NOTE TO USERS

MUSCULOSKELETAL CHANGES AFTER SPINAL CORD INJURY: EFFECTS OF BODY WEIGHT SUPPORTED TREADMILL TRAINING

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Abstract

The overall focus of this thesis was musculoskeletal changes after individuals with spinal cord injury. The bulk of the thesis investigated the impact of a rehabilitation intervention, namely body weight supported treadmill training (BWSTT), on skeletal health and muscle atrophy in individuals with acute and chronic spinal cord injury (SCI). The first two studies in this thesis were methodological in nature. The first revealed that the presence of metal in a dual-energy x-ray absorptiometry scan results in reproducible errors in estimations of bone mineral content and body composition. The second study demonstrated that mid-tibia speed of sound measurements obtained with quantitative ultrasound might not reveal the changes that occur in the skeleton after SCI. These studies impart an important contribution to the area of osteoporosis diagnosis in individuals with SCI. The two longitudinal prospective studies presented here add to the growing body of literature that BWSTT can improve ambulation in individuals with chronic and acute incomplete SCI. Individuals participating in regular BWSTT improved their walking abilities on the treadmill, and some individuals improved their over ground walking abilities. The studies in this thesis are the first to investigate the impact of BWSTT on bone and muscle together in individuals with acute and chronic SCI. BWSTT had a positive impact on muscle, in that significant increases in lean mass and muscle cross-sectional area were observed after training in both acute and chronic SCI. BWSTT did not appear to have an impact on the skeleton, but further research is required to confirm these findings. Ultimately, it appears that individuals with SCI stand to benefit from BWSTT and the benefits are not limited to improved ambulation.

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Glossary of Terms and Abbreviations

ASIA – American Spinal Cord Injury Association
BMC – Bone mineral content, in grams
BMD – Bone mineral density, in grams per centimetre squared if measured using DXA, and in grams per centimetre cubed if measured using computed tomography
BUA – Broadband ultrasound attenuation
BWS – Body weight support
BWSTT – Body weight supported treadmill training
Cr – creatinine, in mmol
CSA – cross-sectional area, in millimetres squared
CT – Computed tomography
DPD – Deoxypyridinoline, in mmol DPD per mmol of creatinine
DXA – Dual-energy x-ray absorptiometry
FES – Functional electrical stimulation
g - grams
I_{max} – Area-weighted maximum moment of inertia, in millimetres to the power of 4
I_{min} – Area-weighted minimum moment of inertia, in millimetres to the power of 4
I_{pol} – Area-weighted polar moment of inertia, in millimetres to the power of 4
MANOVA – multiple analysis of variance
OC – Osteocalcin, in ng/mL
RMSSD – Root mean squared standard deviation
RMSCV – Root mean squared coefficient of variation
SCI – Spinal cord injury
SEM – Standard error of the mean
SOS – Speed of sound, in metres per second
WHO – World Health Organization

1.0 CHAPTER 1: BACKGROUND

1.1 Spinal Cord Injury

1.1.1 Introduction

According to the Canadian Paraplegia Association, over 1000 people per year will sustain a spinal cord injury (SCI) in Canada. Vehicular accidents account for approximately 55% of these injuries, and approximately 18% occur as a result of falls or industrial accidents. Although spinal cord injuries are more common in younger individuals, with 78% occurring between the ages of 15 and 34, the mean age of individuals sustaining SCI is rising due to the increasing number of older individuals experiencing SCI as a result of disease and other medical conditions (27). SCI is a complex problem, in that it can occur in either gender, at any age and at varying levels of severity. The disabilities and morbidities associated with a SCI are unique to each individual. This variability, coupled with the relatively small prevalence of SCI in the population, results in a general lack of awareness about SCI and its many problems and manifestations. Some of these problems are discussed in the next few paragraphs, starting with a brief description of some SCI-related terms.

A SCI can be classified as complete or incomplete. With a complete injury, there is no preservation of motor or sensory function in the fourth and fifth sacral segments. An incomplete injury refers to one where there is preserved motor or sensory function below the neurological level of the injury, and includes the lowest sacral segment. A classification system for injury severity is provided by the American Spinal Injury Association (ASIA) and a person with a SCI can have a score on this classification system of A, B, C or D (refer to Table 1) (47). Brown-Sequard syndrome refers to a lesion producing greater ipsilateral proprioceptive and motor loss with contralateral loss of sensitivity to pain and temperature. Tetraplegia (a preferred term to quadriplegia) refers to damage or loss of function in the neural elements of the cervical segments of the spinal cord, resulting in impaired function in the arms, legs, trunk and pelvic organs. Paraplegia refers to damage or loss of function in the neural elements of the thoracic, lumbar or sacral segments of the spinal cord, and depending on the level of injury, impaired function in the trunk, legs and pelvic organs can occur. In 1999, 53% of injuries to the spinal cord resulted in paraplegia, and 47% resulted in tetraplegia (27).

Table 1: American Spinal Injury Association Impairment Scale

ASIA Classification	Description of Impairment
A = Complete	No sensory or motor function is preserved in the sacral segments.
B = Incomplete	Sensory but not motor function is preserved below the neurological level and extends through the sacral segments S4-S5.
C = Incomplete	Motor function is preserved below the neurological level, and the majority 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 the majority of key muscles below the neurological level have a muscle grade greater than or equal to 3.

E = Normal	Sensory and motor function is normal.

1.1.2 Morbidity After SCI

A person with SCI may experience several secondary physiological and psychological outcomes, the list of which can be extensive. A few of the recognized changes are summarized here. Carbohydrate and lipid abnormalities are a more common occurrence in the SCI population than in non-SCI individuals. In one study, individuals with SCI had higher mean glucose and insulin values during oral glucose tolerance tests than non-SCI individuals (10). Those individuals with SCI who developed carbohydrate disorders did so at younger ages than non-injured controls (10). Reduced insulin sensitivity in individuals with SCI when compared to siblings has also been documented (102). As well, individuals with SCI have been reported to have reduced high-density lipoprotein (HDL) levels. Individuals with tetraplegia had lower HDL levels, and higher total cholesterol to HDL ratios than individuals with paraplegia, suggesting that the extent of lipid changes was related to the level of neurologic lesion (9). Carbohydrate and lipid abnormalities may place individuals with SCI at increased risk for cardiovascular disease.

The remarkable muscle atrophy that occurs after SCI is likely at least partially to blame for the reduced metabolic rates observed in individuals with SCI, as well as the incidence of decubitus ulcers (94;137;163). Autonomic dysreflexia is a potentially fatal condition that can affect individuals with an injury above the 6th thoracic segment (101). In brief, autonomic dysreflexia occurs when stimulation of the bladder or other internal organs causes increased sympathetic nervous system activity, resulting in profuse sweating, bradycardia, headache and severe hypertension, potentially leading to a cerebrovascular accident and death if not properly managed. Spasticity occurs commonly among individuals with SCI, particularly among those with incomplete injuries, and can be problematic (168). Other secondary complications include urinary tract infections, decreased bowel and bladder functions, sexuality concerns and pain (94;200). The occurrence of SCI imposes changes to a person's life which require a tremendous psychological adjustment (143). Recent research has demonstrated that quality of life after SCI is influenced by age, fatigue and the number of health problems a person experiences, as well as whether they have a perception of accelerated aging (130).

SCI is often considered primarily a male problem, as 81% of new injuries occur in males. Osteoporosis is often considered a female disease, yet the largely male population of individuals with SCI often manifests osteoporosis of the lower limbs. This presents an unusual problem for health care providers, who are unable to define fracture risk in this population. The next few sections include a brief description of the form and function of bone and the definition of osteoporosis.

1.2 Bone and Osteoporosis

On a microscopic level bone is composed of both organic and inorganic material. The inorganic component of bone comprises roughly 70% of bone tissue by weight, and is mainly composed of hydroxyapatite, a calcium phosphate mineral. Water accounts for 5 to 8% of bone tissue and the remaining 22 to 25% is comprised of the extracellular matrix, or organic component of bone tissue, which contains type I collagen, non-collagenous proteins and bone cells (115). At the macroscopic level the external shell of a bone is mainly composed of cortical (or compact) bone; 80-90% of its volume is calcified, making it a very dense, calcified tissue (8). The diaphysis, or midshaft of the bone is made up mainly of cortical bone. The bone ends are called epiphyses, and in between the diaphysis and epiphysis is a region called the metaphysis. Towards the ends of the bone the cortical shell thins and the space inside is filled with trabecular bone tissue. Trabecular bone consists of thin, calcified trabeculae that form a latticework-like arrangement. It also contains bone marrow, blood vessels and connective tissue (8). Approximately 80% of the mass of the skeleton is comprised of cortical bone, with the remaining 20% being trabecular bone (8).

The surfaces of bone tissue interface with soft tissue. The external surface of the bone is called the periosteum, and the internal surface is called the endosteum. Most bone tissue turnover occurs on these bone tissue surfaces. Consequently, since the surface area in contact with bone marrow is greater in trabecular bone it is much more metabolically active than cortical bone (8;115). Osteoblasts, osteocytes and osteoclasts are three distinct cell types that are found in bone and regulate its metabolism. Osteoblasts are bone-forming cells that produce osteoid, a protein matrix that is subsequently mineralized. Osteoblasts are also known to initiate bone resorption, and can therefore be considered regulators of bone turnover. Once the synthesis and mineralization of osteoid by the osteoblast is complete, the osteoblast becomes an osteocyte. Osteocytes are connected to each other and to bone lining cells on the surface by cell processes (115). Osteoclasts are multinucleated cells that are active in bone resorption. They synthesize acids and lysosomal enzymes that dissolve hydroxyapatite crystals and digest bone collagen and matrix (8).

Bone remodeling is the removal and replacement of bone tissue by bone cells. It is important for maintaining mineral homeostasis and biomechanical competence of the skeleton. A complete remodeling cycle takes approximately 3-6 months. The bone metabolic unit, or BMU, goes through a cycle of four phases: resorption, reversal, formation and quiescence. Resorption begins with the activation of osteoclasts and the breakdown of bone tissue via acids and lysosomal enzymes. Reversal consists of the formation of a cement line that will cement together the old bone and the new bone to be formed. The resorption cavity created by the osteoclast is then filled by the production and mineralization of osteoid during the formation phase. The end result is a remodeled packet of bone that enters the resting phase, or quiescence (8). Under normal conditions bone resorption and formation are tightly coupled. Osteoblasts attempt to replace the exact volume of bone that was removed by the osteoclast. When these processes become

uncoupled and the amount of bone resorbed is greater than the amount of bone formed, bone is lost. If this continues over time, osteoporosis can result (115). The National Institutes of Health consensus conference has modified the original definition of osteoporosis to include "a skeletal disorder characterized by compromised bone strength, predisposing a person to an increased risk of fracture. Bone strength reflects the integration of two main features bone density and bone quality" (86).

Clinically, the diagnosis of osteoporosis is made using bone mineral density (BMD) measurements obtained using bone densitometry (refer to Chapter 2), and/or the presence of a fragility fracture. A fragility fracture is one that occurs in the absence of major trauma, such as during coughing or as a result of a fall from standing height. Measurements of BMD can be expressed as T-scores (standard deviation units or SD units). The World Health Organization (WHO) defines osteoporosis as having a BMD T-score at the spine, hip or radius that is 2.5 SD or greater below the mean of a healthy young adult reference population. Osteopenia is defined by a BMD T-score at the spine, hip or radius that is 1.0 to 2.5 SD below the mean of a healthy young adult reference population (107).

The skeleton's main functions are to provide mechanical support and attachment sites for muscles and to maintain serum mineral homeostasis by acting as a reserve for calcium and phosphate. It also protects internal organs and houses the bone marrow, the site for hemopoiesis. The structure and amount of bone reflects a balance between its major functions. Therefore, as discussed in the section on bone and exercise later in this chapter, remodeling will remove bone when the mechanical demands on the skeleton are low or mineral homeostasis requires a release of mineral from bone. Instances where uncoupling of bone resorption and formation occur with net losses of bone can result in a reduction of a bone's structural strength and an increased risk of fracture (115). The following sections outline the changes in bone and muscle following SCI.

1.3 Osteoporosis in SCI

1.3.1 Bone Mass Loss After SCI

According to both cross-sectional and longitudinal studies, bone mineral is lost in the lower limbs following SCI (18;62;111;175;201). Cross-sectional studies have revealed that individuals with SCI have significantly reduced lower limb bone mineral density (BMD) when compared to control subjects (73;104;160;174;175). The rate of bone loss has been reported to be rapid and linear in the acute stages, establishing a lower steady state bone mass level 1-2 years after the event (73). However, significant bone loss has been reported many years after SCI indicating that bone loss may not plateau as previous studies had reported (73;160;174).

The magnitude of bone lost after SCI is substantial and has been characterized in several cross-sectional studies. A large cross-sectional study revealed that proximal femur BMD values in individuals with SCI were 16-18% lower than controls and the time course of bone loss was dependent on the age of the individual at the time of injury (174;175). Younger individuals tended to lose more bone following SCI than older individuals. Another large cross-sectional study demonstrated that post-SCI bone

densities at the femoral neck, midshaft and distal femur were 27%, 25% and 43% lower than the respective values among controls (104). A monozygotic twin study demonstrated that total body BMD was reduced by 18% ± 7% in the SCI twins compared to the non-SCI twins, and at the legs and pelvis, BMD values in the SCI twins were reduced by 35% ± 10% and 30% ± 9%, respectively (11). The leg bone loss was significantly associated with injury duration. More recently it was determined that of 41 individuals with SCI, 61% met the World Health Organization (WHO) criteria for osteoporosis and 19.5% were osteopenic (111). The remaining 19.5% of individuals with SCI in that study had normal bone mass, perhaps reflecting the inclusion of a broad range of ASIA classifications and the influence of residual motor and/or ambulatory function. Changes in the microarchitecture of bone after SCI have also been reported. In men with long-standing complete SCI, trabecular bone microarchitecture at the distal femur and proximal tibia was significantly different from control subjects. Moreover, men with SCI had fewer trabeculae and greater trabecular separation, resulting in a lower ratio of bone volume to total volume (133).

Significant loss of lower limb bone after SCI has been confirmed in prospective longitudinal studies (18;40;62;201). In paralyzed limbs, reductions in bone mineral content (BMC) of approximately 4% per month in trabecular bone and approximately 2% per month in cortical bone have been documented during the first year after SCI (201). Trabecular BMD at the tibia has been documented to decrease by 5% and 15% at 6 and 12 months after SCI, respectively, and cortical bone decreased by 7% at 12 months post-injury (62). Within two years of a SCI, mean decreases of 35.3% and 12.9% in the trabecular and cortical bone of the tibia, respectively, have been recorded (40). It is important to point out that there are considerable inter-individual variances in the amount of bone loss that occurs after SCI. Tibia trabecular bone losses within 2 years of SCI ranged from 0.4% to 80%, and cortical bone changes ranged from a 1.7% increase to a 32.7% decrease (40).

