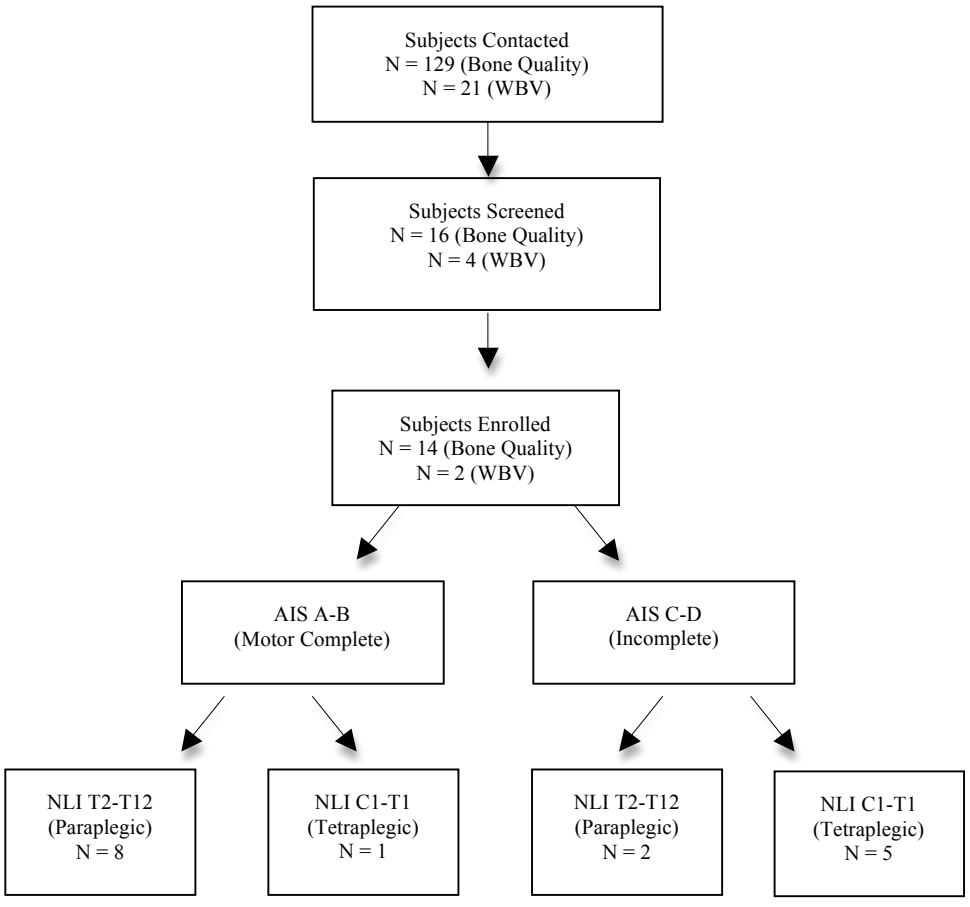

Figure 1: Subject Screening and Recruitment Flow Chart

All participants experienced a traumatic SCI more than 2 years ago from a fall (n=8; 50%), motor vehicle accident (n=6; 38%), assault (n=1; 6%), or gunshot wound (n=1; 6%). The NLI ranged from C3-T12, with 9 AIS A-B and 7 AIS A-D. Average±standard deviation (range) for age was reported as 51.12±12.37 (32 – 76) years, DOI 16.5±7.87 (6 – 29) years, height 1.76±0.09 (1.65 – 1.93) m, and weight 84.41±22.0 (53.1 – 137.44) kg. Demographic (age, sex, height, weight) and impairment characteristics (NLI, AIS, and DOI) of each participant can be found in Table 7.

Table 7: Demographic and Impairment Characteristics

Participant	Sex	Age (years)	Height (m)	Weight (kg)	NLI	AIS	DOI
1	M	51	1.75	88.90	C6	D	6
2	F	72	1.65	72.57	T6	D	28
3	M	44	1.80	77.11	C6	D	25
4	M	54	1.63	65.77	T12	A	12
5	M	76	1.88	111.13	C4	D	11
6	F	52	1.73	74.62	T11	A	22
7	M	44	1.93	101.60	C4	C	19
8	M	67	1.86	99.79	T12	A	13
9	M	34	1.75	58.06	T6-7	A	6
10	M	53	1.73	82.10	T10	A	8
11	M	54	1.80	68.04	C3-4	C	29
12	M	40	1.83	137.44	T6	A	20
13	M	43	1.80	82.10	C4	A	12
14	M	54	1.68	105.7	T9	D	8
15	F	32	1.65	53.1	T4	A	23
16	M	48	1.73	72.6	T12	B	22
AVERAGE	----	51.12±12.37	1.76±0.09	84.41±22.00	----	----	16.50±7.87

NLI = Neurological Level of Injury; AIS = American Spinal Injury Association Impairment Scale; DOI = Duration Of Injury

When exploring the relationship between muscle and bone, data from 29 additional individuals with chronic SCI (DOI >2 years) were included. Forty-one individuals (32 men, 9 women) participated. The NLI ranged from C2-T12, with 13 AIS A-B, and 28 AIS C-D. Average±standard deviation age was 48.7±13.36 years, and DOI 14.22±10.4 years. A summary of the average±standard for demographic (age, sex) and impairment characteristics (NLI, AIS, DOI) can be found in Table 8. There were no demographic differences between the smaller and larger cohort.

Table 8: Demographic and Impairment Characteristics of Larger Cohort

N	41
Ratio men:women	4:1 (32 men, 9 women)
***Age (years)**	48.70±13.36
***DOI**	14.22±10.40
NLI	C2-T12
AIS	13 A-B; 28 C-D

*Reported as mean ± SD; N = Sample Size; DOI = Duration of Injury; NLI = Neurological Level of Injury; AIS = American Spinal Injury Association Impairment Scale.

4.1.1: Sample Size for Body Composition Measures

4.1.1.1: Lean Tissue

Values from pQCT were obtained from 12 participants, as 4 participants were not scanned prior to September 2009. Average±standard deviation was found to be 4914.2±2577.173 mm2 for muscle CSA at the 1/3 proximal tibia (66%). Analysis for muscle density from the pQCT scans were completed for only 8 individuals, as the algorithm used in the Stratec XCT-2000 software was unable to consistently find the contours necessary to determine muscle density among 4 of the participants; average±standard deviation was found to be 53.29±15.54 mg/cm^3 for muscle density at the 1/3 proximal tibia (66%) among the 8 individuals.

4.1.1.2: Adipose Tissue

Surrogates of body adiposity (WC, BMI) were obtained from all 16 participants, average±standard deviation was found to be 99.08±17.85 cm for WC, and 27.00±5.93 kg/m^2 for BMI. Values from DXA for % body fat (31.46±8.95 %) were obtained from 15 participants as one male was >270 lbs (the limit for the DXA scanner).

4.1.1.3: Bone Tissue

Values proximal tibia aBMD (0.45±0.14 g/cm2) were obtained from 15 participants as one male was >270 lbs (the limit for the DXA scanner). Values from DXA for hip aBMD (0.71±0.16 g/cm^2) were obtained from 14 participants as one participant had metal in both hips, precluding accurate assessment. DXA values for distal femur aBMD (0.56±0.22 g/cm^2) were obtained from 14 participants, as one participant had metal plates in both knees. Values from pQCT were obtained from 12 participants, as 4 participants were not scanned prior to September 2009.

Average±standard deviation was found to be 3.57±1.06 mm^2 for cortical thickness at the 1/3 proximal tibia (66%), 538.23±105.16 mg/cm^3 for total vBMD at the 1/3 proximal tibia (66%), 129.22±43.62 mg/cm^3 for trabecular vBMD at the distal tibia (4%), and 170.57±56.90 mg/cm^3 for total vBMD at the distal tibia (4%). A summary of the lean tissue, adipose tissue, and bone tissue body composition values are presented in Table 9. All data was normally distributed.

Table 9: Body Composition after Spinal Cord Injury

Outcome	Able-Bodied Normative Value	Cohort of SCI Average±Standard Deviation
Muscle CSA (mm^2) (n=12)	7019.6±1331	4914.2±2577.173
Muscle Density (mg/cm^3) (n=8)	75.2 (men) 72.4 (women)	53.29±15.54
WC (cm) (n=16)	<102 (men) <88 (women)	99.08±17.85
BMI (kg/m^2) (n=16)	18.5-24.9	27.00±5.93
Body Fat (%) (n=15)	8-19% (men) 21-32% (women)	31.46±8.95
Hip aBMD (g/cm^2) (n=14)	Z-score >-2	50% below Z-score -2; 0.71±0.16
Distal Femur aBMD (g/cm^2) (n=14)	Z-score >-2	100% below Z-score -2; 0.56±0.22
Proximal Tibia aBMD (g/cm^2) (n=15)	Z-score >-2	80% below Z-score -2; 0.45±0.14
Cortical Thickness (66%) (mm^2) (n=12)	**----**	3.57±1.06
Total vBMD (66%) (mg/cm^3) (n=12)	**----**	538.23±105.16
Trabecular vBMD (4%) (mg/cm^3) (n=12)	**----**	129.22±43.62
Total vBMD (4%) (mg/cm^3) (n=12)	**----**	170.57±56.90

WC = Waist Circumference; BMI = Body Mass Index; CSA = Cross Sectional Area; aBMD = areal Bone Mineral Density; vBMD = volumetric Bone Mineral Density; BMC = Bone Mineral Content

4.2: Lean Tissue after Spinal Cord Injury

The number of individuals with SCI who were above and below able-bodied normative values for muscle CSA and muscle density are reported in Table 10, categorized by completeness of injury. It was found that 67% of individuals with SCI had muscle CSA values below the able-bodied norm, and 100% of individuals with SCI had muscle density values below the able-bodied norm. Among individuals with complete (AIS A-B) SCI, 83% and 100% had muscle CSA (n=6) and muscle density (n=3) values, respectively, below able-bodied normative

values. Among those with incomplete (AIS C-D) SCI, 50% and 100% had muscle CSA (n=6) and muscle density (n=5) values, respectively, below able-bodied normative values.

Table 10: Number of Participants with SCI who had Muscle CSA and Muscle Density Values Above and Below Able-Bodied Norms

	Muscle CSA <7019.6±1331 mm^2		**Muscle Density <75.2 mg/cm^3 (Men) <72.4 mg/cm^3 (Women)**	
	+	-	+	-
All	8	4	8	0
AIS A-B	5	1	3	0
AIS C-D	3	3	5	0

+ = At Risk (below normative value); - = Not At Risk (above normative value); CSA = Cross Sectional Area

4.3: Adipose Tissue after Spinal Cord Injury

The number of individuals with SCI who were obese using able-bodied or SCI-specific definitions are reported in Table 11 and Table 12. When using the able-bodied definition of BMI >30 kg/m^2, <20% of the cohort was obese, whereas >60% and >80% of individuals were obese using SCI-specific definitions of BMI >25 kg/m^2 or >22 kg/m^2, respectively. When able-bodied definitions of WC and % body fat were employed, 50% and 87% of individuals with chronic SCI in the present study were obese, respectively.

To elaborate, a larger proportion of individuals with incomplete SCI were obese (71% and 86%) when compared to individuals with motor complete SCI (56% and 78%) using BMI >25 kg/m^2 and >22 kg/m^2, respectively.

Table 11: Number of Participants with SCI Classified as Obese Using Surrogates of Body Adiposity

	Obesity WC >102cm (Men) WC >88cm (Women)		**Obesity BMI >30 kg/m^2**		**Obesity BMI >25 kg/m^2**		**Obesity BMI >22 kg/m^2**	
	+	-	+	-	+	-	+	-
All	8	8	3	13	10	6	13	3
AIS A-B	4	5	1	8	5	4	7	2
AIS C-D	4	3	2	5	5	2	6	1

+ = Obese; - = Not Obese; WC = Waist Circumference; BMI = Body Mass Index

Table 12: Number of Participants with SCI Classified as Obese Based on % Body Fat from DXA (Categorized by Sex, Age, and Completeness of Injury)

	Age	Definition	+	-
Men	20-39	Normal: <20% Overweight/Obese: ≥21%	0	1
	40-59	Normal: <22% Overweight/Obese: ≥23%	9	0
	60-79	Normal: <24% Overweight/Obese: ≥25%	2	0
Women	20-39	Normal: <32% Overweight/Obese: ≥33%	0	1
	40-59	Normal: <34% Overweight/Obese: ≥35%	1	0
	60-79	Normal: <37% Overweight/Obese: ≥38%	1	0
All			13	2
AIS A-B			6	2
AIS C-D			7	0

+ = Obese; - = Not Obese

When % body fat was plotted against BMI for all 15 individuals in the present study (Figure 2), it was found that a BMI of 22 kg/m^2 corresponded with a % body fat of ~29%, a BMI of 25 kg/m^2 corresponded with a % body fat of ~31%, and a BMI of 30 kg/m^2 corresponded with a % body fat of ~34%.

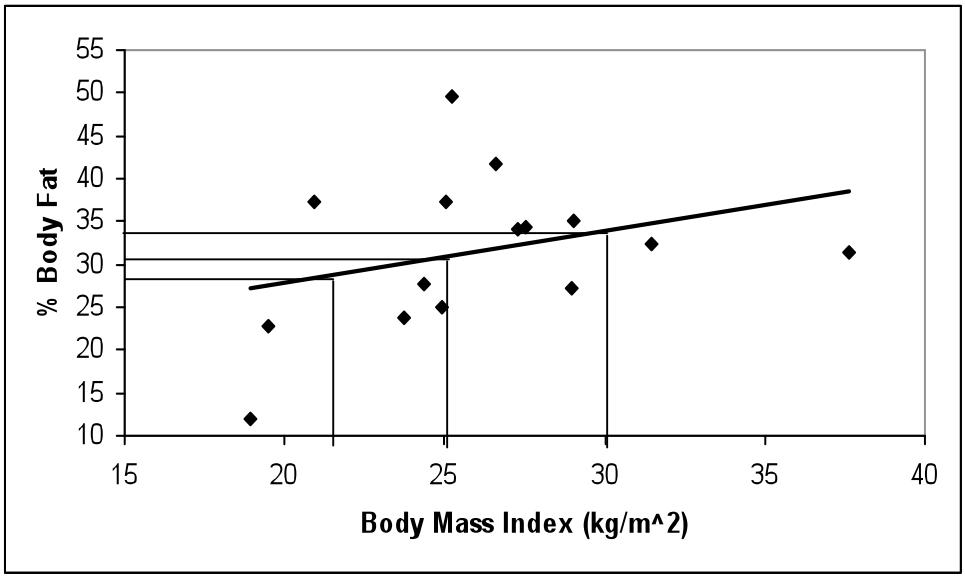

Figure 2: Approximation of % Body Fat Associated with BMI of 22 kg/m^2, 25 kg/m^2, and 30 kg/m^2

4.4: Bone Tissue after Spinal Cord Injury

The number of individuals with SCI who had SLOP or who were at risk of fracture using SCI-specific definitions, respectively, are reported in Table 13 and Table 14. It was found that 50%, 100% and 80% of individuals with chronic SCI in the present study had SLOP based on able-bodied definitions of a Z-score <-2.0 at the hip, distal femur, and proximal tibia, respectively. The distal femur and proximal tibia are skeletal sites most common to fracture after SCI. A larger proportion of individuals with motor complete SCI had SLOP (86%, 100%, and 100%) when compared to individuals with incomplete SCI (14%, 100%, and 57%) using a Z-score of <-2.0 at the hip, distal femur, and proximal tibia, respectively.