Several factors may influence the loss of bone after SCI. The degree of bone loss has been demonstrated to be associated with the degree of post-traumatic immobilization and the time post-injury (39). Early mobilization may reduce the amount of bone lost (41). Individuals with incomplete SCI tend to lose less bone than individuals with complete SCI (42;72;160). A prospective longitudinal investigation indicated that less bone is lost when there is partial recovery. Pelvic and lower limb BMD deficits were 40-45% and 25%, respectively, at one year after SCI, but in those who made a partial recovery the losses were 30% and 10% (201). A cross-sectional study demonstrated that bone mineral densities in individuals with SCI were positively correlated with mobility assessed via a mobility index ranging from complete paralysis to unlimited ambulation (161). Although increased spasticity may preserve muscle mass in individuals with SCI, it does not appear to preserve bone (201).

Bone lost after SCI is site-specific, with the largest decrements visible in the lower limbs. Upper extremity loss is often only noted in tetraplegia. Significant

differences have been noted in upper extremity bone status when comparisons were made between para- and tetraplegic individuals (42;62;177). A 12-month prospective study demonstrated trabecular bone losses of 19% and 6% and cortical bone losses of 3-4% at the radius and ulna, respectively, after SCI in tetraplegic patients (62). Lumbar spine BMD has been documented to be increased, decreased or unchanged after SCI (11;18;39;72;117;160;176). The extent of bone lost within lower limb sites may vary. For example, bone lost at the proximal tibia and distal femur has been demonstrated to be greater than losses at the proximal femur (18;72).

1.3.2 Biochemical Changes After SCI

The bone formation markers bone alkaline phosphatase (BAP), osteocalcin and type 1 procollagen propeptide (PICP) reflect bone matrix maturation, bone matrix mineralization and type 1 collagen synthesis, respectively (24). Osteocalcin levels are low or normal in the first month following SCI, increasing to a peak several months later but often remaining within normal ranges (150;185). PICP levels within normal ranges have been reported up to 3 months after SCI (185). Levels of bone alkaline phosphatase in acute SCI approximately 3 months post-injury were not significantly different from controls (124). However, high levels of alkaline phosphatase have been reported during the first year post injury in individuals with SCI (17).

Markers of bone resorption include: urinary free and total pyridinoline (Pyr) and deoxypyridinoline (DPD) crosslinks, type 1 collagen C-telopeptide (CTX), and N-telopeptide (NTX). Pyr and DPD are molecules that provide stability to collagen and, along with CTX and NTX, are released when collagen is degraded during bone resorption (24). Notable increases in bone resorption markers have been reported to occur as early as 2 weeks following SCI, reaching peak values 2-4 months after injury onset (124;156;184;185). Values did not return to baseline levels at 6 months post-injury, indicating that bone loss is ongoing (156). Histomorphometric data indicate that in the first 16 weeks of immobilization trabecular osteoclastic resorption surfaces increase, returning to normal at approximately 40 weeks. Osteoblastic apposition rate and the thickness of the iliac cortices decrease over 40 weeks of immobilization (131).

Systemic factors known to regulate bone and calcium homeostasis may become altered after SCI. Parathyroid hormone (PTH) regulates calcium absorption from the gastrointestinal tract and calcium mobilization in and out of bone. A cross-sectional study of 40 men with long-standing SCI revealed that PTH levels were significantly lower than in a group of able bodied controls and negatively correlated with injury level (188). In contrast, a large cross-sectional study of 176 individuals with SCI found depressed PTH levels only during the first year post-SCI (175). A smaller study also demonstrated that PTH was not significantly different from the reference range in individuals with long-standing SCI (57).

In the first four months to the first year after injury PTH levels have been reported to be low, eventually returning to normal levels (124;184). 1,25-dihydroxyvitamin D

(1,25-(OH)2 D) is also a regulator of calcium metabolism and may decrease during bed rest and after SCI (124;156;183;205). Depressed levels of 1,25-(OH)2 D have been demonstrated in individuals with long-standing SCI (188). Altered calcium balance is a common consequence of skeletal unloading. Urinary, fecal and serum calcium are significantly increased during bed rest (112;114;205). Changes in total-body calcium during bed rest were significantly correlated with the reductions in total-body bone mineral determined by dual photon absorptiometry (113). Hypercalciuria is often reported after SCI and may be reduced with re-ambulation (99;100). Ionized calcium has been demonstrated to increase into the hypercalcemic range after SCI, remaining there for 6 months with a parallel increase in urinary calcium excretion (156). Predisposing factors for hypercalcemia in acute SCI include: age less than 21 years, higher injury level, complete injury and prolonged immobilization (126). In individuals with long-standing SCI ionized calcium levels were not different from non-SCI controls (188). A study of bone and calcium metabolism in 28 patients demonstrated enhanced bone remodeling during the first year post-injury, where maximal values occur between 3 months and 10 months post-injury. As well, the rate of bone calcium turnover was greater in the non-paralyzed areas than in paralyzed areas during the first 2 months post-injury (17). There was no appreciable loss of bone mineral content at an upper limb site (radius). At 3 months post-injury, elevated bone turnover rates in areas above the level of the lesion decreased and increases in infralesional bone remodeling rates were observed (17).

The impact of alterations in systemic factors regulating bone and calcium homeostasis is reflected in the bone loss that occurs after an SCI. The removal of weight-bearing may initiate alterations in calcium metabolism via decreased PTH and 1,25-(OH)2 D synthesis resulting ultimately in bone loss. Conversely, given that unloading-induced bone loss occurs to a greater extent in the lower extremities, the bone loss may not be solely a result of changes in systemic regulation of calcium metabolism. The slight decreases in PTH and 1,25-(OH)2 D may be a result of increasing calcium concentrations in serum due to increased bone resorption initiated via alternate means. The bone response to reduced weight-bearing may be modulated by systemic factors such as PTH and 1,25-(OH)2 D but it is likely that local factors are primarily responsible for bone changes (139).

1.3.3 Fractures in Individuals with SCI

The bone loss that occurs after a SCI results in an increased risk of fracture in the lower extremities. Individuals with SCI are particularly susceptible to low-energy fractures. A low-energy fracture is one that results from an event that would not normally cause fracture, such as a transfer from bed to chair, or being turned in bed (61;155;189). Fractures of the supracondylar ridge of the distal femur have been termed the "paraplegic fracture", as they are the most common fractures seen in individuals with SCI. Common fracture sites appear to be those around the knee, such as the distal femur or proximal tibia (35;155). The fracture rate in the SCI population has been reported to be from 1% to 6% of patients (35;144;155;189).

Fractures are more likely to occur in individuals with lower than with upper motor neuron lesions and they are more likely in individuals with complete injuries than incomplete injuries (35). It has been estimated that fracture risk increases 2.2-fold for each 0.1 g/cm2 decrement of BMD at the femoral neck and that BMD is the only significant predictor of fractures (111). Complications related to fracture in the SCI population present an additional source of morbidity. Some complications reported in the literature include altered fracture healing, delayed union, malunion and non-union, pressure sores, infection, and osteomyelitis (61;71;144;144;155). As well, diminished pain sensation may delay the seeking of medical advice. Delays of one day to four weeks have been reported (93). Finally, complications and difficulties treating fracture in individuals with SCI may require prolonged immobilization and hospitalization. This may cause further detriment to bone and may result in lost wages, less social interaction and reduced quality of life.

The risk of fracture for individuals with SCI who partake in activities such as functional electrical stimulation, standing frames and treadmill walking has not been studied extensively. A case report documented a femoral fracture that resulted from measurement of maximal isometric quadriceps torque using electrical stimulation (85). Bone mineral density should be monitored regularly in individuals with SCI, particularly in those participating in activities that may increase their risk of fracture.

1.4 Soft Tissue Changes After SCI

1.4.1 Changes in Muscle After SCI

Individuals who have sustained a SCI have less fat-free mass than control subjects (137;145;201). Reductions in muscle can result in decreased metabolic rate and increased fat storage if energy intake is not adequately adjusted relative to energy expenditure (163). Individuals with complete SCI had reduced energy expenditure compared to controls and lesion level was correlated with basal metabolic rate and total daily energy expenditure (136;137). Reduced peripheral sympathetic nervous system activity in individuals with SCI may also contribute to reductions in resting metabolic rate. The potential influence of reduced SNS activity on resting metabolic rate was revealed by the observation that after adjusting for fat-free mass, fat mass and age, resting metabolic rate was still lower in individuals with SCI when compared to control subjects (137).

After SCI there is a rapid and dramatic loss of muscle mass below the level of the lesion. In individuals who were only 6 weeks post-SCI average muscle cross-sectional areas (CSAs) were 18% to 46% lower than in control subjects (28). Prospective study of these patients up to 24 weeks post-SCI revealed further declines in average gastrocnemius and soleus muscle CSAs of 24% and 12%, respectively (28). Similarly, from 6 weeks to 24 weeks post-injury the average decreases in quadriceps, hamstrings and adductor muscle CSAs were 16%, 14% and 16%, respectively. Another prospective study that employed dual-energy x-ray absorptiometry (DXA) to measure fat-free mass documented a 15% loss of lower limb lean mass in the first year after SCI (201). Eight weeks of lower leg cast immobilization resulted in a loss of calf muscle CSA of 25%.

Ten weeks of rehabilitation reversed the loss to a great extent, highlighting the plasticity of skeletal muscle (187). The highest rate of atrophy occurred during the first 2 weeks of immobilization and was greatest in the lateral gastrocnemius. In a large cross-sectional study, individuals with SCI had significantly less DXA-measured lean mass than the reference population for any age group. Advancing age and duration of injury were associated with less percent lean mass (171). In a monozygotic twin study, trunk and leg lean masses were significantly lower in the twins with SCI, whereas arm lean mass was not significantly different when the twin pairs were compared (172). A recent study revealed that in individuals with SCI, fat-free soft tissue contains approximately 15% less muscle tissue than in control subjects. Therefore, using DXA-measured fat-free mass as a surrogate for muscle mass may actually underestimate the muscle atrophy that occurs after SCI (132).

1.4.2 Changes in Fat After SCI

Reports of SCI-related changes in fat mass are not as consistent as the reported changes in muscle. A prospective study in individuals with acute SCI demonstrated trends towards increasing fat mass in the lower limbs after SCI, however large dispersion of individual changes prevented any general conclusions (201). Conversely, a study of monozygotic twins demonstrated that the twins with SCI had more total body fat and percent fat per unit body mass index than the non-SCI twins (172). Another monozygotic twin study failed to demonstrate significant differences in fat mass between SCI and non-SCI twins (11). Several other reports in the literature confirm that fat mass in individuals with chronic SCI is increased relative to controls (145;163;171). Two other studies suggesting that fat mass is not different in individuals with SCI incorporated small sample sizes (137;146).

Several factors may explain the unpredictable nature of fat mass changes following SCI. Several different measurement methodologies have been employed, including DXA, total body electrical conductivity and dilution of $^{3}H_2O$ and $Na_2{}^{35}SO_4$. Although reductions in fat-free mass are an expected consequence following SCI, changes in fat mass may be variable and dependent on the interaction of a variety of patient-specific variables. For example, advancing age has been associated with less lean mass and increased fat mass in individuals with SCI, but is mildly associated with these variables in controls (171). The level and completeness of the injury of all subjects can differ across studies. Activity level may also play an important role. Sedentary SCI subjects were found to have significantly higher percent and absolute body fat mass than active SCI subjects (146). Interestingly, although changes in fat mass and muscle mass may be different among men and women with a spinal cord injury, women are not often studied. One study examined women specifically, however it included only two elite wheelchair athletes who may not be representative of the population of women with SCI as a whole (119).

1.5 Summary of Muscle and Bone Changes After SCI

After SCI there is a rapid and dramatic loss of muscle and bone mass below the level of the lesion. There is considerable inter-individual variability in the amount of muscle and bone that is lost, which may be explained by factors such as level and completeness of lesion, gender, age, duration of injury and the presence of spasticity. One intervention that has potential for improving muscle mass and/or skeletal health in individuals with SCI is exercise. The following section outlines the influence of exercise on bone and muscle in non-SCI individuals, which is followed by a review of exercise interventions for individuals with SCI, and their effects, if any, on muscle and bone mass.

1.6 Exercise as a Means to Improve Musculoskeletal Health

1.6.1 The Effects of Mechanical Loading on the Skeleton

Julius Wolff conceptualized the theory that bone mass and architecture can be remodeled to sustain a certain homeostatic load per unit area, so that changes in the function of a bone will be followed by proportional changes in bone mass and area (204). Frost expanded these concepts, suggesting that mechanisms exist in bone where typical mechanical usage is monitored and the mass and structure of bone are adapted to meet mechanical needs. Bone modeling/remodeling can be turned "on" or "off" depending on the level of mechanical strain the "mechanostat" detects (64). According to the mechanostat theory, bone modeling and remodeling will modify bone mass and architecture, in order to keep the level of strain on bone within an operational range. The physiological loading zone, at where the amount of bone formed and resorbed is relatively equal, is suggested to be at or below 1000 microstrain, but above 50-100 microstrain (65). Bone mass and strength will be increased via modeling if the peak strains on bone exceed the upper threshold, called a modeling threshold. When the typical level of strain is consistently below the lower (remodeling) threshold, the amount of loading is not producing strains sufficient to maintain bone. Disuse-mode remodeling is enhanced and bone tissue is lost until a new, lower level of BMD is reached that is sufficient to withstand the imposed strain and strains are again above the remodeling threshold. Increased mechanical usage above the modeling threshold and in the area of 3000 microstrain are proposed to result in the accumulation of microdamage which can reduce the overall strength of the bone.

The way in which bone cells detect mechanical signals and convert them into a bone adaptive response is still unclear, and has been the subject of several reviews (22;52;182). The network of lacunae and canaliculi in bone, containing osteocytes and matrix, has been proposed as the structure responsible for the sensation and transduction of mechanical signals (22). Osteocytes have been pinpointed as the primary mechanosensory cells in bone because they are ideally situated throughout the lacuno-canalicular network and have been demonstrated to be responsive to mechanical signals. Osteocytes maintain contact with cells on the bone surface and neighbouring osteocytes via gap junctions, and they respond to mechanical loading with increased metabolism, gene activation and the production of growth factors and matrix (22;52). Osteocytes deprived of bone loading became hypoxic after 24 hours of disuse, indicating that

mechanical loading may be essential to maintain nutrient supply and waste removal for this cell population (49). A brief (< 4 minutes) loading protocol prevented osteocyte hypoxia from occurring. Osteocyte apoptosis and/or the removal of osteoclast-inhibiting signals as a result of understimulation of osteocytes have been proposed as mechanisms for the recruitment of osteoclasts to resorb bone tissue (22).

Although bone strain can directly activate bone cells, it has been suggested that mechanical strain on bones indirectly activates bone cells via fluid flow through the canalicular spaces (182). Mechanical loading of bone causes movement of extracellular fluid through the network of lacunae and canaliculi, which can stimulate bone cells via the creation of streaming potentials and/or the generation of shear stress on the osteocyte cell membrane (22;52). The production of signaling intermediaries important in the response of bone to loading has been demonstrated to be shear stress-dependent (4). Mathematical modeling of these cellular mechanisms revealed that fluid shear stress is osteogenic but the mechanosensitivity of bone cells depends on interactions between fluid forces and the viscoelasticity of the cell and/or matrix (92). Loading frequency modulates the proportional relationship between mechanical strain and bone formation, such that formation is increased at higher frequencies.

1.6.2 Does Exercise Have an Effect on Bone?

Animal studies have shown that mechanical loading can increase bone mass (20;78;159). The ability of exercise to increase mechanical strain and enhance bone mass and strength in humans has been explored and debated in recent years. An often-cited study is that of Jones et al (1977), where it was noted that in tennis players the humerus of the dominant arm had a greater cortical thickness than the non-dominant arm (95). Cross-sectional studies of athletes demonstrate that physical activity has a positive impact on bone mass, and that activities involving movements that produce high forces and/or repetitive impacts, such as strength and power training, basketball, running or gymnastics result in higher bone densities (38;110;123). These studies are limited by their cross-sectional nature, in that individuals with higher bone and muscle mass might be more likely to become competitive athletes.

Children who participate in regular physical activity experience greater gains in bone mineral than their inactive counterparts (3). Regular exercise may have a greater osteogenic potential if initiated before puberty, due to the high levels of bone mineral accretion during that time. For example, although the differences in bone mineral content between dominant and non-dominant arms were greater in women who participated in higher levels of squash and tennis than healthy controls, the benefits in the playing arm were two times greater if training was initiated before menarche (98). In adults, the effects of exercise on bone appear to be less dramatic. In a review of the literature, both cross-sectional and longitudinal studies suggested a positive effect of progressive resistance exercise on bone mineral density in adults, but the effects appeared to be specific to the bone sites associated with the active musculature (110). Meta-analyses of the skeletal effects of exercise in pre- and post-menopausal women revealed that although

exercise has a positive effect on the skeleton, it is generally manifest as a prevention of bone loss rather than bone gain (103;192;203). Strenuous aerobic exercise in postmenopausal women had overall beneficial treatment effects at the lumbar spine and femoral neck of 0.96% and 0.90% per year, respectively (203). Both impact exercises (walking, running, aerobics) and non-impact (resistance or strength training, weightlifting) exercises had a positive impact on bone in postmenopausal women at the lumbar spine and femoral neck and in premenopausal women at the lumbar spine. Effects of exercise on bone mass at the femoral neck in premenopausal women were not significant for impact exercise, and there were not enough data for a meta-analysis of the effects of non-impact exercise (192). Randomized controlled trials of exercise training demonstrated an overall prevention or reversal of bone loss of 1% per year in exercisers compared to controls. Overall treatment effects in non-randomized trials were almost twice as high, indicating that non-random allocation of subjects introduces confounding factors resulting in an overestimation of the effects of exercise on bone (203).

The large variability across studies with respect to the type of exercise employed, the duration and intensity of exercise protocols, participant compliance and drop out rates and measurement techniques used, make summarizing the effects of exercise on bone difficult. In one meta-analysis, studies that demonstrated the largest gain of bone at the lumbar spine and femoral neck were also those with the best compliance and incorporated high intensity activities (81;142). It can be said that the potential for mechanical loading in the form of exercise to enhance bone strength is greatest in the growing skeleton. In adults, exercise may possibly prevent age-related bone loss but may not increase bone mass to a great extent, if at all (178).

It is difficult to apply the current knowledge about the effects of exercise on the adult skeleton to bone health in SCI since typical peak strains on the lower limbs are much lower in individuals with SCI than in adults without SCI. Individuals with SCI also have lower initial levels of bone mass than adults without SCI and it has been suggested that bone responses to an exercise intervention may be greatest when the starting level of bone mass is below average (12). Not only are individuals with SCI deficient in regular weight bearing loading, but the muscle atrophy that occurs after SCI reduces the magnitude of the muscle pull on the bone, another source of mechanical loading. It has been suggested that muscle contractions provide one of the largest sources of mechanical loading on bone (64;66;67). In individuals who have some motor function, or who have some degree of spasticity, some of the loading attributed to muscle contraction may be preserved, but in those with flaccid paralysis and/or complete loss of motor function below the injury level, this loading may be lost completely.

1.6.3 What Kind of Loading is Osteogenic?

The nature of the mechanical strain stimulus capable of driving bone adaptation has been explored in recent years, and has been the subject of several reviews (22;23;51). Early research pointed to the importance of the magnitude of imposed strain; using an isolated avian ulna model Rubin and Lanyon demonstrated a graded, dose-response

relationship between the magnitude of the peak strain within bone tissue and the change in bone mass (158). More recent research points to strain rate as a critical factor mediating the skeletal adaptive response. Strain rate and loading frequency have also been implicated as important determinants of bone adaptation, and like strain magnitude, have been demonstrated to be proportional to the bone formation response (180;181). As well, dynamic loading conditions rather than static loading are required for bone adaptation to occur (159).

A dynamic mechanical loading stimulus of sufficient magnitude need only be applied for a short period of time to be osteogenic. Rubin and Lanyon demonstrated that 36 loading cycles per day increased bone mineral content in avian ulnae, and bones subjected to 360 or 1800 loading cycles did not result in greater increases in the amount of bone formed (159). Recent research by Robling and colleagues suggests that bone can become desensitized to loading fairly quickly but its mechanosensitivity may be recovered several hours later, so only a short duration of loading is required for a stimulus to be osteogenic (157). Recovery between loading sessions and/or between loading cycles can improve the bone adaptive response to mechanical loading by allowing for recovery of mechanosensation (23). Bone cells may become less responsive to routine signals, therefore bone adaptation may be governed by bone strains that are unusual (182). Mathematical formulae that predict bone adaptation to mechanical loading have been proposed, incorporating available data on the nature of osteogenic mechanical stimuli (182). In practical terms, important considerations in planning an osteogenic exercise regimen may be the frequency and timing of exercise bouts, as well as ensuring that the exercise delivers strains that have high strain rates and strain magnitudes and are different from those engendered in routine activities.

1.6.4 Exercise Effects on Muscle Mass

Within each muscle fibre is a bundle of myofibrils. After a bout of resistance exercise, there is an increase in myofibrillar protein synthesis in the exercised muscles. There is also a proportionally smaller increase in the degradation rate of myofibrillar protein resulting in a net increase in protein balance (31;149). When bouts of resistance exercise are repeated, as during training, the increases in muscle protein synthesis result in increases in myofibrillar area (120). When the myofibrils reach a certain size, splitting of the myofibril can occur during muscle loading. This also acts to increase the cross-sectional area of the muscle fibre (14). Similar to the increase in modeling that is proposed to occur in bone when loading strains surpass a threshold level, muscles respond to overload by increasing the formation of muscle protein. The result is an increase in muscle fibre size and corresponding increases in the cross-sectional area of the muscle.

Just as the type of loading is likely an important factor governing the bone response to exercise, the type of exercise regimen employed dictates the response in skeletal muscle. Endurance exercise generally results in little or no increase in muscle mass, whereas a well-known adaptation to resistance training is an increase in muscle

size (30;120;127). Resistance training protocols that involve performing exercises with the maximum amount of resistance for a low (3-5) or intermediate (9-11) number of repetitions resulted in muscle hypertrophy after 8 weeks of training, however a lower resistance, higher (20-28) repetition protocol did not (26). Requiring the muscle to produce more force than normal initiates an adaptive process that results in an increased cross-sectional area of the muscle fibres (121;122). The potential for increases in the number of muscle fibres has been discussed, but there is not enough evidence to support this phenomenon in adult humans (120).

When measured at the level of the whole muscle, increases in muscle cross-sectional area (CSA) of the quadriceps femoris have been demonstrated after resistance training. Young men performing progressive resistance training incorporating leg extension and leg press exercises twice per week for 21 weeks experienced increases in quadriceps femoris CSA of 5.6% (2). Similarly, 8 weeks of thrice-weekly resistance training produced increases in quadriceps femoris CSA of 3.3 – 3.6% in young women (90). Increases of 12% and 14% in thigh extensor (included all four quadriceps) area were observed after 10 weeks of thrice-weekly strength training and combined strength and endurance training in young men, however a significantly smaller increase (3%) was observed with endurance training (128). The pattern of change in type II muscle fibre areas in that study was consistent with muscle CSA changes: both strength training and combined strength and endurance training produced significant increases in type II fibre area, however the increase observed in the endurance group was not significant. Resistance training has also been demonstrated to increase muscle CSA in the elderly. Total thigh muscle CSA and quadriceps muscle CSA increased by 11.4% and 9.3%, respectively, after thrice-weekly strength training for 12 weeks in older men (63). Smaller increases (3%-4.5%) in quadriceps muscle cross-sectional area were observed in elderly men (80) and women (167) after 7-12 and 18 weeks of strength training, respectively. Small, but significant increases in type IIA muscle fibre area have been demonstrated after cycle ergometry training in elderly men (29). Among the resistance training studies, the differences in the magnitude of increase in muscle mass is likely due to the intensity of training achieved and/or the pre-training characteristics of the participants. It is evident that the level of muscle overload achieved with progressive resistance training is sufficient to induce muscle hypertrophy and that the level of overload achieved with endurance training is much smaller.

1.7 Exercise Intervention in SCI: Functional Electrical Stimulation in Individuals with SCI

1.7.1 Effects on Muscle

Functional electrical stimulation (FES) can be used to produce isometric contractions (5;50;96), to facilitate gait (69;166), or to produce contractions against resistance during cycling or leg extensions in individuals with spinal cord injury (5;13;16;19;33;50;54;82;91;116;134;147;154;169). During FES cycle ergometry, participants pedal the cycle with the use of coordinated electrical stimulation of several muscle groups (typically the quadriceps, hamstrings and gluteal muscles). A common

practice in FES cycle ergometry interventions is to begin with a muscle-strengthening regimen and to then proceed to the cycle ergometry regimen. The duration of FES cycle ergometry training studies has varied from 8 to 52.8 weeks, and the number of sessions per week is usually 2-3, but has been reported up to 7.

As mentioned previously, individuals with SCI experience lower limb muscle atrophy (50). Despite variability in the intensity, duration, and frequency of the exercise interventions, the positive effects of FES exercise on muscle are fairly well established. FES cycle ergometry is the most commonly used training modality among FES interventions. Lower extremity muscle volume, as measured with computed tomography, increased 10% after 6 months of FES cycle ergometry 2-3 times per week (169). More frequent bouts of exercise may have a greater impact on muscle. For example, males with chronic complete tetraplegia who participated in 7 FES cycle ergometry sessions per week experienced significant increases in lower limb muscle cross-sectional areas of 22%, along with significant increases in whole body percent lean mass and reductions in percent fat mass (91).

FES exercise regimens that include muscle strengthening may be an optimal means of improving muscle mass or preventing muscle atrophy in individuals with SCI. Intermittent high force loading using FES for 2 days per week resulted in significant increases in quadriceps femoris muscle CSA (20% ± 1%) (50). FES muscle strengthening prior to FES cycle ergometry may also be advantageous for increasing muscle. An FES training program that began with quadriceps strengthening and progressed to concurrent arm ergometry and FES cycle ergometry produced significant increases in muscle cross-sectional areas, with no change in adipose tissue. The magnitudes of the increases were as follows: rectus femoris 31%, sartorius 22%, adductor magnus-hamstrings 39%, and vastus medialis-intermedius 31% (162). In fact, the muscle-strengthening component may have the greatest impact. For example, significant increases in quadriceps muscle protein synthetic rate were noted in four paraplegic males after the first 10 weeks of quadriceps muscle strengthening, but the increase in muscle area after transition to a cycle ergometry program was not significantly different from the end of the first regimen (147). Similarly, quadriceps muscle area increased significantly after the first 10 weeks, but did not increase further at the end of the second regimen.

FES-induced isometric contractions can also increase muscle cross-sectional area after SCI (96). In addition, training with FES to produce isometric contractions has been demonstrated to increase maximal force and improve fatigue resistance in individuals with complete SCI (74). However, isometric contractions may not be optimal for preventing or reversing muscle atrophy. FES cycle ergometry, but not isometric contractions with FES, prevented muscle atrophy when performed in the acute phase following SCI (5). In addition to its effects on muscle mass, FES cycle ergometry has been demonstrated to increase muscle fibre area and capillary number in individuals with motor complete SCI (33).

1.7.2 Effects on Bone

In contrast to its well-demonstrated influence on muscle, the effects of FES exercise on bone are inconclusive. Several studies have demonstrated no effect of FES strengthening or cycle ergometry on measures of bone health (13;19;54;116;147). When attempting to quantify the effects of exercise interventions on bone and muscle in individuals with SCI, it is important to acknowledge that the participant characteristics, measurement techniques and the interventions exhibit substantial variation, making comparisons difficult. For example, the response to an FES intervention may vary considerably with age, gender, time post-injury, level of injury, completeness of the injury and functional classification of those involved. There is often variability in the anatomical sites measured, and the techniques employed to perform bone mineral density measurements. Finally, the intensity, frequency and duration of the intervention itself may have an impact on the bone or muscle response. A few studies have demonstrated increases in bone mass after FES-induced muscle strengthening (16) and FES cycle ergometry (135). These studies were longer in duration than many of the other studies, and one employed a higher exercise frequency (5 times per week). In addition, FES may be useful in the prevention of bone loss in the acute stages after SCI (82). However, a recent study of 38 patients with acute SCI failed to demonstrate prevention of bone loss with thrice-weekly FES cycle ergometry (54).