Table 13: Number of Participants with SCI Classified as having SLOP Based on ISCD Z-scores from DXA

	SLOP Hip aBMD Z-score <-2.0		**SLOP Distal Femur aBMD Z-score <-2.0**		**SLOP Proximal Tibia aBMD Z-score <-2.0**	
	+	-	+	-	+	-
All	7	7	14	0	12	3
AIS A-B	6	1	7	0	8	0
AIS C-D	1	6	7	0	4	3

ISCD = International Society of Clinical Densitometry; + = Osteoporotic; - = Not Osteoporotic; AIS = American Spinal Injury Association Impairment Scale; aBMD = areal Bone Mineral Density

Table 14: Number of Participants with SCI Classified as at Risk of Fracture Based on SCI-Specific Fracture Thresholds from DXA and pQCT

	Fracture Threshold at Distal Femur aBMD <0.78 g/cm^2		**Fracture Breakpoint at Distal Femur aBMD <0.49 g/cm^2**		**Fracture Threshold at Distal Tibia vBMD <70 mg/cm^3**	
	+	-	+	-	+	-
All	11	3	7	7	1	11
AIS A-B	7	0	7	0	1	5
AIS C-D	4	3	0	7	0	6

+ = Osteoporotic; - = Not Osteoporotic; AIS = American Spinal Injury Association Impairment Scale; aBMD = areal Bone Mineral Density; vBMD = volumetric Bone Mineral Density

It was found that 79% and 50% of individuals with chronic SCI in the present study were at risk for fracture based on SCI-specific definitions of a fracture threshold at the distal femur <0.78 g/cm^2 and a fracture breakpoint at the distal femur <0.49 g/cm^2, respectively. Further, 1% of

individuals with chronic SCI were at risk for fracture based on the SCI-specific definition of a fracture threshold at the distal tibia <70 mg/cm^3. A larger proportion of individuals with motor complete SCI were at risk of fracture (100%, 100%, and 17%) when compared to individuals with incomplete SCI (57%, 0%, and 0%) using a fracture threshold of <0.78 g/cm^2 at the distal femur, a fracture breakpoint of <0.49 g/cm^2 at the distal femur, and a fracture threshold of <70 mg/cm^3 at the distal tibia, respectively.

4.5: Identifying Risk Factors for Obesity and Sublesional Osteoporosis

Five risk factors for obesity and SLOP after SCI include sex (female), age (≥60 years), DOI (≥10 years), NLI (tetraplegia), and AIS (motor complete). The number of participants with each risk factor is presented as a histogram in Figure 3. The number of risk factors that each participant possessed was matched to the presence of obesity and SLOP. When using ≥3 risk factors to identify those at risk of obesity (BMI >22 kg/m^2) or SLOP (distal femur Z-score <-2), 10 individuals in the present cohort were undetected. When using ≥2 risk factors to identify those at risk of obesity or SLOP, 4 individuals were undetected with no false positives. Twelve participants in the present cohort were at high risk of being obese and/or having SLOP when utilizing the ≥2 risk factors taxonomy.

Among the individuals defined as obese in the present cohort (81% with BMI >22 kg/m^2), 77% had ≥2 risk factors. Among the individuals defined as having SLOP in the present cohort (100% with Z-score <-2.0), 71% had ≥2 risk factors. Among the individuals who were both obese and had SLOP (79%), 73% had ≥2 risk factors.

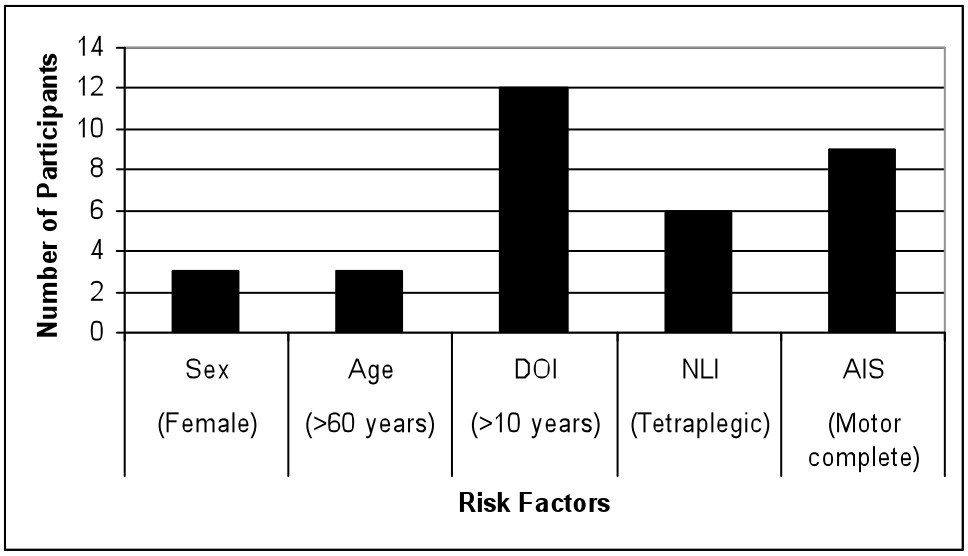

Figure 3: Number of Participants with Each Risk Factor

4.6: Muscle-Bone Unit after Spinal Cord Injury

When exploring the correlations between lean tissue and bone tissue, data from 41 individuals with chronic SCI were included. The muscle and bone characteristics for this cohort can be found in Table 15.

Table 15: Muscle and Bone Characteristics from Larger Cohort

Outcome (n=41)	Average ± Standard Deviation
Muscle CSA (mm^2)	4863.52±2080.40
Cortical Bone CSA 66% (mm^2)	288.35±80.04
Cortical Thickness 66% (mm)	3.48±0.91
Total vBMD 66% (mg/cm^3)	553.49±94.23
Total BMC 66% (mg/mm)	391.26±90.21
Trabecular Bone CSA 4% (mm^2)	1109.65±200.70
Trabecular vBMD 4% (mg/cm^3)	138.97±55.30
Total vBMD 4% (mg/cm^3)	186.74±66.64
Total BMC 4% (mg/mm)	230.03±85.72

CSA = Cross Sectional Area; vBMD = volumetric Bone Mineral Density; BMC = Bone Mineral Content

Weak correlations were found between muscle CSA and cortical bone CSA ($r=0.48$, $p<0.001$), cortical thickness ($r=0.42$, $p=0.005$), and total BMC ($r=0.46$, $p=0.002$) at the 1/3 proximal tibia (66%). These correlations are shown in graph form in Figure 4, Figure 5, and Figure 6, respectively.

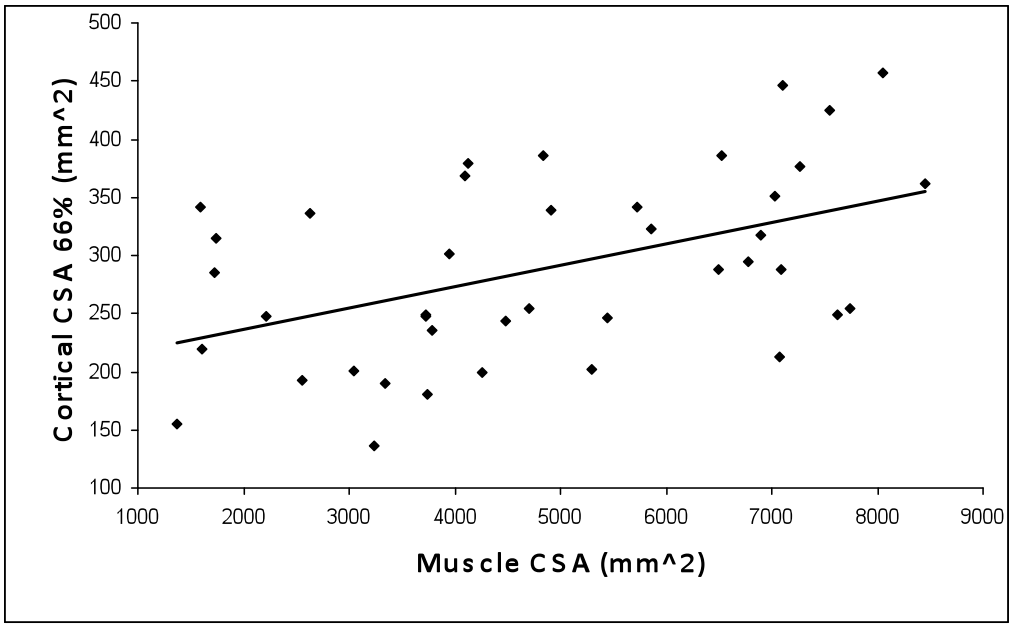

Figure 4: Cortical Bone CSA at 66% (mm^2) vs. Muscle CSA (mm^2); r=0.48; p<0.001

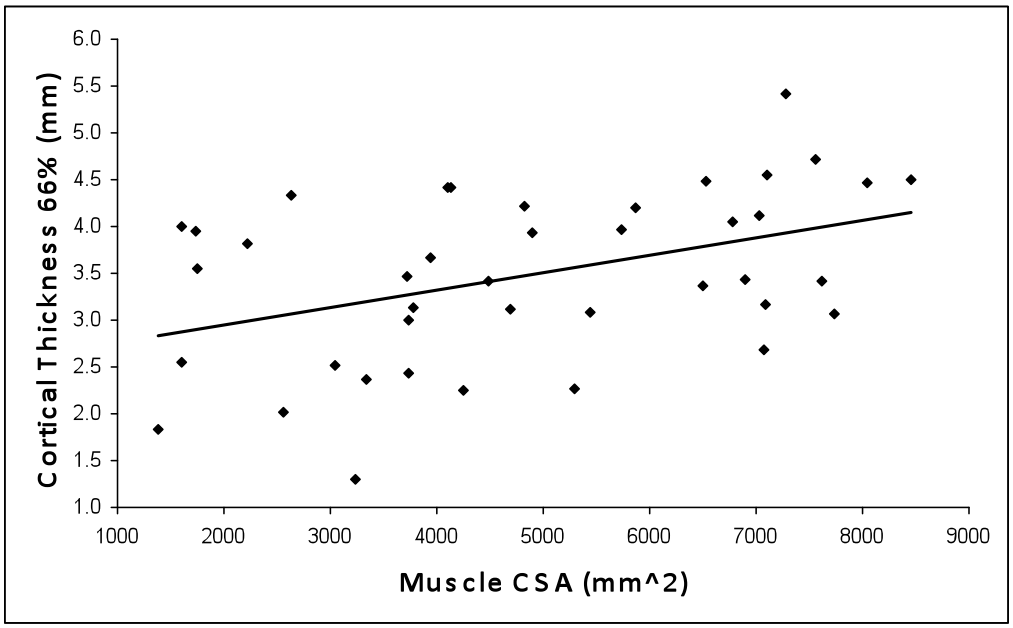

Figure 5: Cortical Thickness at 66% (mm) vs. Muscle CSA (mm^2); r=0.42; p=0.005

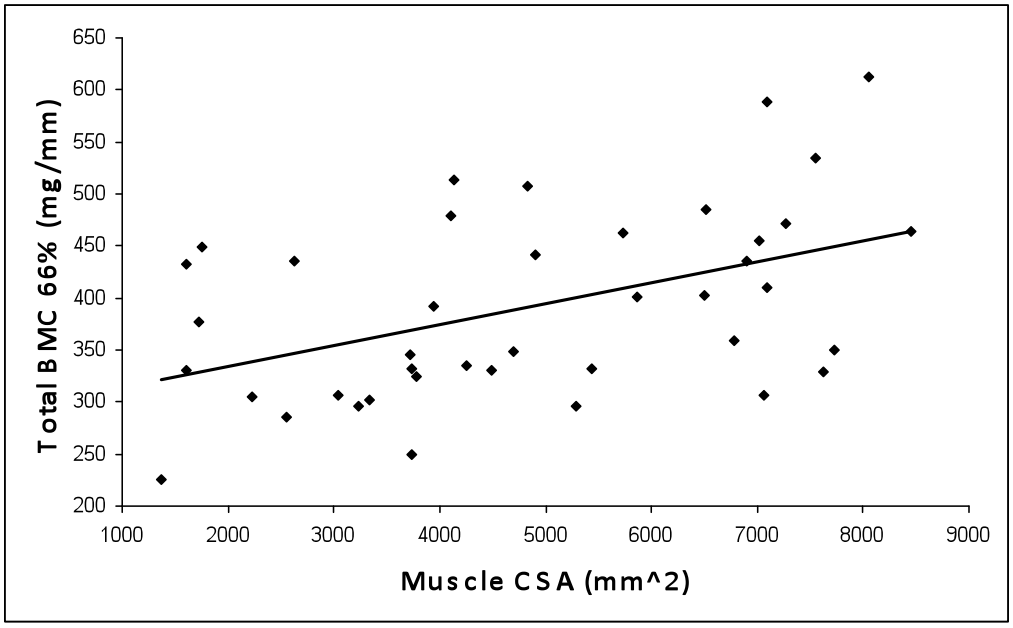

Figure 6: Total BMC at 66% (mg/mm) vs. Muscle CSA (mm^2); r=0.46; p=0.002

Moderate correlations were found between muscle CSA and trabecular vBMD (r=0.55, p<0.001), total vBMD (r=0.54, p<0.001), and total BMC (r=0.57, p<0.001) at the distal tibia (4%). These correlations are shown in graph form in Figure 7, Figure 8, and Figure 9, respectively.