It may be that a minimum effective strain is required in order to stimulate increases in bone density in the lower limbs after a spinal cord injury. Nine months of thrice-weekly FES cycle ergometry failed to increase bone mineral density at the femoral neck, distal femur and proximal tibia in individuals with complete spinal cord injury. However, among those who could achieve a power output of greater than 18 Watts during cycle ergometry (n=4), the average change in distal femur bone mineral density was a statistically significant 17.8% increase (19). Inter-individual variation in the response to FES may also explain the above variability in the bone response to training.

In summary, FES exercise is the most commonly reported method of exercising the lower limbs after an SCI. Randomized controlled trials are necessary to elucidate the exercise intensity, frequency and duration required to produce appreciable changes in lower limb muscle and bone, and to determine the effectiveness of FES exercise in general for reducing negative health outcomes. In addition, determining participant adherence, ease of application and cost-benefit could solidify whether FES exercise is a useful and practical means of physical conditioning for the SCI population.

1.8 Body Weight Supported Treadmill Training After SCI

1.8.1 How and Why

Recovery of functional ambulation after incomplete SCI is often sought in the course of rehabilitation, however the percentage of individuals with SCI who recover ambulatory abilities can range from 15 to 45%(151). An inability to fully support body weight can be a limitation during gait retraining. Conventional rehabilitation focuses on

aspects of locomotion such as weight shifting, balance and stepping, with a gradual progression towards locomotion with weight-bearing. A re-directed approach to rehabilitation of walking abilities, such as body weight supported treadmill training (BWSTT), may allow a greater number of incomplete SCI individuals to achieve functional ambulation.

BWSTT enables patients who are not able to withstand full weight bearing on their lower limbs to walk on a treadmill with partial body weight support, with therapists moving the legs if necessary. BWSTT involves the use of a harness suspended by cables that are attached to weight stacks via an overhead steel frame, so that from 0%-100% of a person's body weight can be supported while they are standing/walking on a treadmill. During the initial stages of training, walking may be facilitated by therapists assisting with proper leg movement, weight transfer and upper body posture, depending on the individual's initial functional abilities (191). BWSTT allows a patient to practice the components of walking without full weight bearing initially and as ambulation improves the body weight support (BWS) can be reduced gradually. The therapist can spend time correcting gait patterns rather than helping to support body weight (88). The application of BWS alleviates the demands of maintaining balance and allows the individual with SCI to assume a more upright position (6;46).

The incorporation of BWSTT as a strategy for improving ambulation in individuals with SCI is based on the idea that neural circuits, or "central pattern generators" exist within the spinal cord that can produce the motor patterns required for locomotion (79). With transection of the spinal cord, the central pattern generator can no longer be activated and/or regulated by higher centres but animal research suggests that the neural networks can be activated by stimuli such as brainstem stimulation, sensory stimuli or bath-applied excitatory amino acids (79). Fictive locomotion, the generation of motor activity at the level of the spinal cord without any actual movement, has been described in an in vitro model of the lamprey (79). After complete spinal transection at the low thoracic level in kittens, walking movements similar to those in an intact cat could be produced (59). Since the kittens' movements occurred spontaneously and in the absence of any afferent input it was concluded that neural networks at the spinal cord level could generate locomotor patterns in response to peripheral input.

Initially, it was thought that the ability to recover locomotor function was related to the age of the animal at the time of transection. Unlike locomotor recovery in kittens, adult cats were not able to support the hind limbs 8 weeks after spinal transection (53). However, adult spinalized cats could support their hindlimbs and demonstrate the main components of the step cycle if placed on a moving treadmill (53). The recovery of locomotion in adult cats after complete spinal transection has been demonstrated after an interactive locomotor training program. The cats' forelimbs were supported on a platform, the hind limbs placed on a treadmill and the tails supported so that the animals had to bear only a portion of their body weight. Adult spinal cats have been trained to walk with appropriate foot placement, completely supporting the weight of the hind

limbs, at different treadmill speeds after 3-4 weeks of the training regimen (7;118) highlighting the importance of training for optimal locomotor recovery.

1.8.2 Clinical Research in BWSTT

The ability to recover locomotor patterns in spinalized mammals led several researchers to examine the application of BWSTT for locomotor training in individuals with SCI (43;44;84;87;89;153;190;196-199). Coordinated leg movements and electromyographic activity of lower extremity muscles can be provoked in individuals with SCI during BWSTT, with little or no increase in the capacity for voluntary muscle activation (44;45). Improvements in locomotor function with BWSTT were not related to the corresponding changes in leg muscle electromyographic activity, providing support for training effects on spinal locomotor centers (45). In support of the central pattern generator theory, it has been demonstrated that electromyographic amplitude was significantly associated with the level of limb loading during assisted stepping, indicating that the human spinal cord may use sensory cues from the lower limbs to create stepping movements (84).

BWSTT may be more effective than conventional methods for recovery of locomotion. Of 36 acute SCI patients who were wheelchair-bound at the onset of BWSTT, 33 (92%) became independent walkers compared to 50% (12/24) of those in a historical control group that had received conventional therapy (198). BWSTT can improve upon the gains attained with conventional rehabilitation. When twelve wheelchair-bound patients who had reached a plateau in functional recovery with conventional rehabilitation were then exposed to BWSTT, nine of the twelve learned to walk (198). BWSTT can also improve ambulation in individuals with chronic SCI. After BWSTT, 20 of 25 wheelchair-bound SCI individuals became independent walkers (199). BWSTT can also improve gait in individuals who possess some degree of functional ambulation (198). In addition, relearning of treadmill stepping is not limited to the clinical setting. Follow-up studies of individuals with SCI who have trained with BWSTT have reported that they continued to use and maintain their new abilities in daily life (199).

Although animal and human research suggests that the basis for the application of BWSTT as a gait retraining intervention is sound, a few weak points need to be addressed. Animal research suggests that appropriate sensory input can facilitate the generation of stepping movements after complete spinal transection. BWSTT has only been demonstrated to improve locomotion in individuals with incomplete SCI. Although lower extremity EMG activity can be provoked with BWSTT in individuals with clinically complete SCI, they do not recover the ability to perform independent stepping with training (43;198). It has been suggested that partial preservation of the function of long ascending and descending spinal pathways is required for neurological control of walking in humans (151).

In addition, the functional characteristics that define who will actually make improvements in ambulatory abilities with BWSTT are not well defined. For example, although a recent study demonstrated that a large proportion of individuals with SCI were able to learn to walk after BWSTT, patients were selected to participate based on specific criteria, including an ability or reasonable potential to use canes or other assistive devices, no severe muscle shortenings and the presence of some voluntary activity in lower limb muscles (198). Case studies comprise a great deal of the BWSTT literature and they have been generally descriptive in nature (25;70;84;87;153). In larger studies, those performing outcome assessments were not blinded, participants were not randomized to conventional or BWSTT treatment, and the time from injury to onset of treatment varied among groups (45;197-199). Randomized controlled trials are required to truly demonstrate the effectiveness of an intervention, and the current research regarding BWSTT as a gait retraining intervention is lacking in this respect (48).

1.8.3 Standing or Walking After SCI: Effects on Bone and Muscle

There are few published studies of standing or walking interventions, and those that are available did not measure changes in muscle and do not allow for any clear conclusions about the effects of these interventions on bone mass. Stewart et al investigated the impact of BWSTT on muscle morphology in a subgroup of the participants that participated in the longitudinal study presented in Chapter 5. In brief, 68 sessions of BWSTT resulted in significant increases in mean muscle fibre area of 25.5±6.3%. After SCI, the phenotype of muscle fibres in muscles below the level of lesion shifts to a higher proportion of type IIx fibres, which are less fatigue-resistant, and there is a reduction in muscle oxidative potential (165). After 68 sessions of BWSTT, there was an increase in the proportion of type IIa fibres and a reduction in type IIax/IIx fibres. Given these findings, it is evident that BWSTT has the potential to partially reverse muscle atrophy in individuals with SCI.

A few studies have examined the impact of standing or walking on the skeleton. Hip BMD measurements were made in individuals with SCI before and after a walking intervention with an ambulation device that combined FES and a modified walker. After 12-20 weeks of training, no significant increase in BMD was observed (141). Individuals with chronic SCI who participated in regular standing (with the use of a standing frame) did not experience any changes in bone mineral density, but the average duration of the intervention was only 135 days (109). A cross-sectional study demonstrated that individuals with complete SCI who had performed standing during the acute phase post-injury, either with long leg braces, a standing frame or a standing wheelchair, had better preserved BMD at the femoral shaft than those who had not. Those who had used long leg braces had higher total proximal femur BMD compared with patients using other standing devices (77). These data are limited by their cross-sectional nature. BMD measurements were made only once at least a year post-injury, and then comparisons between standing and non-standing groups were made. Participants were not randomized to intervention, but self-selected, so the differences between standing and non-standing groups may have existed in the absence of intervention.

Early weight bearing after acute SCI by standing or treadmill walking resulted in no loss or only moderate loss in trabecular bone compared to immobilized subjects, who lost 7-9% of trabecular bone at the tibia (41). Again, these data are limited by the selection criteria used to determine group allocation. The group participating in the walking intervention was comprised exclusively of individuals with an ASIA score of C or D, which could have inflated the BMD values for that group, since individuals with motor incomplete injuries lose less bone. However, the standing group included individuals with scores of ASIA A or B. Ultimately, the control group included only 4 individuals who were excluded from the walking intervention based on predetermined criteria, such as lower motor neuron involvement, other lesions or infirmity, and these criteria might make them more likely to experience bone loss than the weight-bearing group.

1.9 Summary of Background

Spinal cord injured individuals not only lose motor and sensory function, they experience considerable changes in the musculoskeletal system, resulting in osteoporosis and muscle atrophy. Loss of bone and muscle may predispose individuals with SCI to an increased risk of fracture, reduced glucose tolerance or cardiovascular disease, as well as having a negative impact on their quality of life. Exercise interventions may improve muscle and bone in individuals with SCI. Functional electrical stimulation is a method of exercise that has been employed in the SCI population that has demonstrated some success in improving muscle, with less conclusive evidence proving that it has a positive effect on bone. However, certain limitations associated with the use of FES may limit the potential for application of FES across a broad range of individuals with SCI. Body weight supported treadmill training has been explored in SCI with the intention of improving ambulation, but the potential benefits of this technique on the musculoskeletal system have not been explored outside of our laboratory. The lack of consistent findings among previous studies does not negate the potential of standing or walking interventions for increasing bone mineral or attenuating loss after SCI and they do not seem to have a negative impact on bone.

The overall objective of this thesis was to add to the current literature in the area of body weight supported treadmill training, with a specific focus on its impact on muscle and bone. The use of BWSTT in individuals who have sustained a spinal cord injury was investigated to determine whether regular weight bearing loading has an impact on the muscle atrophy and bone loss that occurs in these individuals. Specific hypotheses are outlined in each chapter. In general, it was hypothesized that BWSTT would have a positive impact on muscle and bone in individuals with acute and chronic SCI.

2.0 CHAPTER 2: GENERAL METHODOLOGY

2.1 Overview

The General Methodology chapter provides a description of the equipment and procedures used for the body weight supported treadmill training (BWSTT) intervention, as well as for data collection and analyses. A complete description of the timing of data collection, or any other data that are specific to a particular study, such as statistical analyses methods or participant characteristics, are given in the methods section of the chapters containing each study.

The following four chapters outline four projects conducted within the overall theme of **Musculoskeletal Changes in Spinal Cord Injury.** The first two studies were methodological in nature. The second two studies comprise the bulk of the thesis, and were designed to evaluate the impact of BWSTT on musculoskeletal health in individuals with spinal cord injury (SCI). The purpose of the first methodological study was to evaluate the effects of metal implants on measurements of bone, muscle and fat masses using dual-energy x-ray absorptiometry (DXA), since many individuals with SCI have metal implants, and it was necessary to use DXA to evaluate skeletal status in the participants doing BWSTT. Whole body DXA scans were performed on thirteen individuals without SCI, with and without different sized metal rods placed on the scan table. The distance of the metal rod from the x-ray source, the reproducibility of the metal effect, and the effects of increasing amounts of metal were investigated. Refer to Chapter 3 for a complete description of the study. The second methodological study evaluated the utility of quantitative ultrasound measurements in the assessment of skeletal status in individuals with SCI, since its portability and lack of ionizing radiation make it a potentially valuable diagnostic tool. Fourteen individuals with SCI and ten individuals without SCI had DXA scans performed at the proximal femur and proximal tibia, as well as speed of sound measurements performed at the distal radius and mid-tibia. Comparisons were made between the bone density measurements obtained from the DXA scans and the speed of sound measurements. Refer to Chapter 4 for a complete description of the study.

The third study was a longitudinal study evaluating the effects of thrice-weekly body weight supported treadmill training (BWSTT) on muscle and skeletal health in individuals with chronic, incomplete spinal cord injury. Thirteen individuals participated in thrice-weekly BWSTT for 144 sessions (~12 months), and measurements of bone biochemical markers, bone density and geometry, speed of sound in bone, as well as muscle cross-sectional areas and ambulatory abilities were taken before and after training, with some variables also measured at different time points during the intervention. This study will be referred to as the chronic SCI study, and is described in detail in Chapter 5.

The fourth study was a longitudinal study evaluating the effects of twice-weekly BWSTT on muscular and skeletal health in individuals with acute spinal cord injury. Five individuals participated in BWSTT for 6-8 months (48 sessions), and measurements of bone biochemical markers, bone density and geometry, muscle cross-sectional areas and

ambulatory abilities were measured before and after training, with some variables also measured halfway through the intervention. This study will be referred to as the acute SCI study, and is described in detail in Chapter 6. The following pages describe the BWSTT intervention, and the methodologies used for: bone biochemical markers, dual-energy x-ray absorptiometry, quantitative ultrasound and computed tomography.

2.2 Body Weight-Supported Treadmill Training (BWSTT)

2.2.1 Equipment

The Woodway Loco-system (Woodway USA Inc., Waukesha, WI) is a treadmill with an accompanying suspension system. Weight stacks that are attached to cables can be used to provide graded vertical support for an individual to stand on the treadmill (refer to Figure 1). Participants are fitted into a specialized harness, resembling a parachute harness, while seated in a wheelchair. The harness consists of a belt that attaches around the hips and lower torso, and two thigh straps with anterior and posterior attachments to the torso belt. Once the harness is fitted, the participant is wheeled up a ramp to the treadmill, and the harness is secured to the overhead cables. There are two sets of cables, one that allows for the provision of a chosen amount of body weight support (BWS), and the other that provides 100% body weight support. The 100% support cables are attached to the harness at all times, and are only loosened during training, so that in the event of a fall they will "catch" the participant. Even for individuals capable of supporting their full body weight, the harness is worn for safety. A horizontal bar in front and parallel bars at the sides of the participant are available for additional control of balance while walking.