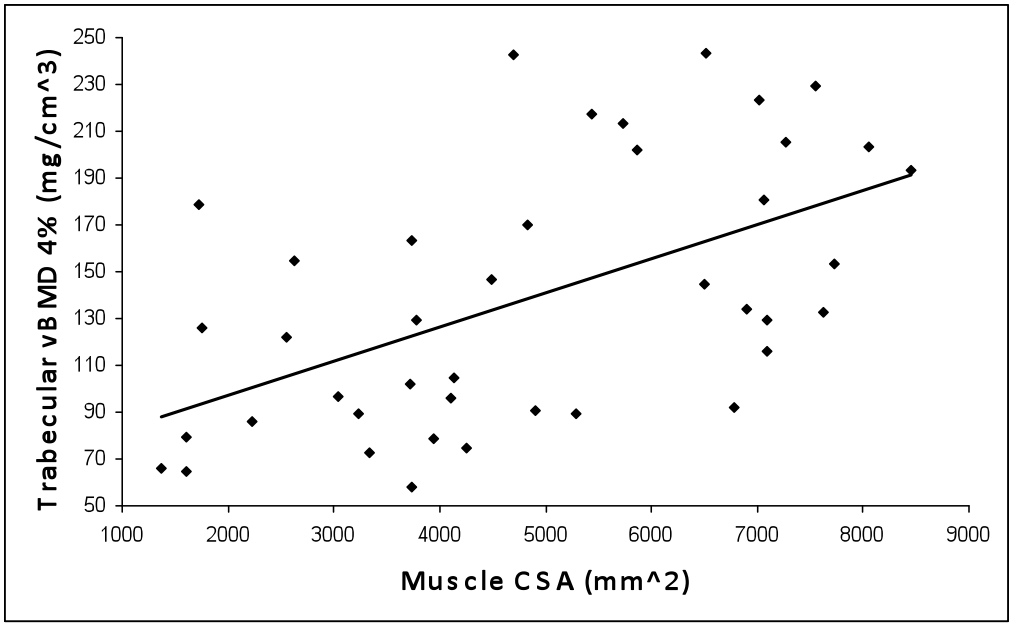

Figure 7: Trabecular vBMD at the 4% (mg/cm^3) vs. Muscle CSA (mm^2); r=0.55; p<0.001

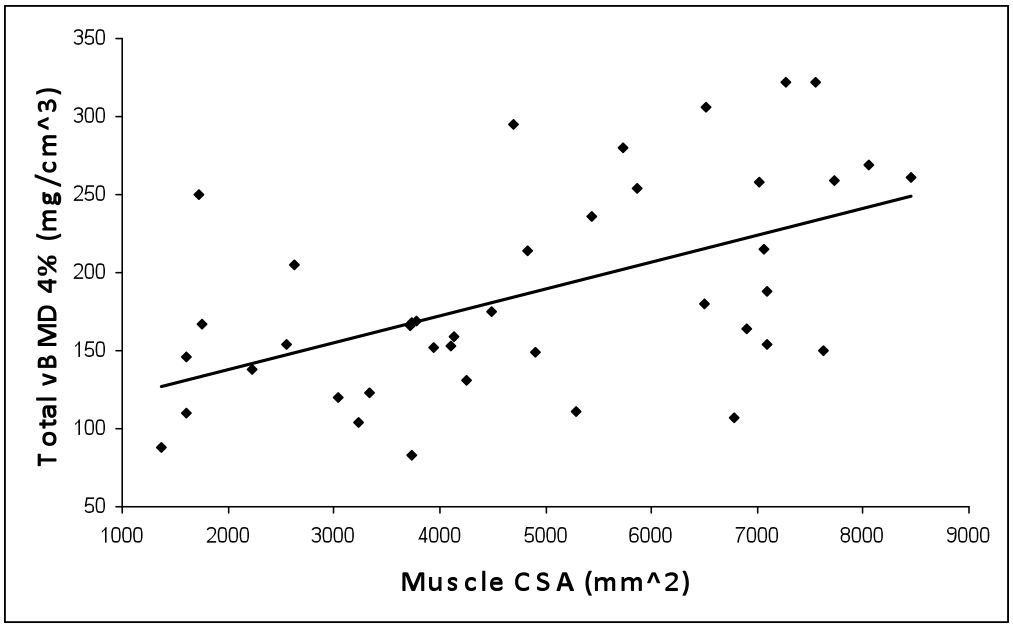

Figure 8: Total vBMD at 4% (mg/cm^3) vs. Muscle CSA (mm^2); r=0.54; p<0.001

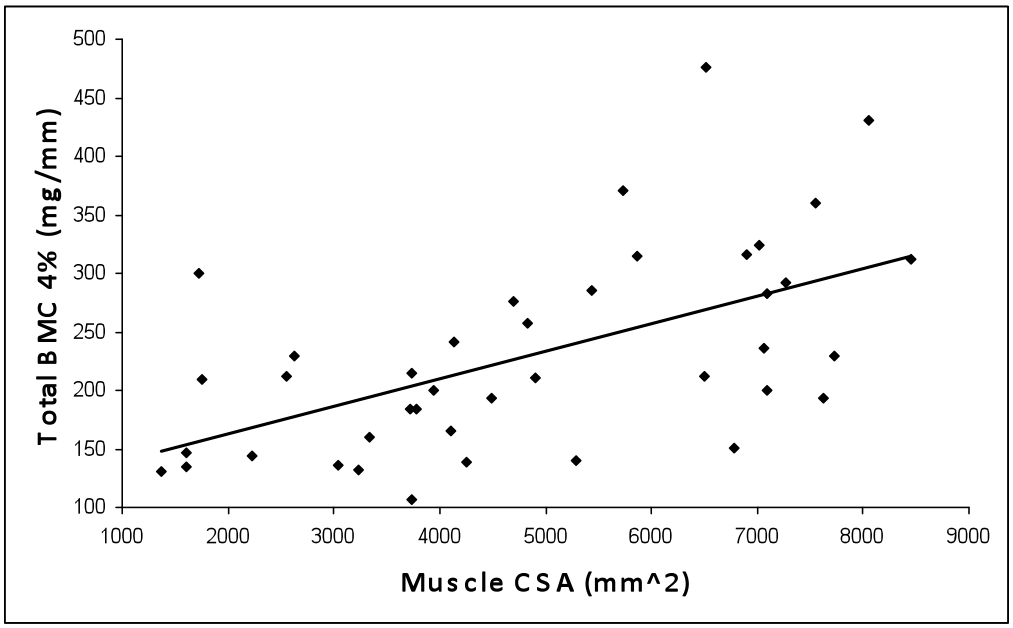

Figure 9: Total BMC at the 4% (mg/mm) vs. Muscle CSA (mm^2); r=0.57; p<0.001

No significant relationship was found between muscle CSA and total vBMD (r=0.29, p=0.06) at the 1/3 proximal tibia (66%), or between muscle CSA and trabecular CSA r=0.0195; p=0.903) at the distal tibia (4%). These correlations are shown in graph form in Figure 10 and Figure 11, respectively.

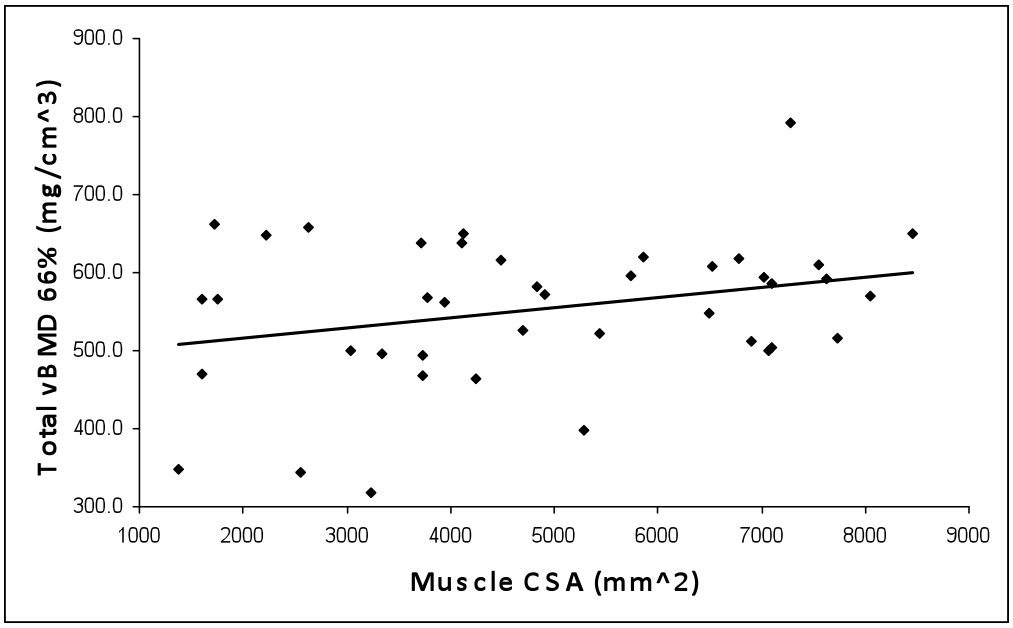

Figure 10: Total vBMD at 66% (mg/cm^3) vs. Muscle CSA (mm^2); r=0.29; p=0.06

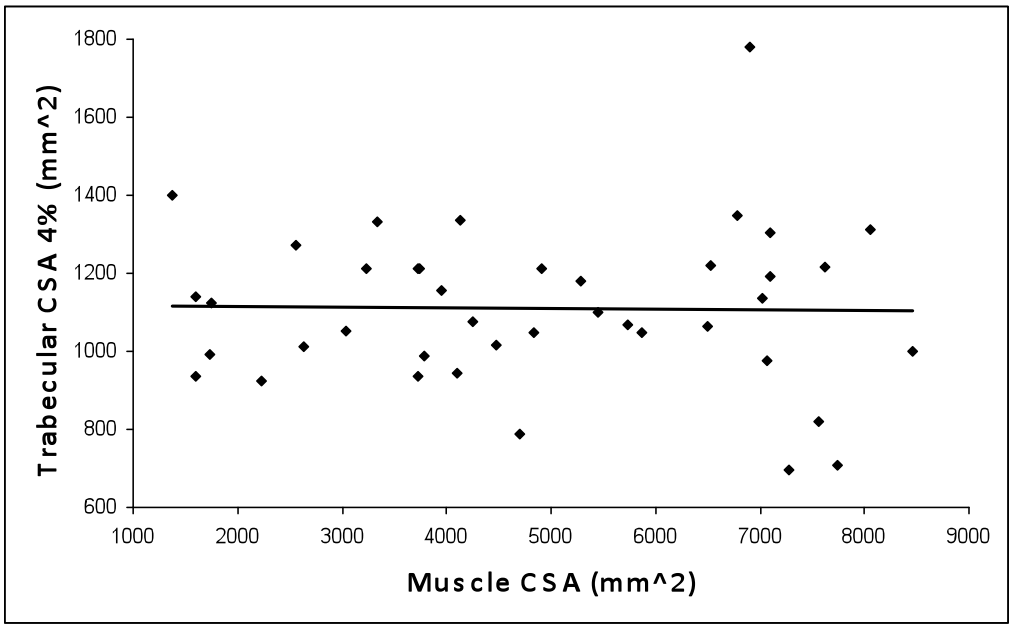

Figure 11: Trabecular Bone CSA at 4% (mm^2) vs. Muscle CSA (mm^2); r=0.02; p=0.90

4.7: Adipose Tissue and Bone after Spinal Cord Injury

When exploring the correlations between adipose tissue and distal femur aBMD, data from 14 individuals with chronic SCI was included. No significant correlations were found between distal femur aBMD and WC (r=0.34, p=0.21), BMI (r=0.39, p=0.16), or % body fat (r=0.03,

p=0.91). These correlations are shown in graph form in Figure 12, Figure 13, and Figure 14, respectively.

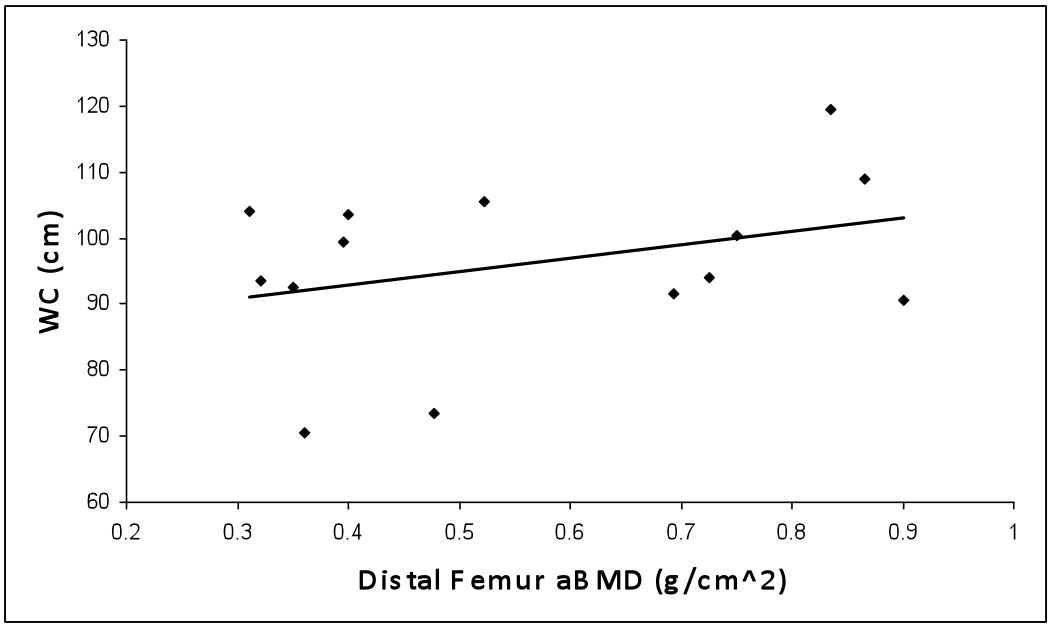

Figure 12: WC (cm) vs. Distal Femur aBMD (g/cm^2); r=0.34; p=0.21

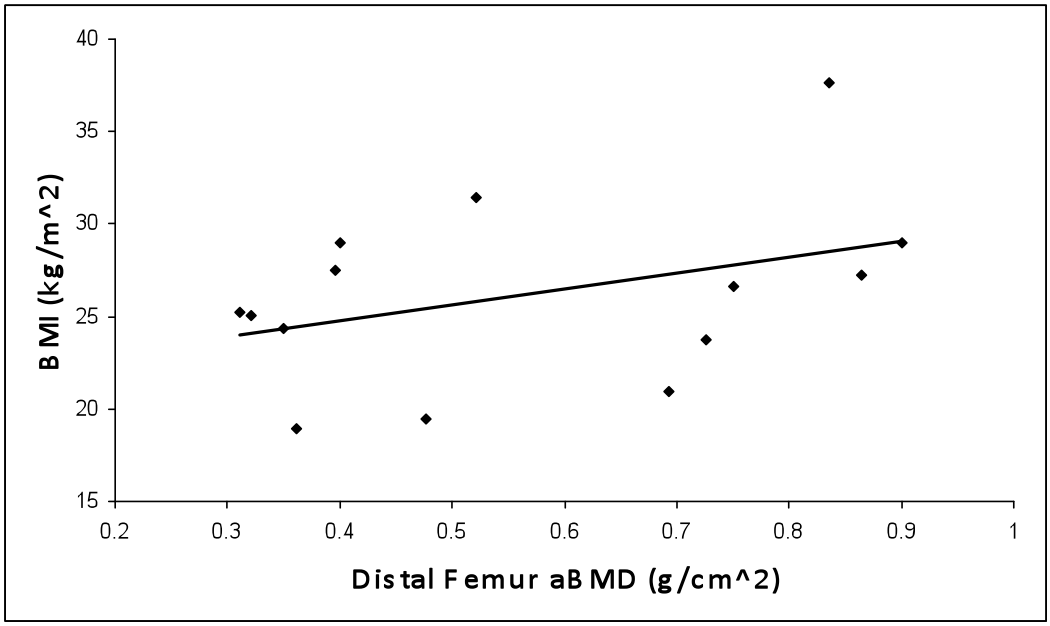

Figure 13: BMI (kg/m^2) vs. Distal Femur aBMD (g/cm^2); r=0.39; p=0.16

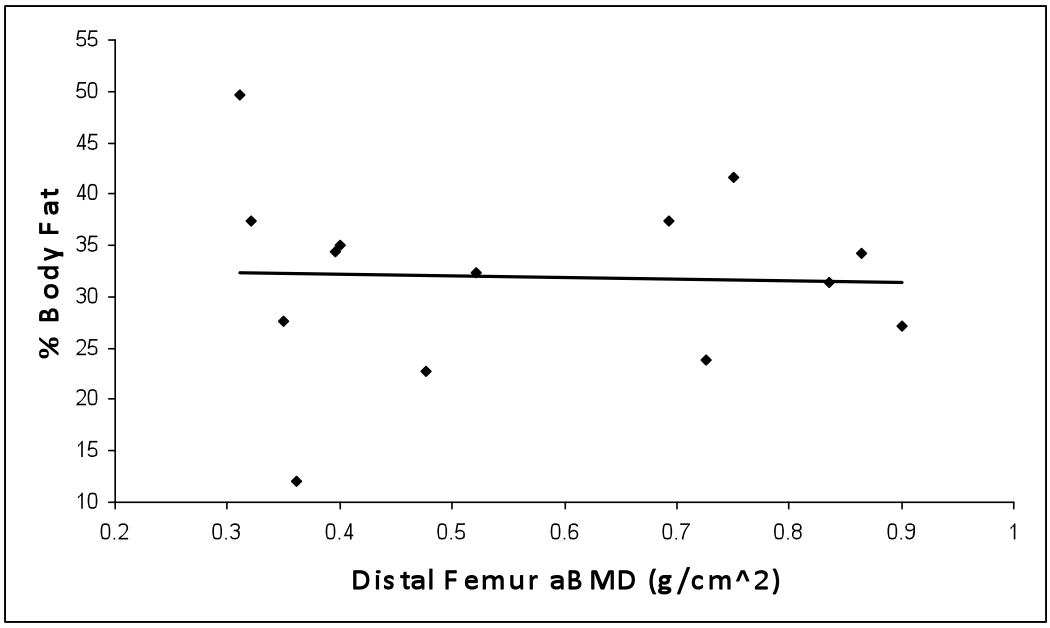

Figure 14: Body Fat (%) vs. Distal Femur aBMD (g/cm^2); r=0.03; p=0.91

When exploring the correlations between adipose tissue and distal tibia trabecular vBMD, data from 12 individuals with chronic SCI were included. No significant correlations were found between distal tibia trabecular vBMD and WC (r=0), BMI (r=0), and % body fat (r=0). These correlations are shown in Figure 15, Figure 16, and Figure 17, respectively. One individual had a % body fat that is not characteristic of an individual with chronic SCI; his % body fat was lower than 2 standard deviations below the mean at 12%. When this outlier was removed, a Pearson Correlation of r=0.56 (p=0.09) was found for the relationship between distal tibia trabecular vBMD and % body fat. This relationship is shown in graph form in Figure 18.

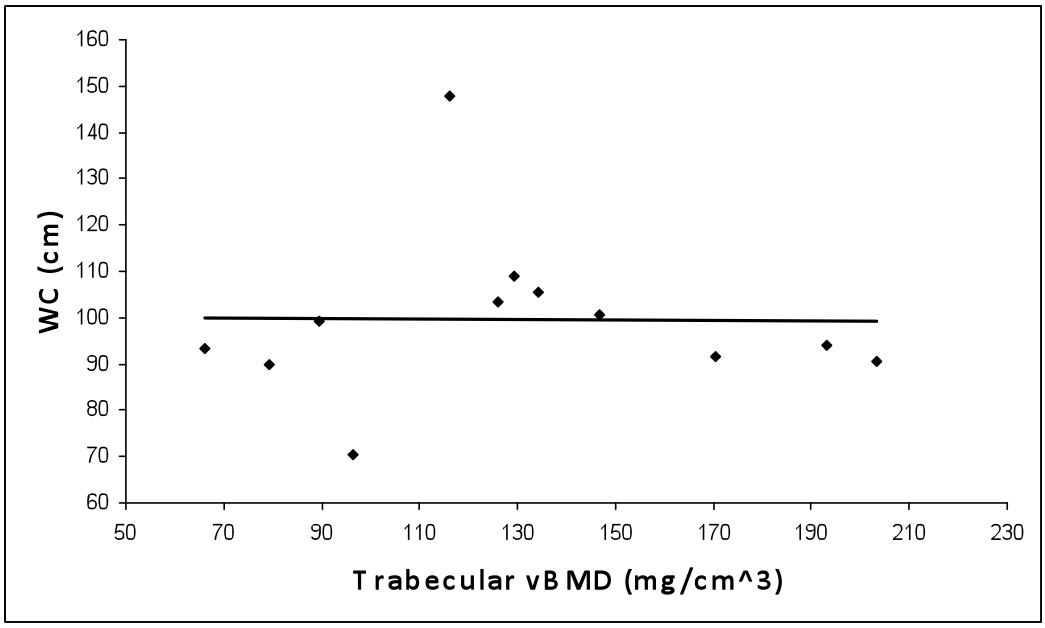

Figure 15: WC (cm) vs. Trabecular vBMD (mg/cm^3); r=0.00

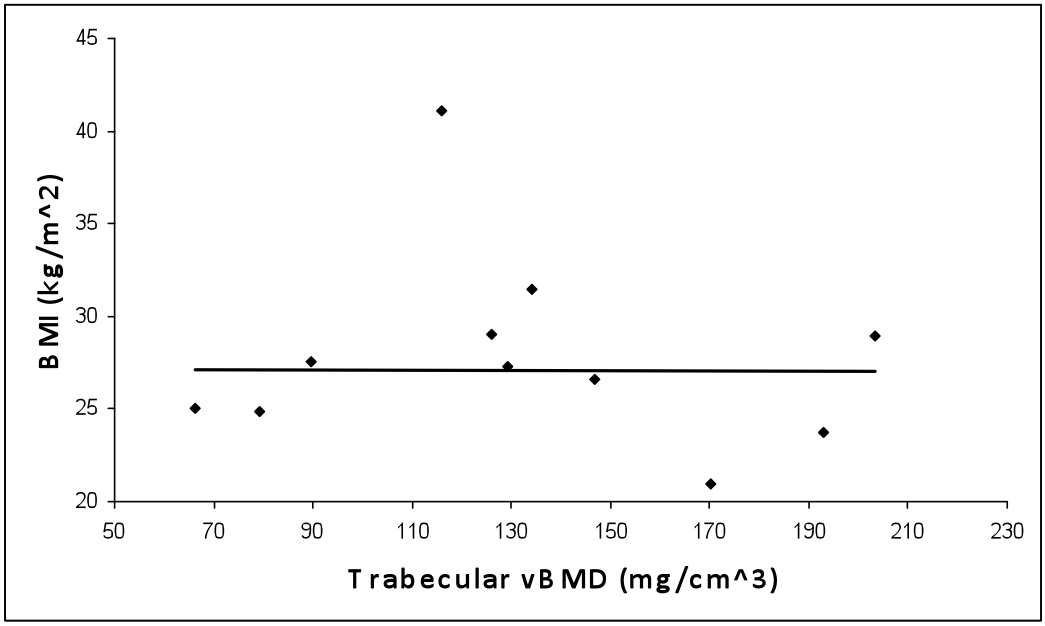

Figure 16: BMI (kg/m^2) vs. Trabecular vBMD (mg/cm^3); r=0.00

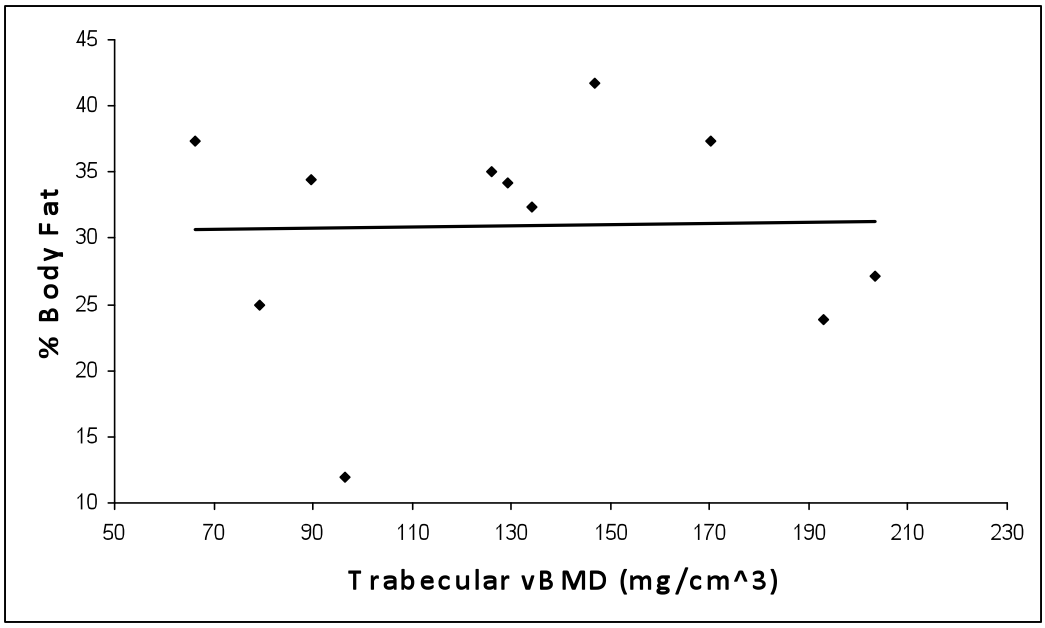

Figure 17: Body Fat (%) vs. Trabecular vBMD (mg/cm^3)**;** r=0.00

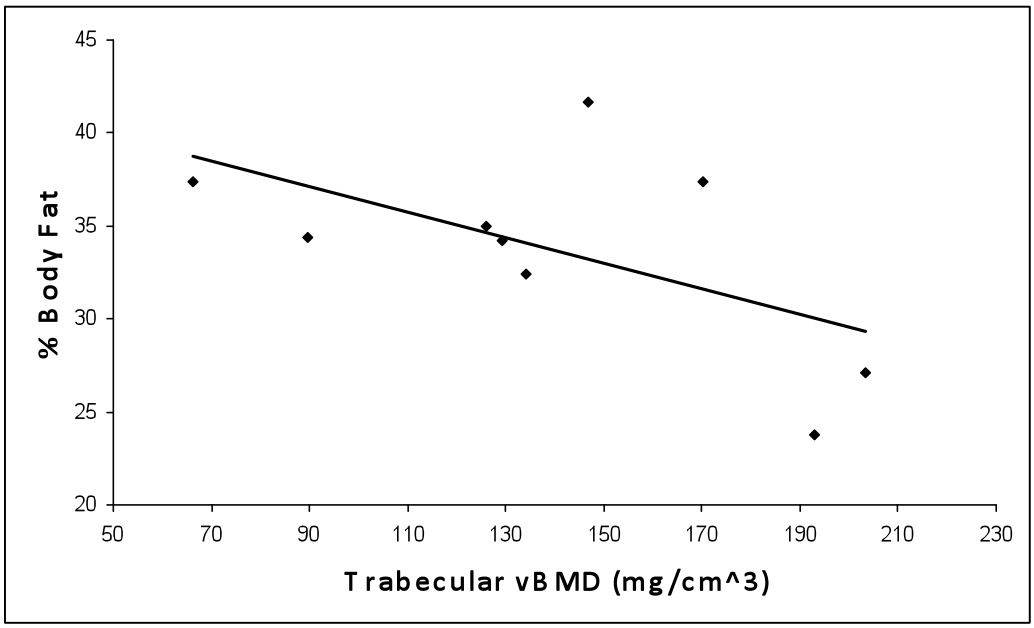

Figure 18: Body Fat (%) vs. Trabecular vBMD (mg/cm^3) with Outlier Removed**;** r=0.56; p=0.09

5: DISCUSSION

Our primary findings were:

1. There was a prevalence of muscle loss, adipose tissue gain, and bone tissue loss among the cohort of individuals with chronic SCI in this study.
2. There was an underrepresentation of obesity among the present cohort when the able-bodied definition of BMI >30 kg/m^2 was utilized; it appeared as though the SCI-specific definitions of BMI >25 kg/m^2 or >22 kg/m^2 were more sensitive in identifying those who were obese.
3. The ISCD Z-scores at the distal femur identified 100% of the individuals in the present cohort as having SLOP, whereas the fracture threshold at the distal femur identified >75% of the individuals in the present cohort as being at risk of fracture.
4. The presence of ≥2 risk factors (female, ≥60 years of age, DOI ≥10 years, tetraplegia, motor complete) identified individuals with SCI in need of body composition screening to subsequently detect those who were obese or who had SLOP. Body composition screening should include % body fat and SCI-specific BMI and WC measures to detect obesity, and distal femur Z-score and fracture threshold to detect SLOP and fracture risk, respectively.
5. Weak to moderate correlations were found between muscle CSA and indices of bone strength, supporting the theory of a muscle-bone unit.
6. No correlations were found between indices of obesity and indices of SLOP. However, when one outlier was removed, a trend towards significance was found between distal tibia trabecular vBMD and % body fat.