2.2.2 Training Protocol

During the first training session, a level of BWS was chosen for each participant so that they could maintain an upright trunk and their knees did not buckle. If the BWS was set too low, participants tended to sit in the harness, which did not facilitate proper gait. The treadmill training strategy focused on proper weight shifting and weight bearing during the loading phase, and on the maintenance of an upright torso. The initial sessions comprised of walking bouts of 5-15 minutes, according to tolerance. Most participants began training with 60% body weight support or greater and walking at treadmill speeds of 0.6 kilometres per hour or less. The maximum number of walking bouts per training session was three. Walking duration during each bout was increased gradually, according to participant tolerance. Resting and exercise heart rates were measured during each session. The participant rested in a wheelchair that was moved onto the treadmill in between sessions.

Kinesiology students assisted in the BWSTT. One stood behind the participant to provide trunk support and help initiate weight shifting, and the others were positioned beside the lower limbs to assist with stepping and limb control. Treadmill speed was chosen based on patient comfort and safety. Both walking speed and BWS were adjusted as the participant improved. Walking speed was adjusted initially and BWS was occasionally increased for a brief period in order to facilitate proper gait at the new speed.

BWS was returned to the previous level once the participant was comfortable at the new speed. Once the participant progressed to their maximum comfortable walking speed, the new goal was to reduce the amount of BWS required. As the level of BWS was reduced, it was often necessary to reduce the speed temporarily so that the participant could become accustomed to walking with less support. Speed was gradually increased as the participant improved his/her walking ability. These procedures were repeated until some participants could walk without BWS, and then the only variable that was adjusted was speed.

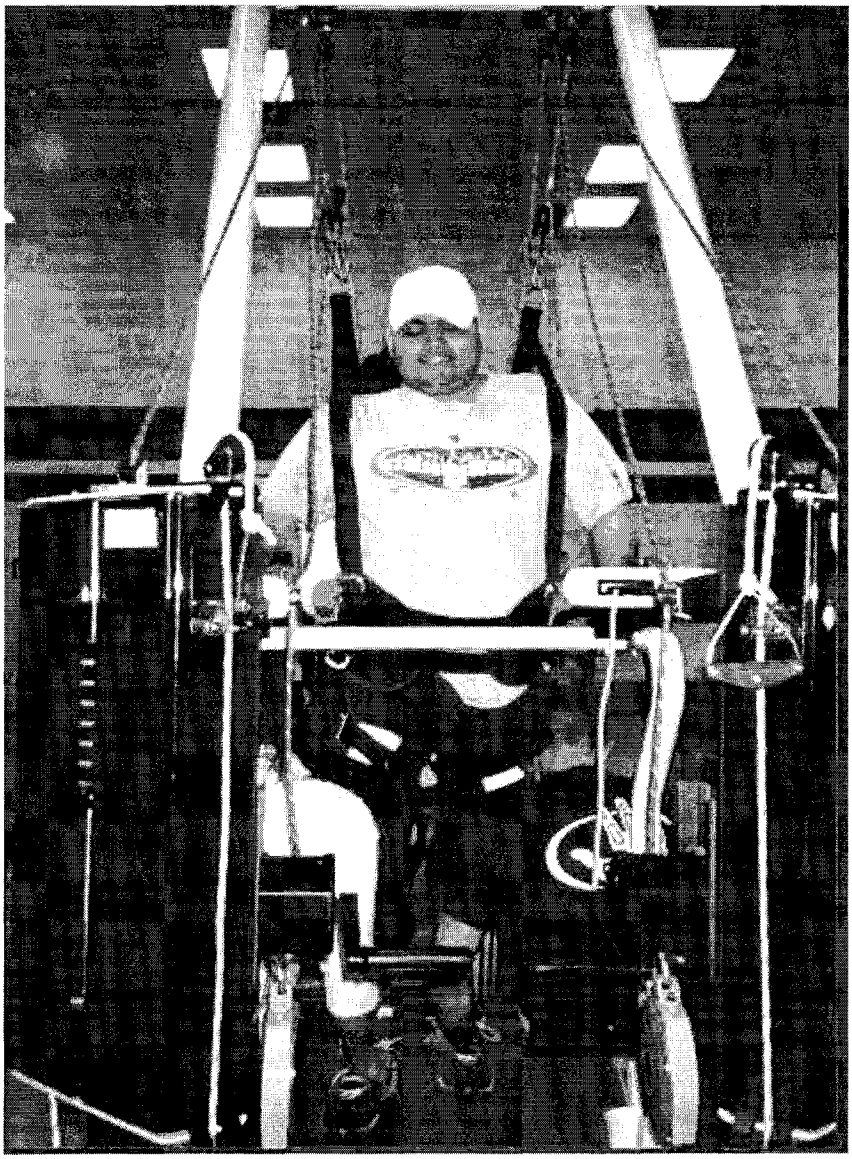


Figure 1: Body Weight Supported Treadmill Training

2.3 Outcome Measures

2.3.1 Treadmill Training and Walking Data

Walking duration per session, number of walking bouts, heart rate and amount of body weight support provided were recorded after each training session. Attendance at each of the scheduled training sessions was recorded. A modified version of a scale developed by Wernig and collegues (198;199) was used to evaluate over ground walking abilities at each 3 month time point. The Modified Wernig Scale is depicted in Table 2. All testing was conducted in the Centre for Health Promotion and Rehabilitation at McMaster University by trained staff.

Table 2: Modified Wernig Scale

Score	Classification
0	No walking capability, even with help of two therapists
1	Capable of walking < 5 steps with the help of two therapists OR along parallel bars
2	Capable of walking ≥ 5 steps with the help of two therapists OR along parallel bars
3	Capable of walking >1 length of the parallel bars, requiring assistance to turn
4	Capable of walking >1 length of the parallel bars, turning independently
5	Capable of walking along railing (< 5 steps) with the help of one therapist
6	Capable of walking along railing (> 5 steps) with the help of one therapist
7	Capable of walking with a rolling walking frame > 5 steps
8	Capable of walking with canes or crutches > 5 steps
9	Capable of walking without devices > 5steps

2.3.2 Bone Biochemical Markers

Osteocalcin (OC) and deoxypyridinoline (DPD) are bone biochemical markers that were used to assess bone formation and bone resorption, respectively. OC is measured in serum and DPD is measured in urine. OC is synthesized by osteoblasts (bone forming cells), and is thought to be involved in the process of bone mineralization. Although OC is generally considered a specific marker of osteoblast function, OC fragments may also be released during bone resorption, so the serum concentration may be a better indicator of the rate of overall bone turnover rather than just bone formation (164). DPD is a collagen crosslink that stabilizes the collagen molecule. During bone resorption, the components of collagen are broken down and released into the circulation, and are also excreted in urine. DPD is found exclusively in bone, is not influenced by the degradation of newly synthesized collagens and its excretion is independent of dietary sources; it is one of the best markers for assessing bone resorption (164).

For the chronic SCI study, first morning urine samples and venous blood samples were taken at baseline, and after 36, 72 and 144 training sessions. For the acute SCI study first morning urine samples and venous blood samples were taken at baseline, and after 24 and 48 training sessions. Serum and urine aliquots were stored at -80°C for later

analysis. Urinary DPD and serum OC were analyzed using competitive enzyme immunoassays (Quidel Corporation, San Diego, California). For the DPD analysis, the assay began with strip-wells coated with a monoclonal anti-DPD antibody. A diluted urine sample and enzyme conjugate (DPD-alkaline phosphatase) were added to each well. During a two-hour incubation (in the dark at 2-8 °C), the DPD in the sample and the DPD-alkaline phosphatase compete to bind with the anti-DPD antibody. The strip-wells were washed with wash buffer, and substrate (p-nitrophenyl phosphate, pNPP) was added. During a second incubation period (1 hour), the bound DPD-alkaline phosphatase hydrolysed the pNPP to p-nitrophenol (p-NP), and at the end of the incubation period the reaction was halted with the addition of NaOH. The optical density of the well was read at 405 nanometres. The optical density is proportional to the amount of pNP formed, which is proportional to the amount of bound DPD-alkaline phosphatase, which in turn is inversely proportional to the amount of DPD present in the urine sample. The optical density of each well was compared to a standard curve created by analyzing DPD standards at the same time as the samples in order to determine the DPD concentration of each sample (36).

The OC competitive enzyme immunoassay differs slightly from the DPD enzyme immunoassay. The OC kit contains strip-wells coated with osteocalcin, and to each well a sample of serum and mouse anti-osteocalcin antibody were added. During a 2-hour incubation period at room temperature, the anti-osteocalcin antibody binds either with the osteocalcin in the sample or the osteocalcin coated in the well. The strip-wells were washed after the incubation, an anti-mouse IgG-alkaline phosphatase conjugate was added to each well, which bound to the anti-osteocalcin antibody (bound to the osteocalcin in the wells) during a second (1 hour) incubation period. The strip-wells were washed at the end of the incubation period, and substrate (p-NPP) was added to each well. During a third (30 minute) incubation period, the IgG-alkaline phosphatase hydrolysed p-NPP to p-NP, and the reaction was halted with the addition of NaOH at the end of the incubation period. In this procedure, the optical density (read at 405 nanometres) is proportional to the amount of p-NP formed, which is proportional to the amount of IgG-alkaline phosphatase present, which is inversely proportional to the amount of osteocalcin in the serum sample. Similar to the DPD kit, a standard curve was created by analyzing OC standards at the same time as the samples, and the optical density of the wells were compared to the standard curve to determine the OC concentration of each sample (36).

The reference ranges for urinary DPD and serum OC provided by the manufacturer (Quidel Corporation, San Diego, California) are listed in Table 3. All samples were processed in duplicate. Urinary DPD data were corrected for urinary creatinine concentration, determined by a modified Jaffe method, where alkaline picrate forms a coloured solution in the presence of creatinine (21). All analyses were conducted in the Exercise and Metabolism Research Group Laboratory at McMaster University.

Table 3: Reference Ranges for Urinary Deoxypyridinoline and Serum Osteocalcin

	Female Reference Range	Male Reference Range
Deoxypyridinoline	3.0 – 7.4 nmol DPD/mmol Cr	2.3 – 5.4 nmol/DPD/mmol Cr
Osteocalcin	3.7 – 10.0 ng/mL	3.4 – 9.1 ng/mL

2.3.3 Bone Densitometry

Dual-energy x-ray absorptiometry (DXA) is a technique used for non-invasive assessment of bone density, in order to diagnose and/or monitor bone disease, most often at fracture-prone sites such as the proximal femur and lumbar spine. It can also be used to measure lean mass and fat mass. Bone mineral content (grams), bone mineral density (g/cm^2), lean mass and fat mass (grams) can be determined for the total body and for sub-regions of the body. The basis for this method is the ability of bone mineral density to predict fracture risk (125).

It is important to define precisely the terms used when describing bone density, especially since two different methods for obtaining bone density measurements were used in this thesis. To obtain a true measurement of bone mineral density, one must measure the mass of bone (minus any non-bone tissue such as marrow) per unit volume. When using DXA, the term bone mineral density (BMD) refers to the mass of bone tissue per unit area, in grams per squared centimetre (55). DXA is limited by the projectional nature of the scan that precludes true, volumetric measurements of bone density. Rather, the BMD measurement obtained using DXA is an areal bone density, combining the influence of both density and geometry on bone strength (186). Computed tomography, however, is a multi-projectional method that allows for volumetric measurements of bone mineral density (to be discussed later).

A review of DXA technology will provide the reader with a better understanding of the differences between techniques for obtaining bone mineral density measurements. Photons emitted from an x-ray source interact with body tissues, such that when the beam of photons is transmitted through tissues and is incident upon a detector, the intensity of the beam of photons has been reduced. The intensity of the beam of photons that reaches the detector is dependent on the original intensity of the beam before any interaction with an object, the mass of the object in its path and the probability that the photons will interact with the particles of the object (140;194). The probability of photon interaction can be represented by the attenuation coefficient for the object, μ, which in turn is dependent on the photon's energy and the atomic composition of the object. If one material is present with a given atomic composition, the mass of the object, m, can be determined if the attenuation coefficient and the original intensity of the photon beam, I_0, are known, simply by measuring the beam after it has been transmitted through the object and been reduced to intensity I, such that:

$$I = I_0 e^{-\mu m}$$

If the object is composed of two materials, then the masses of the individual materials that make up the object cannot be determined by measuring the attenuation of a beam of photons with a single energy, because each material will have its own mass and attenuation coefficient, and both masses would be unknowns. However, if photon beams of two different energies are employed, the attenuation of the higher energy beam relative to the lower energy beam will be different for each material. Two equations like the one above can be written, one for the high-energy beam and one for the lower energy beam, and one equation can be substituted into the other to give the mass of one component, and from that the mass of the other can be determined (55;194). Using the above relationships, the mass of bone mineral and the mass of soft tissue can be determined by relating the measurement of beam intensity to bone and soft tissue by calibration.

In order to determine the contributions of fat and lean mass to the total soft tissue mass, the ratio of fat to lean must be determined. By measuring the ratio of fat to lean at all sites within the body that only contain soft tissue, and determining a weighted mean ratio of fat to lean mass, the soft tissue mass can be further divided into lean and fat masses by comparing the relative attenuation of the high and low energy beams by the soft tissue to calibration materials (55;194).

Using DXA-measured fat-free soft tissue mass as a surrogate for muscle mass may underestimate the muscle atrophy in individuals with SCI. A recent study demonstrated that fat-free soft tissue contains approximately 15% less muscle mass in individuals with SCI than in control subjects (28). Nevertheless, the study did note that a strong relationship exists between fat-free soft tissue mass and muscle mass in both SCI and control subjects.

DXA scans (Hologic 4500A densitometer Bedford, MA, USA) were used to obtain bone mineral density (g/cm^2) measurements of the lumbar spine, right and left proximal femurs, right distal femur and right proximal tibia. In addition, a whole body scan was performed to measure whole body bone mineral density and content as well as muscle mass and fat mass. All bone density measurements were collected and analyzed by the author of this thesis, and scans were performed at baseline and at the end of each training study. Participants reported to the Department of Nuclear Medicine at the McMaster Site of Hamilton Health Sciences for DXA scans, which were followed by computed tomography scans in the Radiology Department. The coefficient of variation for the lumbar spine quality control phantom during the period of the study was 0.52%. The proximal femur, lumbar and whole body scans were analyzed using commercially available software from Hologic. Distal femur and proximal tibia scans were analyzed using a modified lumbar spine protocol, as described previously (138). The positioning device developed for distal femur and proximal tibia scans was not available for five of the subjects in the training study in chronic SCI individuals.