The ratio of men:women at 4:1 in the present study is representative of the SCI population, and there was a fairly balanced proportion of individuals in each category of motor complete (AIS A-B) (n=9) and incomplete (AIS C-D) (n=7) SCI.

5.1: Lean Tissue after Spinal Cord Injury

Two thirds of individuals with SCI had muscle CSA values below able-bodied normative values, consistent with previous reports of drastic reductions in muscle CSA following SCI (1, 2). Individuals with SCI in our study had 35% less muscle CSA than the able-bodied norm,

whereas others have reported a 24-31% lower muscle CSA among persons with incomplete SCI when compared to controls (98). When taking the completeness of injury into account, individuals with incomplete SCI in the present cohort had 6% less muscle CSA than the able-bodied normative values, and individuals with motor complete SCI had 75% less muscle CSA than the able-bodied normative values. In both of these aforementioned studies, three quarters of the participants were AIS D, capable of ambulation in their homes and in the community, perhaps contributing to preservation of muscle CSA. The difference in muscle CSA [6% vs. 24-31%] among individuals with incomplete SCI may be due to DOI [16.5±7.87 years vs. 13±9 months] or varied methodology [pQCT vs. MRI] between the present study and the above-mentioned study, respectively.

The entire cohort had muscle density values below able-bodied normative values. Accordingly, muscle density was low even among the individuals with above norm muscle CSA, implying an increased risk for obesity-related diseases regardless of muscle CSA (92). The low muscle density in this population was expected given a prior report of increased intramuscular adipose tissue (86). We found that individuals with motor complete injuries had 43% less muscle density than those with incomplete injuries. It is likely that the higher muscle CSA and muscle density among those with incomplete injuries was due to preservation from mechanical loading and muscle contraction during activities of daily living and perhaps resistance training. However, even with some muscle preservation, individuals with incomplete SCI have muscle density values below able-bodied norms. Decreased muscle quantity and quality puts individuals with both complete and incomplete SCI at risk for obesity and SLOP. For example, reductions in muscle CSA after SCI can result in a decreased metabolic rate and increased adipose tissue storage (88), and muscle atrophy has been suggested to be the largest contributor to obesity-related complications including hyperglycemia, peripheral insulin resistance, and type II DM, consequently contributing to an atherogenic internal environment (91, 92). In addition, the loss of muscle CSA results in a decrease or cessation of physiological loading on bone; based on the mechanostat theory, this results in decreased bone strength and osteoporosis (30).

Interventions that may preserve or improve muscle quantity/quality include standing, electrically stimulated cycling or resistance training, and walking exercises. Exercise with electrical simulation appears to prevent atrophy and/or increase muscle mass (315-317), but the impact of standing or walking exercises on muscle has not been well established (318-320). It is

difficult to confirm the utility of these exercises for individuals with SCI due to several methodological limitations such as measurement techniques, skeletal muscle measurement sites, and study design. Future work should take into account these limitations to facilitate practical and clinically relevant interventions for preserving and/or improving muscle mass.

To our knowledge, this is the first time muscle density has been reported among individuals with SCI using pQCT technology. Some difficulty arose when analyzing the pQCT scans for muscle density. The pQCT software was designed to analyze bone characteristics, and was manipulated to provide muscle outcomes. As such, the Stratec XCT 2000 software was not sensitive enough to analyze muscle density among the entire cohort of individuals with SCI in the present study. In other words, the algorithm used in the software was unable to find the contours necessary to distinguish muscle density from adipose tissue or bone in 4 of the 12 pQCT scans. The software was able to provide muscle density values among 8 participants (7 men, 1 woman). No pattern was found among the scans that were analyzed vs. those that could not be analyzed. It may be possible to use the pQCT images and analyze them for muscle density using SliceOmatic software, developed specifically for tissue segmentation and body composition analysis. The person performing analysis can visually separate SAT from muscle and then use the segmentation tools to separate muscle from IMCAT, or a composite of both, to determine muscle density in Hounsfield Units. However, the pQCT images must be converted into files that SliceOmatic software can recognize. Future work should determine a means of converting the pQCT files, as well as ascertain an equivalent density value between pQCT and SliceOmatic software (mg/cm^3 vs. Hounsfield Unit).

5.2: Adipose Tissue after Spinal Cord Injury

A high prevalence of obesity was found among the present cohort of individuals with SCI; our findings are consistent with previous reports in that at least two thirds of individuals with chronic SCI are obese (13, 14). With regards to completeness of injury, it was found that a greater proportion of individuals with incomplete SCI were obese when compared to motor complete SCI; however this difference was marginal. Other studies have reported a greater adipose tissue gain and lean tissue loss among individuals with motor complete SCI (67-69). A rationale could be that ~90% of the participants in the current study with motor complete SCI were paraplegic and ~70% with incomplete SCI were tetraplegic. Therefore, the individuals with

motor complete paraplegia may have had the capability of greater independence, accomplishing activities of daily living, and participation in athletic endeavors when compared to individuals with incomplete tetraplegia.

Use of several accepted screening methods has confirmed a prevalence of obesity among individuals with SCI. Therefore, screening for obesity in this population is important, and subsequent action plans for intervention to prevent or reduce obesity-related complications are necessary.

SCI-specific screening tools must be identified and/or combined with selected able-bodied tools, as able-bodied definitions for any given tool may not accurately categorize obesity. For example, when the able-bodied obesity definition of BMI >30 kg/m^2 was employed, <20% were obese despite the fact that over three quarters of the participants had a % body fat in the obese range. A linear regression analysis of % body fat vs. BMI using the present cohort of individuals with SCI showed that a BMI of 22 kg/m^2 was associated with ~29% body fat, and a BMI of 25 kg/m^2 was associated with ~31% body fat. These values of % body fat clearly fall within the definition of overweight/obesity for men of ≥23% body fat, and are close for women of ≥35% body fat. Other studies have found similar discontinuity between able-bodied definitions and SCI diagnosis (33, 54, 190).

Whole body % fat is a dependable means of screening for obesity, as it is a more direct measure of body adiposity and the guidelines can be applied to any population. Two of the 15 participants were "normal" when using the able-bodied definition of % body fat; the same participants plus an additional male were "normal" when applying the SCI-specific definition for obesity of BMI >22 kg/m^2. This additional person (NLI C3-4, AIS C, DOI 29 years) deemed "normal" (BMI 20.92 kg/m^2) had a % body fat of 37.4%. The high % body fat in this individual may be due to the lengthy DOI. Given the large range of functional ability among individuals classified as AIS C, this person may have had limited mobility below the level of lesion (high cervical), contributing to an adverse body composition. The discontinuity of these obesity definitions, even at a lowered BMI cutoff of >22 kg/m^2, raises the persistent concern that BMI is not an ideal screening tool. When using WC as a screening tool, 8 of the 16 individuals were "normal", 6 of which were obese when using % body fat.

Ideally, % body fat should be used as the primary screening tool for obesity among this population. However, SCI-specific BMI and WC are more cost and time effective. Therefore, a

combination of cautiously interpreted SCI-specific BMI and WC may be used to detect obesity after SCI when % body fat is unavailable or infeasible.

Considerable evidence supports the improvement in health outcomes even after modest weight loss in the general population (321). However, the value of such interventions specific to the SCI population is limited. Dietary interventions utilized by the able-bodied population may not appropriately address the special health (124, 322-324) and nutrition (325-327) needs of individuals with SCI. One study assessed the effectiveness of employing a 12-week intervention program including education on nutrition, exercise, and behaviour change, and reported small but significant differences in measures of obesity (BMI, WC, increased HDL-c) (328). The program did not strictly control for diet or exercise intervention, although it included both. Limited evidence exists that exercise interventions such as body weight supported treadmill training or functional electrical stimulation can improve indicators of obesity (329, 330). Aerobic and functional electrical stimulation exercise training may lead to a loss of adipose tissue in parallel with an increase in lean tissue, and it has been reported that habitual physical activity can lead to numerous health benefits that reduce the risk for multiple chronic conditions (331-333). Further, a recent study reported an association between greater leisure time physical activity and lower chronic disease risk among individuals with SCI (334). Pharmacologic or surgical intervention for obesity in SCI has not been reported in the literature (13). The scope of research on interventions specific to the persons with SCI is narrow; future research must evaluate the effectiveness of obesity interventions for this population.

5.3: Bone Tissue after Spinal Cord Injury

One hundred percent and 80% of the participants in the present study had SLOP at the distal femur and proximal tibia, respectively, when employing the WHO criteria of a Z-score <-2.0. These are the skeletal sites common to fracture. When employing a SCI-specific aBMD distal femur fracture threshold (below which fracture begin to occur) and fracture breakpoint (the value at which the majority of fractures occur), almost 80% and 50% of the participants were at risk of fracture. These findings agree with previous studies reporting that over 90% of fractures occur at the distal femur and proximal tibia in the SCI population (22, 72, 73). Only 1% of the current cohort was at risk of fracture based on SCI-specific vBMD distal tibia fracture threshold. The considerable discrepancy between detecting individuals at risk of fracture in the present study

using fracture threshold definitions at the distal femur (g/cm^2) vs. distal tibia (mg/cm^3) may be due to differences in population across studies. Only individuals with motor complete injures were included in the study proposing a distal tibia fracture threshold, whereas the study proposing a distal femur fracture threshold as well as the present study included individuals with complete and incomplete injuries. The completeness of injury has implications on lower extremity body composition such that individuals with motor complete injuries experience greater bone tissue loss (70, 71). Therefore, the participants in the study including only those with motor complete injuries may have had lower distal tibia vBMD, and subsequently the derived fracture threshold may be too low to identify individuals with incomplete injures as being at risk of fracture. Alternatively, the current cohort may simply be at lower risk of fracture than the group in which the threshold was derived, so fewer individuals in the current cohort met the criteria. In addition, a shortcoming of study suggesting the distal tibia fracture threshold is that the bone status was not measured at the time of the fracture; often the fracture(s) had occurred several years prior to the vBMD measurements (35). Therefore, it is difficult to attribute a causal association between distal tibia vBMD and fracture; perhaps the suggested distal tibia fracture threshold is not an accurate estimate of fracture threshold in the present population. A prospective study determining predictors of fracture risk and assessing fracture incidence in a more representative sample is necessary.

It was found that individuals with motor complete SCI had lower aBMD at the distal femur and proximal tibia, as well as lower vBMD at the distal tibia when compared to those with incomplete SCI. These findings are consistent with the literature stating that individuals with complete SCI tend to lose more bone than those with incomplete SCI (70, 71). Therefore, SLOP and possible subsequent fractures are more common among individuals with complete injuries (72). The ability of muscle contraction, weight bearing, or even ambulation among individuals with incomplete injuries may account for the higher BMD when compared to those with complete injuries.

Based on the prevalence of SLOP in the present cohort, we propose that a distal femur Z-score and distal femur fracture threshold should be used to screen individuals with SCI for SLOP. Future studies should include a post-SCI fracture history to determine the prevalence of fracture among those individuals with aBMD values below the fracture threshold/breakpoint. A recent study published that health screening, fracture risk assessment, and determination of knee

region BMD is needed to select patients for treatment (335). The first step is to check for medical history and concomitant medications; any conditions or changes in medications should be treated before moving onto the next step. Investigating lifestyle factors (caffeine intake, smoking history, alcohol intake, mobility change) is the second step; education on any or all lifestyle factors affecting bone health should be addressed. Nutrition and bone factors (knee region BMD, prior fragility fracture) should then be assessed and treated with calcium and/or vitamin D supplements, education on fracture prevention, rehabilitation interventions, or bisphosphonate therapy (335). The findings from the present study of the importance in using SCI-specific aBMD fracture thresholds at the distal femur or proximal tibia is a critical part to this paradigm for treatment of SLOP and/or fragility fracture risk after SCI.

Literature on interventions for bone health has shown preservation of bone or increases in BMD (257, 336-342), but none have reported sustained benefits once the intervention was completed. A regular physical activity program is recommended for any individuals with SCI. Some examples include: an aerobic and resistance training program at an accessible gym with knowledgeable instructors, regular standing in a standing frame, functional electrical stimulation, or body weight supported treadmill training. There is a need for developing interventions for SLOP after SCI that have clear guidelines taking into account NLI and AIS.

5.4: Identifying Risk Factors for Obesity and Sublesional Osteoporosis

As mentioned previously, five risk factors of sex (female) (275, 276), age (≥60 years) (153, 277), DOI (≥10 years) (275), NLI (tetraplegia) (67, 69, 103), and AIS (motor complete) (67-69, 235) have implications for the development of obesity and SLOP after SCI. Based on the data from the present cohort, ≥2 risk factors detected the majority of individuals who were at risk of obesity and SLOP while avoiding false positives. With a larger cohort, relative risk of each factor should be taken into account to better identify those at risk of obesity and SLOP.