2.3.4 Quantitative Ultrasound

The standard method for evaluating fracture risk and diagnosing osteoporosis is DXA measurements of hip and spine bone mineral density (BMD). Accessibility to DXA machines is often limited to larger cities, so alternative methods for assessing bone status would be useful. Newer techniques, such as quantitative ultrasound (QUS) may provide a portable, more economical alternative to DXA that is also free of ionizing radiation. Ultrasound has been used in the engineering field to measure the mechanical properties of materials (55). The Sunlight Omnisense Ultrasonometer (Sunlight Medical, Rehovot, Israel) is capable of measuring the speed of sound (SOS) conduction through bone, which is considered an index of bone strength (see Figure 2). The ability of the Omnisense to measure SOS at the phalanx, radius, tibia and metatarsal makes it an attractive alternative to DXA for osteoporosis screening. Ultrasound is especially attractive for individuals with SCI since it is portable, does not require the individuals to transfer onto a scanning table, and measures a site relevant for individuals with SCI. However the ability of QUS to evaluate skeletal status is not as well established as for DXA, especially in the SCI population. Consequently, SOS and BMD measurements in individuals with SCI were compared in order to evaluate whether they demonstrated similar abilities to assess skeletal status in this population. The results of this study can be found in Chapter 4.

Speed of sound at the radius and tibia were measured with an ultrasonometer (Sunlight Omnisense; Sunlight Ultrasound Technologies Ltd.). Both sites were measured according to the instructions provided with the ultrasonometer. For each measurement, ultrasound gel was placed on the surface of the probe in order to improve probe-tissue coupling, and was reapplied as necessary. Ultrasonic waves at a frequency of 1.25 MHz were transmitted to the bone from a transducer in the ultrasound probe, and the speed of conduction through bone was measured. A measurement cycle lasted approximately 15 seconds and 3-5 measurement cycles were required at each site. Software particular to the ultrasonometer digitized and analyzed the signal, taking soft tissue into account. A good coupling between the probe and the skin was indicated by the sound of a tone during the measurement cycle. If the tone ceased, the operator corrected the positioning. Long- and short-term precision for the Omnisense has been reported previously (106). In cases of lower limb edema, the tibia measurement had to be repeated several times in order to move some of the edema and allow access of the sound waves to the bone surface underneath. Quantitative ultrasound was not used in the acute SCI study. For the chronic SCI study, SOS measurements at the distal radius and mid-tibia were taken at baseline, and after 36, 72, 108 and 144 training sessions.

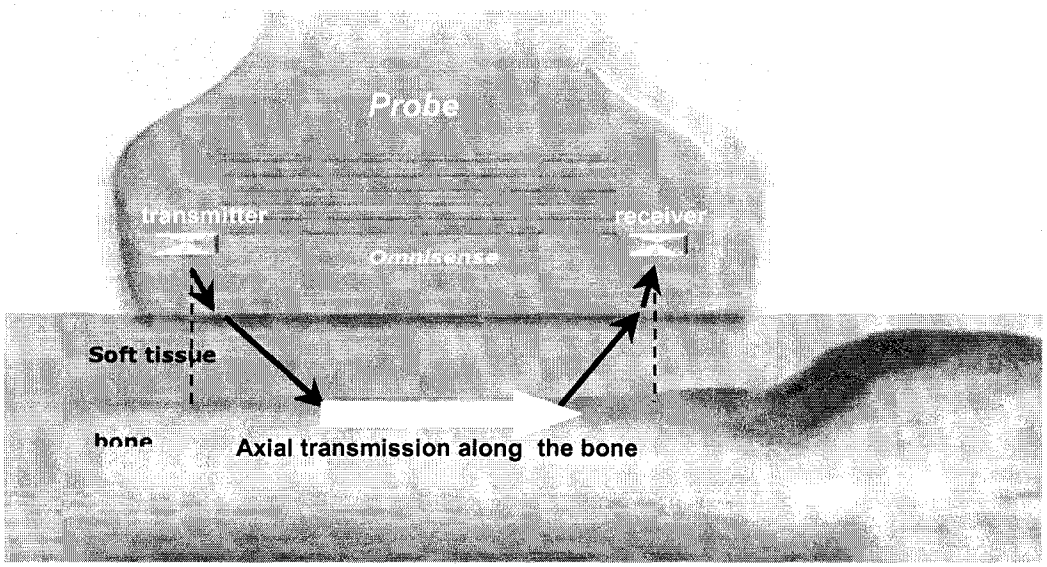


Figure 2: Omnisense Ultrasonometer Technology

2.3.5 Computed Tomography

The ability of a bone to withstand loading and resist breaking depends on variables such as the size of the bone, the geometry of the bone and the bone's material and tissue properties (60). A bone's ability to resist deformation, or its stiffness, is a function of its elastic modulus, or the intrinsic stiffness of the material, and the distribution of bone mineral around the axis of applied load, or the areal moment of inertia (60;179). DXA is limited in its use as a surrogate for whole bone strength, as it does not differentiate between bone density and bone geometry (60).

Computed tomography (CT) can be used to produce an image of a transverse cross-section of the body. Finely collimated x-ray beams are passed through all points of the cross-section, or slice, of interest at multiple angles. The attenuation of each of these x-ray beams represents the summed attenuations of each volume element, or voxel through which the x-ray passes. The attenuation data is collected and processed by a computer, which contains an algorithm to reconstruct the image, producing a series of CT numbers, where each number represents the linear attenuation in each voxel within the slice. The CT number is the linear attenuation coefficient expressed as an integer relative to the linear attenuation coefficient of water at the kilovoltage of x-ray beam used. A display device is then used to produce an image of the slice, where each of the voxels is displayed in a shade of gray (or colour) relative to its CT number (140).

Computed tomography (CT) allows for measurements of true, volumetric bone density in grams per cubic centimetre. As well, CT can be used to obtain measurements of bone geometry, namely cross-sectional moments of inertia. Maximum and minimum moments of inertia represent the distribution of material around the axes of maximum and minimum bending strength, respectively. The polar moment of inertia represents the distribution of material around the central axis. Bending stiffness is determined by the elastic modulus (intrinsic stiffness of the material in bending) and the areal moment of

inertia around the relevant axis. Torsional stiffness is determined by the shear modulus (intrinsic stiffness of the material in torsion) and the polar moment of inertia. Therefore, although clinical assessments of skeletal status often employ DXA measurements of BMD, independent analyses of bone density and bone geometry are extremely valuable in assessing bone strength, and can be performed with computed tomography. In addition, CT can be used to obtain site-specific measurements of muscle cross-sectional area (CSA, mm^2).

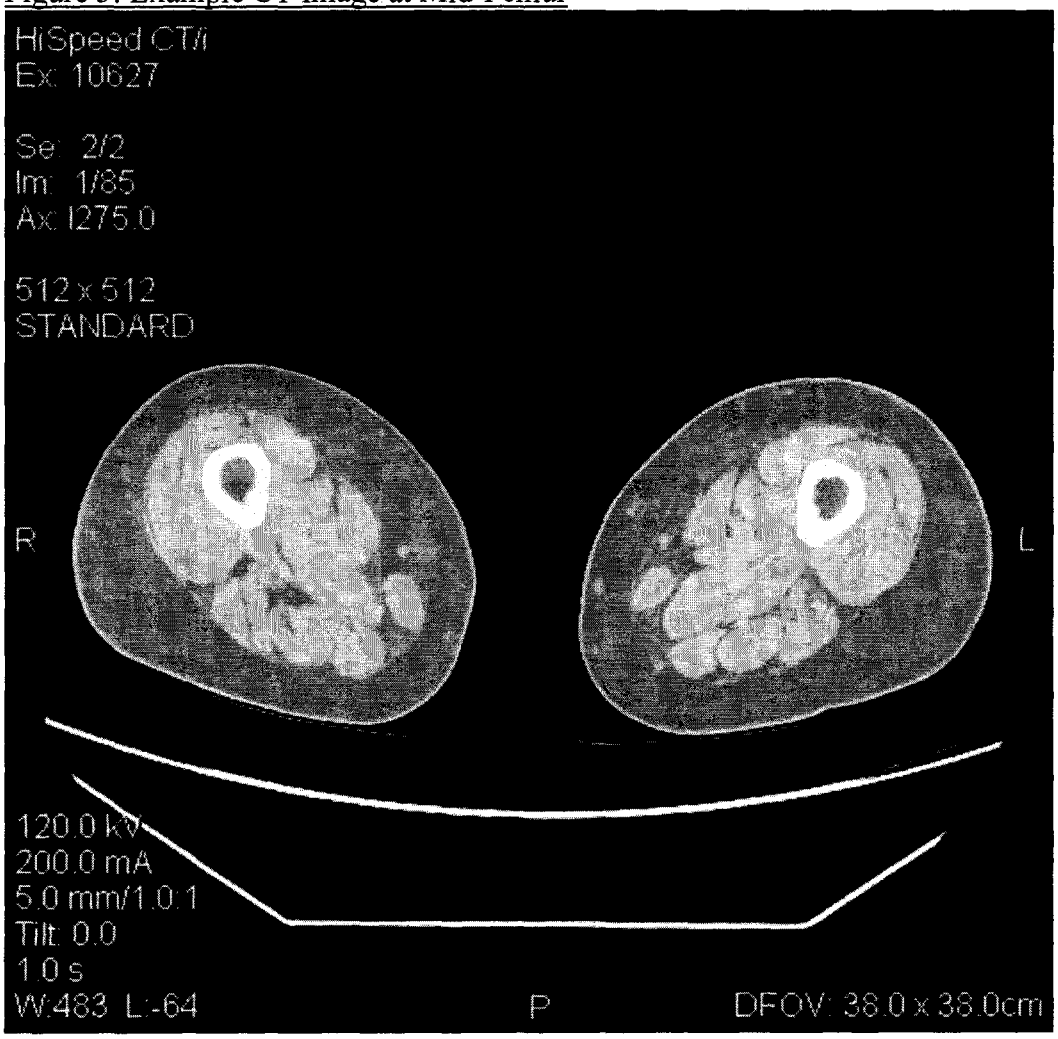
A General Electric CTI Scanner (GE, Milwaukee, Wisconsin, USA) was used to scan all participants before and after training. A scout scan was taken of the lower limbs in order to determine the lengths of the femur and the tibia. For the chronic SCI study, helical scans were taken from mid-femur to mid-tibia. The helical scan was started 5 mm proximal to the mid-femur level and ended at 5 mm distal to the mid-tibia level. Mid-femur was defined as the midpoint between the head of the femur and the medial joint line of the knee. Mid-tibia was defined as the midpoint between the medial tibial plateau and the lateral malleolus. Based on the chronic SCI study, it was decided that slices at two sites would be sufficient for the 6-month study in acute SCI individuals. For the acute SCI study, a 5 mm slice was taken at both the mid-femur and at the point of maximal lower limb muscle cross-sectional area, defined to be 66% of the length of the tibia, starting from the distal end and measuring proximal. The system parameters used were as follows: slice thickness 5 mm, pixel matrix 512 x 512, and exposure factors of 120 kV, 200mA and standard reconstruction algorithm.

Muscle cross-sectional areas were assessed at the mid-femur and 66% tibia sites. In the helical scans, the mid-femur slice was generally the second slice in each series, and the 66% point was calculated using the length of the tibia and the number of tibial slices in the series. To ensure accurate positioning before and after training, the tibial plateau was used as an anatomical landmark, and the slice containing it was identified. Any differences in leg length, or differences in leg positioning at baseline and after training, could be corrected for based on differences in the slice containing the left and right tibial plateaus in each helical scan. CT scans were analyzed using a validated software program (BonAlyse 1.3, BonAlyse Oy, Jyvaskyla, Finland), according to the software manufacturer's instructions. The thresholds –270 to –101 Hounsfield Units (HU) were used to identify fat, and the thresholds –101 to 270 HU were used to identify muscle. For both the thigh and lower leg sites in each participant, the right and left leg muscle CSAs were averaged.

BonAlyse was also used to calculate bone cross-sectional areas (CSA, mm^2) and volumetric bone mineral density (vBMD, mg/cm^3) without bone marrow at the mid-femur and 66% tibia. Once again, reproducibility in positioning was verified using the tibial plateau as an anatomical landmark. The thresholds for the outer and inner border of the bone were 280 mg/cm^3 and 70 mg/cm^3, respectively. Maximum (I_{max}) and minimum (I_{min}) cross-sectional moments of inertia, as well as the polar cross-sectional moment of inertia (I_{polar}) were calculated for femur and tibia slices. The reproducibility of the muscle

and bone data measured using computed tomography is listed in appendix A, and raw data is listed in appendix B. The protocol used to validate the conversion of Hounsfield units from the computed tomography scans to volumetric bone density is outlined in Appendix C. For both the thigh and lower leg sites, the values obtained for the right and left legs were averaged for each variable in each participant.

Figure 3: Example CT Image at Mid-Femur



3.0 CHAPTER 3: EFFECTS OF METAL IMPLANTS ON WHOLE BODY DXA MEASUREMENTS OF BONE MINERAL CONTENT AND BODY COMPOSITION

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3.1 Abstract

Objective: The purpose of this study was to evaluate the influence of metal implants on body composition as measured by x-ray based dual-photon absorptiometry.
Materials and Methods: Four whole body dual-photon absorptiometry scans were performed on 13 participants with metal rods either present or absent during the scans. The influence of the amount of metal (50, 100, and 150 grams), the proximity of the metal rod to the x-ray source and the reproducibility of any metal-induced effects were evaluated by altering the position and/or the size of the metal rod used.
Results: The presence of metal rods weighing 100 or 150 grams significantly increased reported total body mass and bone mineral content ($p<0.05$). Soft tissue mass was increased when the scan included the 100 gram rod ($p<0.003$). The proximity of the metal to the x-ray source did not have a significant influence on the body composition changes induced by the metal. The effects of the metal rods on body composition variables were reproducible.
Conclusion: The presence of metal rods inflated body composition variables measured by dual-photon absorptiometry, however, the effects will be reproducible during repeat scans of an individual patient. Metal had the largest impact on total body BMC, inducing errors of 1.5 to 3%.

3.2. Introduction

Dual-energy x-ray absorptiometry (DXA) is a technique for the non-invasive assessment of bone status, in order to diagnose and/or monitor bone disease. It provides measures of bone mineral content (BMC) in grams and projected bone area. The result is usually expressed as bone mineral density (BMD) in grams per centimeter squared. DXA can also provide an estimate of body composition from a whole body scan, which allows partition of body mass into mineral, lean and fat tissue. When another material is introduced, the ability of DXA to partition the tissues into the three constituent components will be impaired, particularly if the new material has a distinct mass attenuation coefficient, different from that of either bone or soft tissue. Under normal diagnostic conditions, the presence of additional materials in the scan area is not expected. However, metal will be introduced when the individual being scanned has implanted metal rods or fixation plates.

The presence of metal in a whole-body DXA scan will be a concern when evaluating bone status in the spinal cord injured population. Men and women with a spinal cord injury (SCI) experience a rapid and dramatic loss of bone in the first few months after injury, placing them at increased risk for low-energy fracture, particularly in the lower extremities (73;93;93;201;201). In order to be able to monitor bone status in individuals with SCI, accurate and precise DXA measurements are necessary. When using DXA to evaluate bone status and body composition in the SCI population, it is important to know how metal implants will influence x-ray attenuation, the subsequent partitioning of body mass into bone and soft tissue, and the allocation of soft tissue mass into its lean and fat components. It is also important to evaluate whether the influence of the presence of metal on body composition is linearly related to the amount of metal present, as orthopaedic implants will be of various sizes. If we know the extent to which metal will influence DXA measures, we can evaluate individuals with metal implants more accurately by adjusting the acquired data for the metal effect. If the presence of metal implants has a significant effect on the quantification of body composition, it would be useful to know if any errors attributable to the metal are reproducible. That is, if metal has an effect, does it have the same effect every time an individual is scanned? If so, then for monitoring with repeat scans it is not necessary to adjust for the metal effect, as the amount and position of metal will be constant for every scan. In addition, the spatial relation between the location of the metal in the body and the x-ray source may also be important particularly for fan beam x-ray densitometers.

The present investigation examines the influence of metal implants on DXA-measured bone and soft tissue indices, with the intent of answering the following four questions:

1. Is there a significant effect of metal on the evaluation of bone and/or soft tissue with DXA?
2. Does the measured effect of metal increase linearly with increasing amounts of metal?

3. Will the metal have the same effect, if any, each time a person with a metal implant is scanned?
4. Does it matter how close the metal is to the x-ray source?

3.2.1 Hypothesis

It is hypothesized that the attenuation of the metal will be interpreted by the DXA software as bone, and the addition of metal in a whole body scan in the area of the lumbar spine will increase the reported amount of bone mineral and the reported total body weight compared to a condition when no metal is included. It is also hypothesized that the presence of metal in the scan area will not change the apparent amount of total soft tissue in the scan area. In addition, it is hypothesized that the division of soft tissue compartments into fat and muscle will not be affected by the presence of metal in the scan area.

3.3 Methods

3.3.1 Participants

Volunteers over the age of 18 were recruited from the local university population in accordance with the policies of Research Ethics Board for McMaster University and Hamilton Health Sciences. Six women and seven men volunteered, with a mean age of 24 years, and mean body weights of 57.4 kg and 78.4 kg, respectively. Informed consent was obtained prior to beginning the investigation. Exclusion criteria included the presence of metal implants or the possibility of pregnancy.

3.3.2 Protocol

Four whole-body DXA scans (Hologic 4500A densitometer, Bedford, MA, USA) were taken for each participant, all during the same visit. The coefficient of variation for the lumbar spine quality control phantom during the period of the study was 0.52%. Participants were allocated to one of two groups. In the first group (3 males, 3 females), the effect of including metal on body composition variables was investigated. In the second group (4 males, 3 females), the effect of increasing amounts of metal was investigated. The participant lay supine on the scanning table during each scan, and did not move from the table between scans, in order to minimize potential error. For scans where metal was included, the metal was placed underneath the participant, on top or immediately adjacent to the lumbar spine, except for one condition, where it was placed on top of the torso, in the same projected location adjacent to the lumbar spine. This position was chosen to simulate the position of implanted metal rods. All scans, including placing of regions of interest, were analyzed according to the manufacturers' specifications.

Effects of Increasing Amounts of Metal

To determine whether the presence of metal would produce errors in measured body composition that increase linearly in magnitude with increasing amounts of metal, participants were scanned with no metal and with 3 different sized metal rods. The rods were manufactured from 300 low vacuum metal (LVM) stainless steel. The largest rod

weighed 150.77 grams, was 15cm long and 1.25 cm in diameter. The medium sized rod was 100.58 grams, 10 cm long and 1.25 cm in diameter. The small sized rod was 50.25 grams, 5 cm long and 1.25 cm in diameter. The conditions of measurement with and without metal will be referred to as NO METAL, LARGE, MEDIUM and SMALL, respectively.

Effects of metal placement and reproducibility

In order to investigate whether the location of a metal rod in the x-ray beam is important, participants were scanned once with no metal, once with the LARGE metal rod posterior to the torso close to the x-ray source and once with the LARGE metal rod anterior to the torso and close to the x-ray detector. To test the reproducibility of DXA in the presence of metal, the scan with the metal rod beneath the subject was repeated. The conditions will be referred to as NO METAL, BACK1, FRONT and BACK2, respectively.

From the whole-body DXA scans, whole-body bone mineral, lean body mass and fat mass were assessed. Total body weight was determined by summing the masses of bone mineral, fat and lean compartments as determined by DXA, and is reported in kilograms. Changes in body mass, soft tissue (ST) and bone mineral content (BMC) are tabulated in grams (g). Since the DXA machine automatically partitions soft tissue (ST) into lean mass and fat mass, ST was determined by adding lean and fat masses for each condition.

3.3.3 Statistical Analyses

Mean and percent changes were calculated for total body mass, total body bone mineral content, soft tissue mass, lean mass and fat mass. Each metal condition was compared to the NO METAL condition using two- tailed paired t-tests, where all comparisons were determined a priori. Significance is reported at $p<0.05$. To determine if the addition of metal introduced a significant source of variance in body composition variables, the intra-class correlation coefficient was calculated for each variable. All analyses were performed with SPSS for Windows, Release 11.0.1 or Microsoft Excel. Raw data is listed in Appendix D and statistical analyses are given in Appendix E.

3.4 Results

Effects of Increasing Amounts of Metal

Table 4 presents the mean increases in total body weight, soft tissue mass and bone mineral content when metal rods are introduced into a whole body scan. Total body weight, as determined by DXA, was significantly increased in the presence of the LARGE ($p<0.027$) and the MEDIUM ($p<0.001$) metal rods. When compared to the absence of metal, the changes did not reach significance ($p<0.058$) when the SMALL metal rod was present.

Table 4: Mean (±SEM) Increases In Body Composition Variables When Metal is Added to a Whole Body Scan.

	Weight of Metal (g)	Total Body Weight (g)	Soft Tissue (g)	BMC (g)
LARGE	150.8	324.5 (361.0)*	262.9 (319.7)	61.4 (43.5)*
MEDIUM	100.6	441.4 (243.1)*	407.3 (222.6)*	34.0 (40.4)*
SMALL	50.3	171.7 (246.9)	152.9 (225.5)	18.8 (35.6)
BACK	150.8	263.5 (183.8)*	192.9 (170.8)*	70.5 (13.6)*
FRONT	150.8	203.2 (191.0)*	153.8 (191.7)*	49.4 (21.8) *

** significantly different from NO METAL, $p<0.05$*

Figure 4 shows that reported total body BMC was significantly increased for the LARGE ($p<0.005$) and MEDIUM ($p<0.034$) conditions when compared to NO METAL, but the SMALL metal rod did not have a significant effect on reported bone mineral content. The results shown in Figure 4 could be fitted by a regression analysis to a linear equation with a slope of 0.397 g BMC/ g metal and an intercept of –1.4 g BMC. Thus for every 10 grams of metal included in a whole body scan the reported whole body BMC will be falsely increased by almost 4 grams.

Figure 5 depicts the changes in ST when metal is introduced into the scan area. In general, ST increases in the presence of metal. There was a significant increases in ST in the MEDIUM ($p<0.003$) condition when compared to the NO METAL condition, however the increase failed to reach significance for the LARGE ($p<0.07$) condition. Soft tissue mass in the SMALL condition was not significantly different from the NO METAL condition.

Lean mass and fat mass, as measured by DXA, only showed a statistically significant change for the MEDIUM condition, where lean mass was significantly larger compared to the NO METAL condition ($p<0.008$). Even in this case the percentage increase was only 0.72 %. Table 5 provides the percentage changes of body composition variables in all metal conditions.

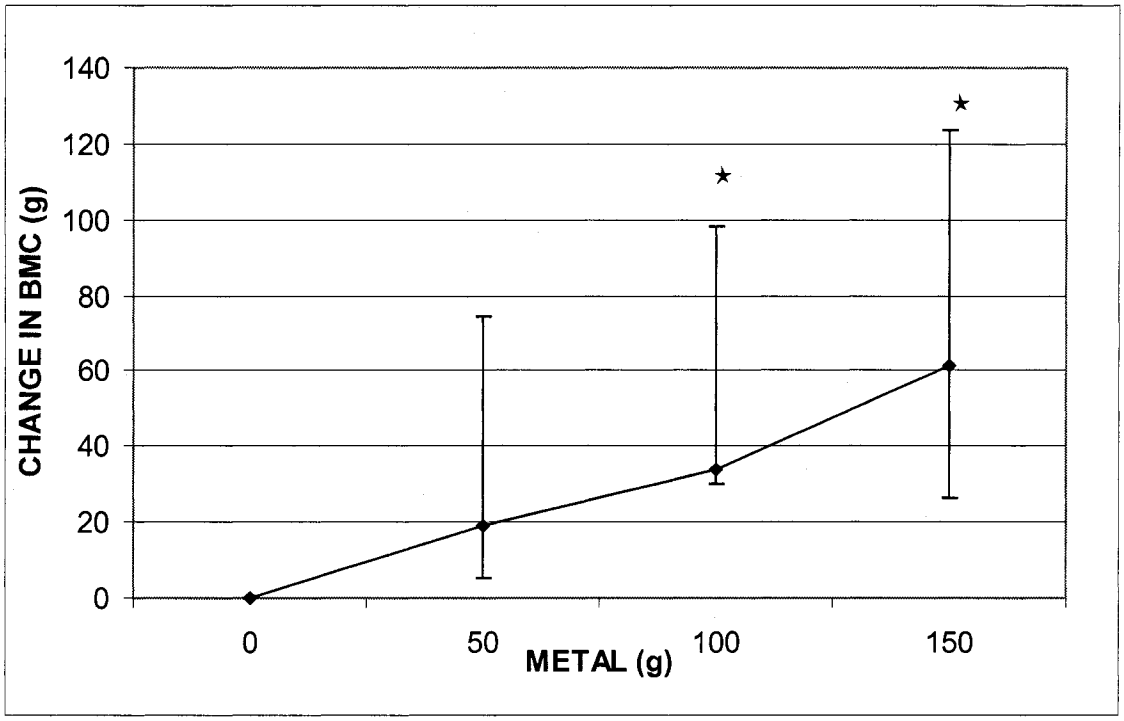

Figure 4: Mean (±95% C.I.) BMC Change With Increasing Metal.

* Different from NO METAL, p<0.034

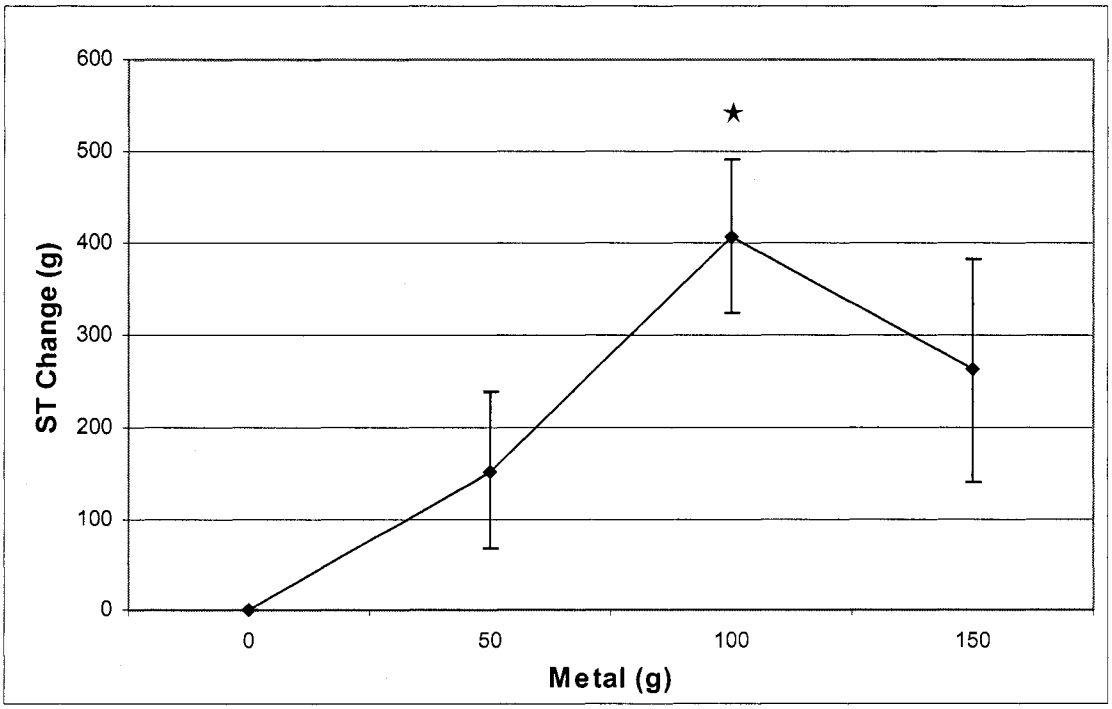

Figure 5: Mean Change in Soft Tissue Mass with Increasing Metal Mass (±SEM).

* Different from NO METAL, p<0.03.

Table 5: Mean (±SEM) Percent Increases in Body Composition Variables For All Metal Conditions

	Total Body Weight (%)	Soft Tissue (%)	Fat Mass (%)	Lean Mass (%)	BMC (%)
LARGE	0.5 (0.5)	0.4 (0.5)	1.4 (2.1)	0.3 (0.7)	2.5 (1.8)
MED	0.7 (0.4)	0.6 (0.3)	0.8 (3.0)	0.7 (0.5)	1.5 (1.8)
SMALL	0.3 (0.4)	0.3 (0.3)	0.7 (3.4)	0.4 (1.0)	0.8 (1.5)
BACK	0.4 (0.3)	0.2 (0.1)	1.0 (1.7)	0.3 (0.7)	3.0 (1.2)
FRONT	0.4 (0.3)	0.3 (0.1)	0.9 (3.3)	0.1 (1.0)	2.2 (1.2)

Effects of metal placement and repeat scans including metal

There were no significant differences for total body weight, BMC, ST or lean or fat mass when BACK 1 and BACK 2 were compared. Therefore, any further comparisons were made using the mean of BACK 1 and BACK 2.