Based on the data from the present cohort, we propose that individuals with ≥2 risk factors should be screened for obesity using % body fat from DXA. Percent body fat cutoffs proposed by Gallagher et al., 2000 should be used to classify overweight/obesity. Future work should confirm if these cutoffs can be generalized to all populations, including individuals with SCI. If a DXA scan is not possible, a combination of a cautiously interpreted SCI-specific BMI >22 kg/m^2 and a WC >102 cm for men and >88 cm for women should be used to screen for obesity. Future

work should determine if SCI-specific WC is necessary. Individuals with ≥2 risk factors should also be screened for SLOP and risk of fracture using a distal femur Z-score <-2.0 and distal femur fracture threshold of <0.78 g/cm^2 from DXA. Diagnosing obesity or SLOP early provides the opportunity to intervene in an attempt avoid cardiovascular events (i.e. stroke, heart attack) or fracture.

5.5: Muscle-Bone Unit after Spinal Cord Injury

The theoretical background for a muscle-bone relationship is provided by the mechanostat theory, which proposes that bones adapt their strength to keep the strain caused by physiological loads close to a set point (30). Since the largest physiological loads are caused by muscle contractions, there should be a close relationship between muscle size/strength and bone strength (3).

Based on the theory of the muscle-bone unit in addition to the physiology of muscle and bone after SCI, we would expect to see associations between muscle CSA and bone size/shape variables, but not necessarily between muscle CSA and BMD, at the 1/3 proximal tibia site (66%). This is because the 66% site is made up mostly of cortical bone that is less metabolically active than trabecular bone; therefore, the reduction or cessation of physiological loading from muscle contractions at this site are expected to result in cortical thinning, but not necessarily a reduction in cortical BMD. In addition, a reduction in cortical wall thickness is characteristic of bone loss after SCI, whereas a reduction in cortical vBMD is not (245). Our findings are consistent with this theoretical and physiological explanation for a relationship between muscle CSA and bone size/shape variables (cortical thickness, cortical CSA) at the 1/3 proximal tibia, and agree with a previously reported relationship between muscle area and cortical area at the shaft of the radius among able-bodied men and women (307).

A hypothesis presented by Frost in 1992 asserted that variations in the ratio between cortical bone area and muscle area involve different types of osteoporosis, with differing fracture risks (343). Although the definitions proposed are infrequently used and not verified through prospective studies, the type of osteoporosis may provide information as the importance of muscle loss on bone status of an individual or group. “Harmonic osteoporosis”, or fragility or disuse osteoporosis, can be defined as a cortical bone area to muscle area ratio of >0.05. This kind of osteoporosis implies that there is a harmonic and concordant loss of muscle and bone.

The second is “disharmonic osteoporosis”, or true osteoporosis, which can be defined as a cortical bone area to muscle area ratio <0.05. It has been suggested that this kind of osteoporosis primarily involves bone, and thus may be linked with a higher fracture risk (343). The present cohort was diagnosed with “harmonic osteoporosis” with a cortical area/muscle area ratio of 0.0593. This agrees with the muscle-bone unit theory, as “harmonic osteoporosis” implies that with muscle loss, there is a harmonic and concordant loss of bone. However, the ratio is on the cusp, suggesting that “disharmonic osteoporosis” may also be present. This is reasonable among the present cohort, as individuals with SCI experience denervation to the lower extremities, perhaps contributing to a disproportionate loss of muscle and bone. In other words, the presence of “disharmonic osteoporosis” suggests that bone loss after SCI is not only due to muscle loss.

With regards to the distal tibia site (4%), based on the theory of the muscle-bone unit and on the physiology of muscle and bone after SCI, we would expect to see associations between muscle CSA and BMD or BMC. This is because the 4% site is made up mostly of metabolically active trabecular bone, and is the site of muscle attachment; therefore, the reduction or cessation of muscle contractions will result in a corresponding decrease in BMD at this site. In addition, a reduction in trabecular vBMD is characteristic following SCI (245). There may also be an association between muscle CSA and cortical CSA, however the scanning technology used in the present study does not have high enough resolution to detect changes in cortical thickness at this site. Our findings correspond with this rationale in that associations were found between muscle CSA and BMD or BMC at the distal tibia; these findings are consistent with previous research among able-bodied persons (3).

Given that weak to moderate associations were found between muscle and bone, it is likely that other factors distinct from muscle CSA contribute to bone adaptation in an atrophied state. Some of these other factors include: 1) mechanical loading – most of the participants were incomplete (AIS C-D) and could therefore perhaps weight bear or ambulate, and the participants who were motor complete (AIS A-B) may have spasticity and/or used a standing frame on a regular basis; 2) endocrine function – bone active hormones such as estrogen or testosterone; 3) vascular function; and/or 4) neurological function – bone denervation below the level of the lesion, contributing in part to “disharmonic osteoporosis”.

Nonetheless, the results indicate that muscle atrophy contributes to a reduction in bone strength. The relationship between muscle and bone is clinically important, as muscle strength is

potentially amenable to rehabilitation intervention. The limitations of this analysis include the small sample size (n=41) of a large diversity of age and neurological impairment, the lack of adjustment for potential confounders, and the lack of a measure of functional muscle strength. Future research in this area should incorporate a measure of muscle strength and/or quality, and adjustment for potential confounders such as age, sex, serum hormone levels, DOI, NLI, and AIS.

5.6: Adipose Tissue and Bone after Spinal Cord Injury

No association was found in the present study between indices of obesity (WC, BMI, % body fat) and indices of SLOP (distal femur aBMD, distal tibia trabecular vBMD) among the cohort of individuals with SCI. However, the able-bodied literature suggests that a relationship is biologically plausible. Therefore, the lack of association found may be due to: 1) the limitations in our study of a small sample size and diversity of neurological impairment, both of which increase the risk of type II error, 2) the possible protective effects of hormones and/or limited weight bearing, or 3) simply because an association does not exist.

To elaborate on the second reasoning, some weight bearing or ambulation in addition to a hormonally mediated effect may provide rationale for the attenuation of a relationship in the present cohort. Several studies have reported that increased mechanical loading from excess body weight may provide a protective effect on bone (41-43). Fifty percent of the participants in the present study had motor complete (AIS A-B) SCI and thus were unable to weight bear or ambulate, while the other 50% had incomplete (AIS C-D) SCI and thus may have been able to weight bear or ambulate. If the individuals with motor complete SCI experienced spasticity or accomplished standing on a regular basis, and if the individuals with incomplete SCI were able to weight bear or ambulate, these activities may have provided a partial preservation of BMD from gravitational or mechanical loading.

Further, numerous studies have reported that excess adipose tissue may have a protective effect on bone due to hyperinsulinemia (46, 292) and enhanced production of estrogen (47-51). The hormones insulin and estrogen contribute to increased bone mass. The majority of adults with chronic SCI in the present study had excess adipose tissue, and therefore may have been in a state of hyperinsulinemia and/or had enhanced serum estrogen that may have provided a bone protective effect. In fact, a recent study reported positive associations between adipose tissue

mass and lower extremity bone mass among persons with chronic SCI, which they attributed to hormonally mediated effects (344).

It is possible that some gravitational or mechanical loading in combination with the potential protective effect of enhanced hormones that contribute to increased bone mass among this population resulted in a null association between adipose tissue and bone. Further, the relationship between obesity and osteoporosis has been reported to be dependent on physical activity (stronger among sedentary persons) (299), and therefore the lack of adjusting for physical activity may have impacted our results.

On the other hand, the body composition changes that occur after SCI in combination with the drastically reduced gravitational or mechanical loading provide grounds to support an inverse relationship between adipose tissue and bone. For those individuals with SCI who were unable to weight bear or ambulate, a reduction of gravitational and mechanical forces may attenuate the association found between body weight and bone mass. In addition, the presence of outliers may have influenced the association between adipose tissue and bone in the current cohort. The uniform removal of outliers was not planned a priori. Only one participant was identified as an outlier when using the criteria of a data point more than 2 standard deviations from the mean. This participant had a % body fat more than 2 standard deviations below the mean % body fat of the present cohort; this is uncharacteristically low of an individual with SCI. A combination of genetic factors, physical activity level, dietary intake, socioeconomic status, or DOI may account for the atypical % body fat in this individual with chronic SCI. When the outlier was removed, a trend towards an inverse association emerged, suggesting that with a larger sample size an inverse association may be present.

It is well known that muscle and bone positively respond to mechanical challenges, and that adipose tissue increases with inactivity. On a cellular level, adipogenesis is thought to be the default pathway for mesenchymal stem cells that do not receive simulation to differentiate into other cells of osteoblasts or myocytes (345). It has been proposed that load bearing in the form of low magnitude mechanical signals may inhibit the differentiation of mesenchymal stem cells into adipocytes, as well as have an anabolic effect on bone (346). An animal study reported that after 15 weeks, adipogenesis was inhibited by 27% among mice exposed to loading when compared to controls. This loading suppressed adiposity as measured by both adipose tissue mass and adipose

tissue volume (347). Further, another animal study reported a down-regulation of PPARγ by 27% following 6 weeks of low magnitude mechanical stimulus (348).

These results suggest that it is possible that mechanical loading may facilitate the suppression of mesenchymal stem cells into adipocytes. Therefore in the absence of load bearing activity, such as after SCI, mesenchymal stem cells may differentiate into adipocytes over osteoblasts or myocytes. The outcome is that in the long run, adipocyte production will increase while osteoblast production decreases, providing a possible rationale for an inverse relationship between adipose tissue mass and bone mass in the absence of weight bearing.

Recognizing a relationship between obesity and SLOP is clinically important, as obesity and fracture risk are potentially amenable to rehabilitation intervention. In addition, understanding the relationship may provide insight into mechanisms explaining why some people lose more bone than others, and may lead to future examination of other mechanisms for protection of bone. Future studies exploring the obesity-SLOP relationship among individuals with SCI will need to include an adequate sample size, control for variables such as DOI, NLI, and AIS, and account for any spasticity/regular standing among individuals with AIS A-B and any weight bearing/ambulation among individuals with AIS C-D.

5.7: Limitations and Future Directions

The disproportion of individuals in each subcategory (motor complete vs, incomplete SCI) based on NLI was a limitation in the present study. Only 1 individual with motor complete tetraplegia and only 2 individuals with incomplete paraplegia were included in the present study. Future studies should ensure a large enough sample size in both groups of individuals with motor complete (AIS A-B) and incomplete (AIS C-D) SCI to stratify based on NLI. Further, it would be valuable to have enough participants to stratify based on DOI, as DOI influences body composition changes.

Underpowered studies can bias towards type II error, accepting the null hypothesis when the null hypothesis is false. The sample size in the present study is small, and includes an outlier. These two factors can have a large impact on assumptions drawn. For example, an $r=0$ was found between trabecular vBMD and % body fat among the present cohort of individuals with chronic SCI. However, when one outlier was removed, an $r=0.56$ ($p=0.09$) was found, suggesting that this outlier may have contributed to a null hypothesis. In addition, the small sample size

prevented a regression analysis evaluating muscle-bone and adipose tissue-bone relationships controlling for confounders.

Physical activity is an important determinant of body composition, and the lack of a physical activity measure in the present study is a limitation. Obtaining a larger cohort of individuals with chronic SCI and including a measure of physical activity may contribute to the assessment of chronic disease risk in this population. A recent study conducted by a group who developed a self-report physical activity measure specific for individuals with SCI, reported an association between increased leisure time physical activity and lower chronic disease risk in adults with SCI (334). It may be interesting to look at the relationship between chronic disease risk and participation in all forms of physical activity, instead of only leisure time physical activity. In addition, it would be valuable to include an assessment of dietary intake among individuals with chronic SCI. Few studies have been conducted on dietary interventions after SCI, and to the knowledge of the author, no published studies to date show that a dietary intervention improves body composition.

Finally, it would be valuable for future studies to include a direct measure of VAT using MRI in place of the indirect WC measure in the present study. The VAT value could be used to characterize VAT accumulation following SCI, determine obesity and obesity-related disease risk, and provide an index of obesity to associate with osteoporosis.

5.8: Conclusions

The clinical assessment of body composition in the present study demonstrates a high prevalence of obesity and SLOP among individuals with SCI. SCI-specific definitions for these chronic diseases are limited and not widely recognized. The definition and methods used to assess obesity, SLOP, and fracture risk will affect the number of people identified as at risk, so it is important to develop SCI-specific risk assessment methods that best identify high risk individuals. We propose that an individual should obtain a body composition assessment for detection of obesity and/or SLOP if he or she has ≥2 risk factors (female, ≥60 years, DOI ≥10 years, tetraplegia, and motor complete). The body composition assessment should include a % body fat measure from DXA to identify the presence of obesity. A combination of a carefully interpreted SCI-specific BMI and WC measure may contribute to the assessment, as these measures are easily obtained, and the location of adipose tissue is important. The body

composition assessment should also include a distal femur Z-score and distal femur aBMD measure from DXA to detect SLOP and risk of fracture.

Weak to moderate correlations between muscle and bone were found, even among this relatively small sample, providing support that muscle activity may contribute to bone strength. In addition, the analyzed muscle-bone relationship may provide rationale for future differential diagnosis between "harmonic" or disuse osteoporosis, in which the bone/muscle relationship is usually maintained, and "disharmonic" or other types of osteoporosis in which the proportionality between bone mass and muscle mass may be affected by endocrine, vascular, or neurological influences. No relationship between adipose tissue and bone was found in the current study.

The research presented here provides a comprehensive picture of the body composition changes that occur after SCI in the context of the risk of chronic diseases. The results presented here can inform future initiatives to develop risk assessment methods and targeted treatment strategies.

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APPENDICES

Appendix A

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

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

Bone quality in individuals with chronic spinal cord injury

Lyndhurst Centre 520 Sutherland Drive Toronto, Ontario M4G3V9

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

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

Visit to Lyndhurst

- 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 draw 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 (30-60μSv) 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 be reimbursed $40 per visit each year ($120 in total over the course of the study) to assist with transportation costs to Lyndhurst or Chedoke. For participants traveling to Hamilton from the Toronto area (>50km), transportation is provided and you are welcome to have someone accompany you on the trip. For those wishing to use their own transportation for travel between Hamilton and Toronto, the stipend will be increased to $140 per visit.

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 the 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 PARTICICIPANT/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
Sponsor: Canadian Institutes of Health Research

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

Appendix B

Participant Information Package and Consent Form

TITLE OF STUDY:

Intermittent Whole Body Vibration for Treatment of Sublesional Osteoporosis after Spinal Cord Injury:
Phase II – Safety and Efficacy

INVESTIGATORS:

Dr. B.C. Craven (MD)[1,2], M. Alizadeh-Meghrazi (MSc candidate)[2], J. Totosy de Zepetnek (MSc candidate)[3], L. Giangregorio (PhD)[3], S.L. Hitzig (PhD)[1], M. Miyatani (PhD)[1], A. Morris (PhD)[1], M. Popovic (PhD)[1,2], and L. You (PhD)[2].

STUDY DOCTOR: Dr. Cathy Craven x6122

RESEARCH COORDINATORS: Jude Delparte (MSc)[1] x6359

delparte.jude@torontorehab.on.ca

RESEARCH STAFF: Stephanie Hadi[1,3]

hadi.stephanie@torontorehab.on.ca

SPONSOR:

This study has been funded by the Ontario Neurotrauma Foundation.

DEVICES:

The vibrating platform was manufactured by WAVE® Manufacturing Inc., Windsor, ON

The standing frame, Easy Stand 5000, was manufactured by Altimate Medical Inc., Morton, MN

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[1]Toronto Rehabilitation Institute
[2]University of Toronto
[3]University of Waterloo

INFORMED CONSENT

You are being invited to participate in a research study. This information package explains the purpose of this study, provides information about the study devices, the tests and procedures involved, any possible risks and benefits, and your rights as a research participant.

Please read all the pages in this package carefully. You may take as much time as you wish to make up your mind about whether or not to take part in the study. Ask any questions you may have before signing it. Please ask the study staff to explain anything you do not understand or would like to know more about.

You will be asked to sign the consent form at the end of this package if you are willing to participate. You will be given a copy of this information package and consent form to keep. It may take you 30 minutes or more to read this form.

INTRODUCTION

Osteoporosis of the hips and knees is expected to develop in 85 – 90% of people with traumatic spinal cord injury (SCI). In people with SCI, osteoporosis occurs mostly in the hip and knee regions. Normally, your body continually builds and breaks down bone through a process called bone remodeling or bone turnover. After SCI, this process is interrupted causing the leg bones to become less dense (low bone mineral density) and more prone to fracture. Osteoporosis is a problem we do not see or feel until our bones break. The most common cause of fracture in people with SCI is rolling in their bed or during car transfers. There is no commonly accepted way to treat osteoporosis and prevent fractures in people with SCI.

Treatments for low hip and knee region bone density after SCI include: standing, walking, Functional Electrical Stimulation, bisphosphonates (bone strengthening drugs), calcium and/or vitamin D supplements. Lifestyle modifications, such as smoking cessation or avoidance of alcohol, also help reduce the loss of bone. One prior drug study has shown to increase bone mineral density among subjects with SCI and low hip and knee region bone mineral density.

PURPOSE OF STUDY

The main goal of this pilot study is to see if standing in a standing frame on a vibrating platform 10-13 times a month has positive effects on bone turnover, bone mineral density and bone strength among people with SCI. We also want to see if standing on a vibrating platform has similar positive effects on body fat, leg muscles, leg blood flow, and heart disease risk, as other forms of exercise. The results of this pilot study may inform the design of a larger multi-centre study (therefore allowing for a larger study population and primary and secondary outcomes).

Research into the impact of standing on a vibrating platform on bone health is beneficial in post-menopausal women and children with disability. Standing on a vibrating platform increases blood flow, and improves muscle strength during and after exercise in able-bodied subjects and elite athletes. Other benefits reported in able-bodied subjects include: decreased body fat, increased metabolism, increased muscle activity, and increased blood flow.

No prior studies looking at the benefits of standing on a vibrating platform have looked at its effects on bone, fat and muscle in people with SCI. During the study, many tests will be done to see what harms or benefits, if any, vibration has on your health, bones, fat, heart disease risk, blood flow and muscles. Most of the measurements will look for changes in your legs over time (9 months).

THE VIBRATING PLATFORM

The vibrating platform used in this study is a modified version of a platform that is available for purchase in Canada called WAVE® (WAVE Manufacturing Inc., Windsor, ON; wavexercise.com). This device has been custom-fitted with a standing frame (EASYStand 5000, Altimate Medical Inc., Morton, MN) to allow people with SCI to be able to stand on the vibrating platform.

STUDY REQUIREMENTS & TIME COMMITMENTS

If you take part in this study, you will be asked to come and stand on a vibrating platform with the help of a standing frame about 3 times per week for 40 weeks (9 months) at the Lyndhurst Centre (520 Sutherland Dr., Toronto ON). You will stand for 45 minutes at a time. The platform will vibrate on and off at 1-2 minute intervals during this time period (45 minutes).

Before you begin the study, we will need to make sure that it is safe for you to participate. You will: 1) be asked questions by phone interview; 2) allow us to review your health record; 3) be examined by one of the study doctors; and 4) have a blood test before we can tell if it is safe for you to be in the study.

If the study doctor thinks it is safe for you to take part and you do not stand on a regular basis, you will need to go through a tilt-table training program. This tilt-table training program involves up to 5 tries to get you and your body used to standing again without getting dizzy. After passing the tilt-table training, you can start the vibrating sessions. If you are unable to successfully complete the tilt table training, it will not be safe for you to be in the study and you will be withdrawn from the study.

During this study, we will be conducting many tests to see if there are short-term and long-term benefits or side effects of standing on a vibrating platform. These tests include: blood test, bone density test of your whole body and legs, CT scan of your legs, blood flow test, and measures of leg spasticity,

nerve and muscle activity. A table summarizing the timing of each test is attached to the back of this information package (p.15).

All of the tests will be conducted at Lyndhurst Centre except for one. We will arrange free transportation to the McMaster University Medical Centre via taxi 3 times (at the start, middle and end of the study) for a CT scan of your legs. The Centre is located in Hamilton, ON, at 1200 Main St. West.

Usually your study visits will take about one hour. However, at the start, 3-4 month time point, and at the end of the study, some visits will take up to 3 hours.

YOUR ROLE IN THE STUDY

Being a research subject takes a lot of your time. We will work hard to make it easy for you to be in the study. At times during the study we will ask you to:

- Tell us about your past and current medical history, medications, over the counter medications and supplements.
- Tell us about any new health problems you get during the study.
- Tell us when you can or cannot come for visits and how to get a hold of you.

This information helps to ensure your safety during the study.

STUDY CRITERIA

To be in this study, we need to make sure that you meet our criteria for scientific and safety purposes. In order to take part, you must:

- Be male
- Be between 20 and 55 years of age
- Have an injury due to trauma (i.e., car accident, fall, diving, gunshot, etc.) for at least 2 years
- Have a spinal cord injury between T2 and T10 which is motor complete (ASIA Impairment Scale of A or B)

For safety reasons, we will ask you not to be in the study if:

- You weigh more than 250 lbs (113kg)

- You are less than 5’6” (168 cm) or more than 6’2”tall (188 cm)

- You have now or have had in the past, any of the following conditions:
    - uncontrolled autonomic dysreflexia
    - blood clots (deep vein thrombosis)
    - pressure sores on your legs or ankles
    - dizzy spells when standing (orthostatic hypotension)
    - abnormal heart beats (cardiac arrhythmias)
    - heart valve problems
    - an unhealed broken bone
    - a sliding vertebrae (spondylolisthesis)
    - joint implant (hip or knee replacement)
    - blood sugar problems (diabetes)
    - gallstones
    - pacemaker
    - cancer
    - kidney stones
    - seizures
    - frequent migraines
    - rheumatoid arthritis
    - dislocated hip

- You have a condition that makes it hard for you to stand safely:
    - Bone spur in the hip or knee region
    - Very stiff ankles, knees, or hips

- You are in another study which will affect how we interpret the study results (e.g. Body Weight Supported Treadmill Training or Functional Electrical Stimulation)

If you have not yet done so, a Research Coordinator or a member of the research staff will go over these criteria with you during a telephone interview. Since you may not know if you have any of these conditions, you will need to see a study doctor at a screening visit before participating. At this visit, some of the above-listed criteria will be compared to your medical records.

SCREENING VISIT

A screening visit will be done for you and the study doctor to see if it is safe for you to be in the study. At this visit, you will meet the Research Coordinator and a member of the research staff. They will make sure that all of your questions about the study are answered. They will ask you to sign the consent form at the end of this information package. After talking about the study and signing the consent form, the screening visit will take 2-3 hours.

During or after the screening visit you will be asked to:

- Fill out a few surveys – these ask about your age, gender, type of SCI, education & employment, medications, functional abilities (SCIM questionnaire), medical history, frequency and severity of muscle spasms

(SFSS form), type and frequency of health issues related to your SCI (SCI-SCS questionnaire), and activity levels (PARA-SCI Questionnaire).

- Answer questions about your medical history.
- Have a blood test to ensure your hemoglobin and vitamin D blood levels are in the normal range.
- Be examined by a study doctor to verify the type of SCI you have, grade any pressure sores (NPUAP form), and record the range of motion and spasticity in your legs (if any).
- An ultrasound of the kidneys and bladder to ensure you do not have any kidney stones or gallstones.
- An X-ray of the spine to ensure that you do not have any loose or broken hardware.

If you had an ultrasound of your kidneys in the 3 months before the study or spine X-ray 6 months prior, you do not have to have these tests.

Once all the test results (bloodwork, X-ray and ultrasound) come back, we will let you know if you are suitable/eligible for the study and when you can try the tilt-table program, if needed.

TILT-TABLE PROGRAM

The tilt-table program will help your body get used to standing again without becoming dizzy or lightheaded. You will have up to five tilt table sessions over a two-week period. While on the tilt table, we will check your heart rate, blood pressure and symptoms. If after five tries your blood pressure is too low, your heart rate is too high, or you feel too dizzy, you will not be able to be in the study. Each tilt-table session will be less than one hour. If you complete the tilt table program in two or three visits, the training will stop.

VIBRATION SESSIONS

Each time you come, you will transfer into the standing frame and then the platform will vibrate you. The vibration will occur at 45 cycles per second (45Hz), and move you vertically 0.6mm for 1 minute at a time followed by 2 minutes of rest until 45 minutes have gone by. You will be asked to come to 3 vibration sessions a week or 10-13 times per month for nine months (40 weeks). We hope you will come to a total of 120 vibration sessions. Preliminary data in 10 able-

bodied men and 4 SCI subjects has shown that the vibration settings used in this study are safe for use with people with SCI.

Your blood pressure (BP) and heart rate (HR) will be monitored before, after and every 15 minutes during the sessions. If your BP or HR goes out of range, we will ask you to take a break from the vibration and may ask you to stop the vibration session that day if your BP or HR does not come back to normal. Someone will be present at all times to assure your safety and help you with transfers, if needed. Each visit, we will ask you if you had any of the following side effects during the vibration sessions:

- Inner ear troubles
- Dizziness
- Blurred Vision
- Pain
- Itching
- Headache

All participants in the study will be given a free pair of shoes (Crocs™) to wear during the vibration sessions. These shoes are being used to make sure that everyone participating in the study has a similar material on their feet between them and the vibrating platform. The shoes are yours to keep after the study.

If you miss a few sessions due to illness, you can stay in the study. If you miss too many sessions so that your study data is hard to interpret, you will no longer be in the study. A study team member, or Dr. Craven, will talk to you about this issue if it comes up.

ENTRY AND EXIT MEASUREMENTS

Since we want to see how your bone, muscle and fat changes during the study, you will be asked to have many tests at the beginning and end of the study. The following tests/actions will be done over the course of 2 or 3 visits before and after the 9 months of vibration (total of 4-6 measurement visits):

- Two-dimensional bone and body composition scan (DXA)
- Three-dimensional bone, fat, and muscle leg scan (pQCT)***
- Blood test to look at bone health and metabolism
- Urine test of bone turnover (urine sample)
- Assessment of spasticity (Ashworth, SFSS questionnaire, pendulum test)
- Assessment of blood flow in your trunk and legs (PWV)
- Nerve activity (H-Reflex)
- Muscle activity (EMG)
- Measurement of your weight, height and waist circumference

*** Please note that pQCT will be conducted at McMaster University Medical Centre (1200 Main St. West, Hamilton, ON) which is about 85km from the Lyndhurst Centre.

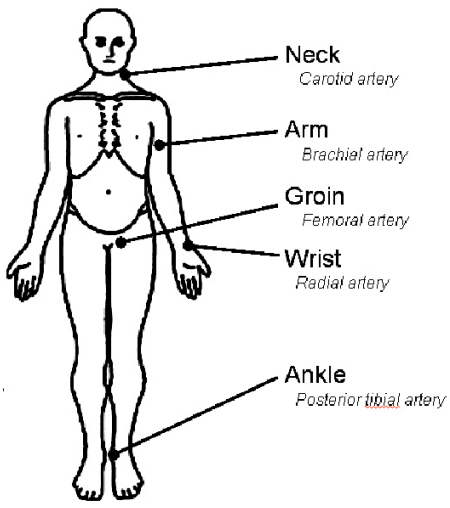
BLOOD DRAWS AND URINE SAMPLES

Blood and urine will be collected on days when you are coming for a vibration session and will add about 15 minutes to your visit. We will draw your blood and ask for a urine sample to test at the start of the study, 3 months, 6 months, and 9 months (end of study).

On the days that you come in for blood work, we will ask that you fast (no eating) starting at 11:00pm the night before. All blood measures will be done between 10:00am and 12:00pm. We will ask that you bring a small snack so that once the blood work has been completed, you may eat a little bit before the vibration session. If you have diabetes, we will allow for you to take some of your medications with water and a piece of bread. At the start of the visit, we will take up to five tubes of blood (30mL = 6 tablespoons) while during other visits we will take four tubes of blood (25mL = 5 tablespoons).

We will ask you to bring in your first morning urine to us. We will give you a container to collect it in before you need it. Urine samples will be used to look for indicators of bone health. Blood drawn will be analyzed for indicators of sugar and lipid metabolism and bone health. We will give you a list of all the indicators upon request. You will not see the blood and urine results until after the study is over. We will give you a list of all the results after the study is over, if you would like.

MEASUREMENTS OF BLOOD FLOW



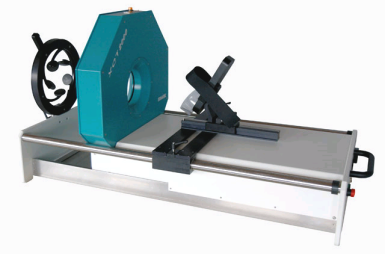
Blood flow will be measured by using a method called pulse wave velocity (PWV). PWV is the speed at which blood pressure waves travel in the arteries within your body. It is a simple, painless way of measuring the stiffness of your arteries. For this test you will be asked to fast (no eating) for 6 hours and to avoid alcohol, caffeine, and nicotine for 12 hours before the test. You will also be asked not to do any heavy exercises during this time period. During the PWV test, you will rest on your back for about 20 minutes in a quiet room. Two small sensors will be held on your skin to determine the flow rate between 1) your neck and groin, 2) groin and ankle, and 3) your upper arm and wrist.

PWV will be measured before and after vibration at the start, end and 3 month time points. Each time, PWV will be measured 2 times per visit: before vibration and again 20 minutes after vibration. PWV will also be measured before the regular vibration sessions start. It will be measured in response to standing in a standing frame for 45min without vibration. On days when PWV is measured, you will be at the Lyndhurst Centre for 2 more hours than a regular visit.

TWO-DIMENSIONAL BONE AND BODY COMPOSITION

You will be asked to have a whole body bone density and body composition scan at the start, 4 month time point and end of the study. Bone density scans are x-rays that measure how much bone mineral you have in your bones. 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 onto the scanning table. If you are not able to transfer yourself, we will use a lift. You will not feel anything when the scanner is on. This test will take about 45 minutes. The machine does the work to measure your muscle and bone mass from one set of scans.

THREE-DIMENSIONAL BONE, MUSCLE & FAT COMPOSITION



These visits will take place at McMaster University Medical Centre (Hamilton, ON). The visit will take about 30 minutes for the scan plus your travel time to Hamilton. The study will pay the cost for each of the three visits for you to go to Hamilton via wheelchair taxi and take you home after the scan is done. These tests will be done at the start, at the 4-month time point, and at the end of the study.

During this visit, you will have one set of three scans that measure the shape and structure of your bones with a scanner called peripheral quantitative computed tomography (pQCT shown). For these scans, your leg will be positioned in the scanning device and held there with a strap at the ankle and plastic holder at the knee. A researcher will take three scans, one at your ankle, the second at mid-calf, and the third at the widest cross-section of your calf. We will conduct the scans while you are seated in a chair or your wheelchair. You will not feel anything during the scans. People with thick calves or severe spasticity may find this test hard or impossible to do.

If the diameter of your right calf is greater than 14 cm, or you have a metal implant in your right calf, or have broken your shinbone in the past, or have severe leg spasms and are allergic to lorazepam (Ativan), you will not be able to have the peripheral quantitative computed tomography test done. This will not affect your ability to be in the study.

During the peripheral quantitative computed tomography scans, it may be difficult for the technologist to position you if you have leg spasms. If you have severe leg spasms, you will be given a small dose of lorazepam (0.5-1.0 mg below the tongue) to prevent spasms while the scan is taking place. 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. If needed, the lorazepam will be prescribed for you by Dr. Craven and given to you by her or the hospital pharmacist on the day of your scan.

These safety measures are taken to reduce your chance of injury if a spasm occurs when your leg is in the scanning device. You do not have to agree to take lorazepam. However, we may decide not to do the scan if your spasticity limits our ability to position you safely.

If you need lorazepam to reduce your leg spasms during the pQCT scans, there is some 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 be provided to you at no cost. After taking lorazepam, the study staff will monitor you for an hour or so, to make sure you do not have any side effects.

If you do not take lorazepam regularly, you should not drive or perform other tasks that require alertness for two hours after taking lorazepam. Also, you cannot take lorazepam if you are currently taking the fungal medications called Ketoconazole or Itraconazole.

H-REFLEX

An electrode will be placed on the skin of your calf muscle to measure the Hoffman reflex or H-reflex. The H-reflex is a measure of how the muscle reacts to a small electric current. To measure this reflex, we will stimulate the nerve with an electric current that will gradually increase in intensity until your muscle contraction is visible or you ask us to stop. During this test you may feel a stinging sensation behind the knee where the electrode is placed. H-reflex will be measured at study entry and exit and takes about 15 minutes to do.

MUSCLE ACTIVITY

We will look to see how your leg muscles respond to vibration from the platform. To measure your leg muscle activity, four pairs of electrodes or sensors will be placed on your skin over the front and back of the thigh and calf (upper and lower leg). To make sure we get a good signal, your skin will be prepared by washing it with a cleansing gel and then rubbing it gently with fine sandpaper. The sensors will be connected to an electromyogram (EMG) which will record the electrical activity of your muscles when standing and vibrating. You will be asked to wear suitable clothing for the study (i.e. athletic clothing, track pants/shorts, and not jeans). Muscle activity will be measured in months 1 and 9 and will add about 30 minutes to your regular vibration session on these two days.

SPASTICITY ASSESSMENTS

Three separate spasticity tests will be performed: Modified Ashworth Scale, SFSS and Pendulum test with shape tape. The three tests will be done before and after your vibration session at the start, 3 month time point and end of the study. The MAS test is scored by a trained rater using a 5-point scale. You will lie on your back on a plinth while they test the spasticity of your legs, including the hip abductors, hip adductors, hamstrings, quadriceps, and ankle plantar and dorsiflexors. During the Pendulum test with shape tape, we will tape a cable to the side of your leg, drop the lower half of your leg off the edge of a table, and watch it swing until it stops, a computer will record the rate your leg drops and how quickly it stops moving. The SFSS is short questionnaire that you will be asked to answer about how often and how bad your spasms are. The three tests take 15 minutes to complete.

WAIST CIRCUMFERENCE

A measuring tape will be used to measure your waist while laying down. You will be asked to roll up your shirt so that the measurement can be taken in line with your lowest rib by a research staff member. This will take 1-2 minutes to do the measurement and record the value. We will measure this at the start of the study, after 4 months, and at the end of the study.

ACCELERATION

We want to know how far vibrations from the device travel up your legs. This has been measured in able-bodied people who have normal muscle tone in their legs. We are interested in finding out if the vibrations travel through the body differently in people with SCI who have increased muscle tone in their legs. To do this, we will use four accelerometers. These accelerometers are small boxes connected to a computer that measure movement. They will be attached to your

leg at the ankle, knee, hip and forehead with the use of tape and a headband. We will do this in the first month and the ninth month.

INTERVIEW

At the start of the study, you will be asked to do a 30 minute interview about your reasons for being in the study and your expectations of the device. Again at the end of the study, you will meet with the same interviewer who will ask you about your experiences during the study, if you find it has benefited you, and if you have any comments about the device.

POSSIBLE BENEFITS

We cannot promise any personal benefits to you from your participation in this study. However, your participation may help other people with SCI in the future. Individuals with SCI who have participated in standing programs have told us they benefited. They told us they had reduced spasticity, improved bowel and bladder function, fewer digestive complaints, less pain and lower limb swelling, and better hip range of motion.

POSSIBLE RISKS AND DISCOMFORT

Because no one has done a study like this one, we may not know about some risks. There are some risks we do know about, they are listed below:

Vibration

Vibration causes side effects. The known side effects of vibration include: inner ear problems, dizziness, headache, worsening of existing lower limb spasticity, fracture, or short-term loss of hearing. Although we think it is possible but it has never been seen before, your hardware could loosen. If this happens, the Study Doctor will refer you to a spine surgeon.

If you have had in the past or develop any of the following conditions it will not be safe for you to be in the study: chronic migraine headaches, kidney stones, cochlear implant, deep vein thrombosis, irregular heart beat, cancer, heart valve disease, rheumatoid arthritis, gallstones, pacemaker, joint implant, diabetes, pregnancy, or seizure.

There may be some extra risks when you are vibrated without having eaten for a while. These include light-headedness, dizziness, and fainting because of low blood sugar. During each vibration session, we will have juice and cookies handy in case you start feeling like this.

Radiation

The bone density (DXA) and computed tomography (CT) scans involve small amounts of radiation. The level associated with the scans proposed in this study is ~26μSv for each set of bone density scans and ~1μSv for the CT scans. Since the bone density and CT scans are performed three times during the study the total dose is ~80μSv. This is about the same as getting one chest X-ray (30-60μSv). Compared to what you usually get exposed to naturally over a year (2500 μSv), this is very little. Radiation exposure adds up but the total radiation dose from the study scans is low and has minimal risk.

Standing

Possible side effects related to standing include, but are not limited to the following: autonomic dysreflexia, low BP, pain, a broken bone, skin breakdown or pressure sores, and swollen feet and/or ankles.

There is a chance that you might get a pressure sore on your knees or feet during the study. We will regularly check your knees and feet for pressure sores and if present record their severity with the National Pressure Ulcer Advisory Panel Pressure Ulcer Scale. If you develop a sore we will refer you to our skin and wound clinic for advice and monitoring.

Blood Draws

You may get pain during the blood draws or a bruise at the site where the needle goes in. There is a small risk of infection after a needle stick. You may need to have more than one needle stick to draw your blood.

Pulse Wave Velocity

You might be uncomfortable during the PWV testing as a staff member will place the ultrasound device on your neck and groin area. We will try to make this as comfortable as we can. Very few people were worried by this in prior studies.

Electrodes

Sometimes, we have to shave a small area of the skin where the electrode is applied if you have hairy legs.

Again, there may be some side effects of which we are likely unaware. We have used vibration and standing in a previous study with rare serious side effects (1 in 60 patients). Please report any side effects or discomforts if they occur to Dr. B.C. Craven at 416-597-3422 x6122 or any of the study staff members.

YOUR RIGHT TO ASK QUESTIONS

You may ask questions about this consent form or the study, now or at any time during the study. If you have questions about this study or if there are any problems or injuries associated with this study, you may call Dr. Craven at (416) 597-3422 x6122.

If you have questions regarding your rights as a research subject that you wish to discuss with someone not involved with the study but who has reviewed the study, you may call Dr. G. Tardif, Chair, Research Ethics Board at (416) 597-3422 x3730.

COST OR PAYMENT TO YOU

There will be no charge for taking part in this study or for any of the tests performed. Taking part in this study will not change the way you pay for your health care. You will not be paid to take part in this study. You will get $10.00 per study visit to cover some of your transportation expenses to Lyndhurst Centre (travel/parking). You will receive payment for each visit at the end of each month of participation in the study. If you decide to drop out of the study, you will be paid for the visits you attended.

RIGHT TO WITHDRAW FROM STUDY

Taking part in this study is your choice (voluntary). You may refuse to be in this study. You have the right to withdraw from this study at any time, and for any reason. Withdrawing from this study will not affect your medical care or your ability to take part in future research at Toronto Rehab.

It is also important for you to know that the investigators may decide not to use some of your data from the study if there is not enough data to analyze. Additionally, if at any point the study doctor(s) decides that starting or continuing in this study would be harmful to you, you will be asked to withdraw from the study.

If you decide to withdraw from the study before completion, you may be asked if we could collect some final data. We hope that you will at least agree to have a spinal X-ray to make sure that the vibration did not loosen any hardware.

If you should decide to withdraw from this study or are asked by your family doctor to leave the study, you are encouraged to contact the study doctor Dr. B.C. Craven immediately at (416) 597-3422 x6122 or the Research Coordinator at (416) 597-3422 x6359.

CONFIDENTIALITY

Your study 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 Toronto Rehabilitation Institute Research Ethics Board may review 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.

Dr. B.C. Craven or a delegate will store a paper and electronic (computer) copy of your results for seven years. These results will not be part of your hospital chart. Dr. B.C. Craven or a delegate will review your medical records, if necessary, to verify the information collected. Sponsors and manufacturers will not have access to the data generated in this study until it is in its published form.

At your request, we will share the study purpose and or your study results with your treating physician during or after the study.

A copy of the attached consent form will be inserted in your hospital chart.

STUDY VISITS

			Week							
		Screen	**0**	**1**	**13**	**17**	**20**	**26**	**40**	**41+**
Screening										
	Verbal Consent	X								
	Pre-screening Form	X								
	Informed Consent	X								
Safety Assessments										
	Medical History (HO)	X								
	Physical Exam (Contractures)	X								
	Kidney Ultrasound	X								
	Bladder Ultrasound	X								
	Spinal X-ray	X								X
	Serum Screening (blood test)	X								
	Tilt-table	X								
	Pressure sores score	X								X
	Current Medications	X	X	X	X		X	X	X	X
	Adverse Events	X	X	X	X		X	X	X	X
	Accelerometry			X					X	
Bone Outcomes										
	Bone Biomarkers		X		X			X		X
	DXA – bone scan		X			X				X
	pQCT – bone scan		X			X				X
Metabolic Syndrome and CAD										
	MetS Biomarkers (blood test)		X		X					
	Pulse Wave Velocity		X	X			X			X
	Blood pressure/Heart Rate	X				X				X
Adiposity										
	pQCT – muscle and fat scan		X			X				X
	DXA - body composition scan		X			X				X
	Waist Circumference	X				X				X
	BMI (height, weight)	X				X				X
Spasticity (muscle stiffness)										
	Ashworth test		X		X					X
	Pendulum Test		X		X					X
	Stiffness Questionnaire		X		X					X
Neuromuscular Function										
	sEMG (muscle activity sensors)			X					X	
	H-Reflex			X					X	
Questionnaires										
	SCIM-III	X								X
	SCI-SCS	X								X
	PARA-SCI		X							
Qualitative Interview										
	SCI Subjects		X							X
	Researchers									X

CONSENT STATEMENT

TITLE OF STUDY: Intermittent Whole Body Vibration for Treatment of Sublesional Osteoporosis after Spinal Cord Injury: Phase II – Safety and Efficacy

I have read this consent form. My questions regarding this study have been answered. I voluntarily consent to take part in this study involving the treatment and procedures described above, with an understanding of the known possible risks that might occur, and recognizing that not all such risks may be completely known. I am aware that I may not benefit directly from taking part in this study.

By signing this consent form I am not giving up any legal rights. I also understand that nothing in this consent form is intended to change any applicable federal, provincial or local laws regarding informed consent. I will receive a copy of this consent form to keep.

I hereby consent to participate.

_____

My Name (Please print)

_____ _____

My Signature (Date)

_____

Printed Name of Person Obtaining Informed Consent

_____ _____

Signature of Person Obtaining Informed Consent (Date)