Total body weight was significantly increased when the rod was positioned either above the subject (FRONT, $p<0.02$) or below the subject (BACK, $p<0.009$), when compared to the NO METAL condition. Reported whole body BMC was also significantly increased in both metal conditions ($p<0.001$). Soft tissue mass was significantly increased when the metal was placed below the subject ($p<0.04$) or above the subject ($p<0.05$). There was no statistically significant effect of metal at either location on lean mass or fat mass. There were no significant differences for total body weight, BMC, ST or lean or fat mass when FRONT was compared to the mean of the two BACK conditions. Intra-class correlation coefficients for BMC, body weight, fat mass and lean mass were all at least 0.99, indicating that the variance in all metal conditions was mainly due to between subjects variance.

3.5 Discussion and Conclusions

The aim of this study was to determine the influence of the presence of metal upon the results of whole body measurements of body composition variables determined by x-ray based dual photon absorptiometry. Whenever more than 50 g of metal was introduced into a whole body scan field of view, DXA-determined body weight increased significantly. The greatest observed mean increase in total body weight amounted to only 0.65%. Since the weight of the largest metal rod represents about 0.2% of adult body weight, the greater fractional increase observed with DXA serves to illustrate the importance of the presence of materials of higher atomic number and density than soft tissue or bone mineral.

Total body bone mineral content increased linearly with the mass of added metal. It is to be expected that given the typical mass of a small set of metal implants in an SCI patient (~ 80 g), falsely elevated levels of whole body BMC will be observed. It is only

when less than about 50 g of metal is present that there will be no statistically significant influence upon whole body bone mineral mass. According to regression analysis, for every 10 grams of metal present the reported whole body BMC may be falsely elevated by almost 4 grams. Increases in BMC when 100 grams of metal or greater were in the image field of view ranged from 1.50 to 3%.

Since the major contribution to whole body mass is soft tissue mass, it is not unexpected that the observed increases in soft tissue mass were only marginally less than the whole body changes. As with total body bone mineral, statistically significant increases in total body soft tissue mass were noted when more than 50 g of metal were included in the scan, indicating that small amounts of metal may not produce appreciable changes in the reported soft tissue mass. The greatest mean increase observed in soft tissue mass was 0.62%. The ranges of DXA-measured soft tissue mass changes were narrow, indicating that any errors in the measurement of soft tissue mass attributable to the metal were no larger than the expected measurement error. It is of note that the inclusion of the medium-sized metal rod produced a relatively larger change in soft tissue than the large rod, however it is likely due to the expected measurement error.

The measured soft tissue mass is divided into lean and fat by examination of the attenuation of the two x-ray beams through body locations occupied solely by soft tissue. Consequently it would be anticipated that the presence of metal, which would be confined to image regions containing mineral, would not influence the partitioning of soft tissue into lean and fat, and the apparent increase in soft tissue mass would be proportionately distributed between the lean and fat compartments. Although in this study the effects of metal on DXA-measured lean and soft tissue mass were not statistically significant, the potential errors introduced by the metal in the partitioning of lean and fat mass cannot be dismissed. The method errors for lean and fat masses are typically 1.4% and 1.8% respectively (32). Although the mean percent increases in fat and lean mass in our metal conditions were within expected measurement variability, individual changes in the measured fat mass could be larger than the mean measurement error.

When the whole body scan with the metal positioned underneath the subject was repeated, no significant differences were observed for total body weight, BMC, soft tissue mass or lean and fat mass. The excesses of total body BMC during repeat scans including metal (BACK1 and BACK2) were 63.9g and 77.2g. The difference between these two values reflects the difficulty of measuring the small difference (~70g) between two large numbers. Therefore, although the presence of metal may have an influence on the accurate measurement of body weight, soft tissue and BMC by DXA, the change in these variables is reproducible. When repeating measurements in a single subject who has metal implants, the impact of the metal on body composition will be comparable across scans, so the body composition variables can be reported without adjustment for the metal effect. The reproducibility, calculated as method error, of total body BMC measured by DXA is typically 1.6 % (32). According to the intra-class correlation coefficients calculated for each of the body composition variables, the majority (99%) of

the variance across trials was due to between subjects variance rather than variance between trials (or between metal conditions). Therefore, even when NO METAL and BACK conditions were compared, the variance in body composition variables due to the metal was reproducible.

In order to determine whether the proximity of the metal rod to the x-ray beam influenced the measurement of body composition variables, subjects were scanned with the metal above and below the torso (FRONT and BACK conditions, respectively). When the FRONT and BACK conditions were compared, there were no significant differences in total body weight, BMC, ST or lean or fat mass, indicating that the proximity of the metal to the x-ray source does not have a significant influence on the measurement of body composition variables.

In summary, it can be said that the presence of metal implants may inflate body composition variables measured by DXA. However, the largest changes are noted in the measurement of whole body BMC, where 100 and 150 grams of metal were able to induce significant average BMC errors of 1.50 to 3 percent. The bone remodeling process is slow, resulting in small changes in bone over time. In order to be able to detect BMC changes, high precision is required. However, in repeat scans of the same individual, the errors in body composition induced by the metal are reproducible, and will not reduce the ability to detect change.

4.0 CHAPTER 4: SPEED OF SOUND IN BONE AT THE TIBIA: IS IT RELATED TO LOWER LIMB BONE MINERAL DENSITY IN SPINAL CORD INJURED INDIVIDUALS?

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4.1 Abstract

Objective: The purpose of this cross-sectional study was to evaluate bone mineral density (BMD) at the hip and tibia, and speed of sound (SOS) at the radius and mid-tibia in individuals with spinal cord injury (SCI) and a sub-group individuals without SCI.
Methods: In 14 individuals with SCI and 10 non-SCI individuals, proximal femur and tibia BMD were measured using dual energy x-ray absorptiometry, and radius and tibia SOS were measured with an ultrasonometer. T-scores were calculated using healthy reference databases. Inter-relationships between measurement techniques were determined using Pearson correlation coefficients. P values less than 0.05 were considered statistically significant.
Results: The average ages of the SCI and non-SCI groups were 33±9 and 27±6 years, respectively. Lesion level ranged from C4 to T12 and average time post-injury was 12 years, with a range of 1.6 to 25 years. Using the WHO criteria for osteoporosis, 9 of 14 SCI subjects were osteoporotic at the hip, with the remainder in the osteopenic range. Tibia SOS T-scores were in the osteoporotic range for one subject with SCI, and two were in the osteopenic range. All individuals without SCI were within the normal range for SOS T-scores. Hip BMD and tibia SOS were significantly correlated ($r = 0.46$, $p<0.01$). Hip BMD and tibia BMD were more strongly correlated ($r = 0.80$, $p<0.0005$). Tibia BMD was not significantly correlated with SOS at the tibia ($r = 0.35$, $p=0.09$). Radius SOS T-scores were positive and not significantly correlated with any lower limb variable.
Conclusion: Lower limb bone mass is reduced in spinal cord-injured individuals, but SOS at the mid-tibia is not. It remains to be determined whether ultrasound measurements can predict fracture in the SCI population.

4.2 Introduction

Lower-limb bone loss is an expected consequence following spinal cord injury (SCI). A cross-sectional study of bone changes following SCI estimated bone mass at the distal femur after an SCI to be 22%, 27% and 37% lower than controls at 3, 4 and 16 months post-injury, respectively (73). A large cross-sectional study documented post-SCI bone density at the femoral neck, midshaft and distal femur to be 27%, 25% and 43% below controls, respectively (104). Dramatic reductions in bone mass may predispose individuals with SCI to fracture, particularly in the lower limbs. A recent study demonstrated that among 41 men with SCI, 25 were osteoporotic and 8 were osteopenic at the femoral neck according to the World Health Organization criteria for osteoporosis, and 14 of these subjects had sustained a fracture after the SCI (111). Lower-limb fractures occurring in SCI individuals are often a result of trivial injuries or falls that would not normally cause a fracture, demonstrating the severity of osteoporosis (93). Delays in obtaining medical care after fracture, and/or misdiagnoses of fracture in the SCI population have been reported (93;170;170).

The standard method of evaluating fracture risk and diagnosing osteoporosis is via measurements of hip and spine bone mineral density (BMD) using dual-energy x-ray absorptiometry (DXA). Accessibility to DXA machines is often limited to larger cities, so alternative methods for assessing bone status would be useful. Newer techniques, such as quantitative ultrasound (QUS) may provide a portable, more economical alternative to DXA that is also free of ionizing radiation. The Sunlight Omnisense Ultrasonometer (Sunlight Medical, Rehovot, Israel) is capable of measuring the speed of sound (SOS) conduction through bone at the phalanx, radius, tibia and metatarsal. The ability of the Omnisense to measure SOS at the tibia makes it an attractive alternative to DXA for osteoporosis screening, since it is portable, does not require a transfer onto a scanning table, and measures a site relevant for individuals with SCI. However the ability of QUS to predict fracture is not as well established as for DXA, especially in the SCI population. The current study evaluated the relationship between hip BMD, as measured by DXA, and SOS, as measured by QUS, at the tibia and radius in individuals with chronic SCI. In order to evaluate the relationship between SOS and BMD across a large range of BMD values, a group of non-SCI individuals with normal bone mass was also included in the study. Since fractures of the proximal tibia are common in the SCI population (93), proximal tibia BMDs were also compared to tibia SOS.

4.2.1 Hypothesis

It was hypothesized that tibia SOS would be related to BMD measures at the hip and tibia, and that individuals with SCI would not only have low bone mass, but also have low SOS at the tibia.

4.3 Methods

4.3.1 Participants

14 spinal cord-injured subjects and 10 non-spinal cord injured subjects were recruited in accordance with the policies of the McMaster University Research Ethics Board. Informed consent was obtained prior to beginning the investigation. Exclusion criteria included the presence of metal implants in the measured leg or hip, or the possibility of pregnancy.

4.3.2 Protocol

SOS values at mid-tibia and distal third radius of each subject were measured using the Omnisense Ultrasonometer (Sunlight Ultrasound Technologies Ltd), which can be used to measure SOS at several skeletal sites. Ultrasonic waves at a frequency of 1.25 MHz are transmitted to the bone from a transducer in the ultrasound probe, and the speed of conduction through bone is measured. Software particular to the ultrasonometer digitizes and analyzes the signal, accounting for soft tissue. Long- and short-term precision for the Omnisense has been reported previously (106). For individuals with SCI who had lower limb edema, two or three measurements at the tibia were often necessary in order to obtain a satisfactory signal.

Total proximal femur BMD and proximal tibia BMD were measured using dual-energy x-ray absorptiometry (DXA, Hologic 4500). Hip BMD was measured according to standard protocol. Tibia BMD was measured using the Hologic 4500 lumbar spine protocol, with modifications to the methods of analysis, described previously (138). The fibula was excluded from the analysis. The subject's lower leg was placed onto a specialized positioning device, which held the leg in 5 degrees of knee flexion and approximately 10 degrees of internal rotation. The scan distance was kept the same as that for the lumbar spine protocol. The scan was started at a point 20 cm below the superior aspect of the patella. The size of the region of interest to be analyzed was calculated relative to the width of the tibial epiphysis, so that the region of interest was proportional to body size.

4.3.3 Statistical Analyses

Criteria analogous to those developed by the World Health Organization (WHO) for the interpretation of femoral neck BMD in postmenopausal women were applied to individual tibia and hip BMDs to categorize the participants as normal, osteopenic or osteoporotic (1;97). The Hologic 4500 contains a database of normal hip BMD values for a healthy reference population that were used for calculation of T-scores in the participants. The Sunlight Omnisense Ultrasonometer also contains a reference database of normal radius and tibia SOS values that were used for T-score calculation. For tibia BMD, we have collected sufficient reference data to calculate T-scores. Pearson product moment correlations were calculated to determine if significant relationships existed between variables measured at the hip, radius and tibia using x-ray attenuation and ultrasound propagation. The p values considered to be associated with statistical

significance were those less than 0.05. Raw data and statistical analyses are given in appendices F and G, respectively.

4.4 Results

The average age of the participants with SCI (11 males and 3 females) was 33±9, and the lesion levels ranged from C4 to T12. According to the American Spinal Injury Association (ASIA) the neurological impairment level for one individual was ASIA A, three were ASIA B, eight were ASIA C and two were ASIA D. The average time post-injury was 12 years, with a range of 1.6 to 25 years. The non-SCI participants (6 males and 4 females) averaged 27±6 years of age.

Applying the WHO criteria for osteoporosis to the hip BMD data, 9 of 14 SCI subjects were osteoporotic at the hip, and 5 were osteopenic. All of the non-SCI individuals had normal hip BMD according to WHO criteria. If the WHO criteria for osteoporosis were applied to the tibia BMD data, for the individuals with SCI, 7 subjects would be considered osteoporotic, 3 would be osteopenic and the 3 others had T scores between –0.233 and –0.946. None of the non-SCI individuals had low tibia BMD. Average BMD data are presented in Table 6.

Table 6: Mean (±SEM) bone mineral density at the hip and tibia, and SOS at the radius and tibia in individuals with and without SCI

	Total Hip BMD (g/cm^2)	Proximal Tibia BMD (g/cm^2)	Radius SOS (m/s)	Tibia SOS (m/s)
Males, non-SCI (n=6)	1.131 (±0.060)	1.217 (±0.073)	4184 (±42)	3988 (±29)
Males, SCI (n=11)	0.661 (±0.040)	0.670 (± 0.052)	4197 (±33)	3928 (±28)
Females, non-SCI (n=4)	0.985 (±0.037)	0.996 (±0.019)	4246 (±36)	4029 (±14)
Females, SCI (n=3)	0.678 (±0.017)	0.610 (±0.068)	4285 (±136)	3955 (±33)

None of the subjects with SCI had a tibia SOS T-score in the osteoporotic range. Two subjects had osteopenic T-scores. Radius SOS T-scores were positive and averaged 0.74 and 1.1 in males and females with SCI, respectively. Among the non-SCI individuals, one male had a tibia SOS T-score of –1.4, all others were within the normal range. Average SOS data is also presented in Table 6.

Hip BMD and tibia SOS were significantly correlated ($r = 0.46$, $p<0.024$), as shown in Figure 6. Hip BMD and tibia BMD were more strongly correlated ($r = 0.83$, $p<0.0005$), as shown in Figure 7. BMD at the tibia was not significantly correlated with

SOS at the tibia (r = 0.299, p=0.09, Figure 8). Radius SOS T-scores were not significantly correlated with any lower limb variable (p>0.05).

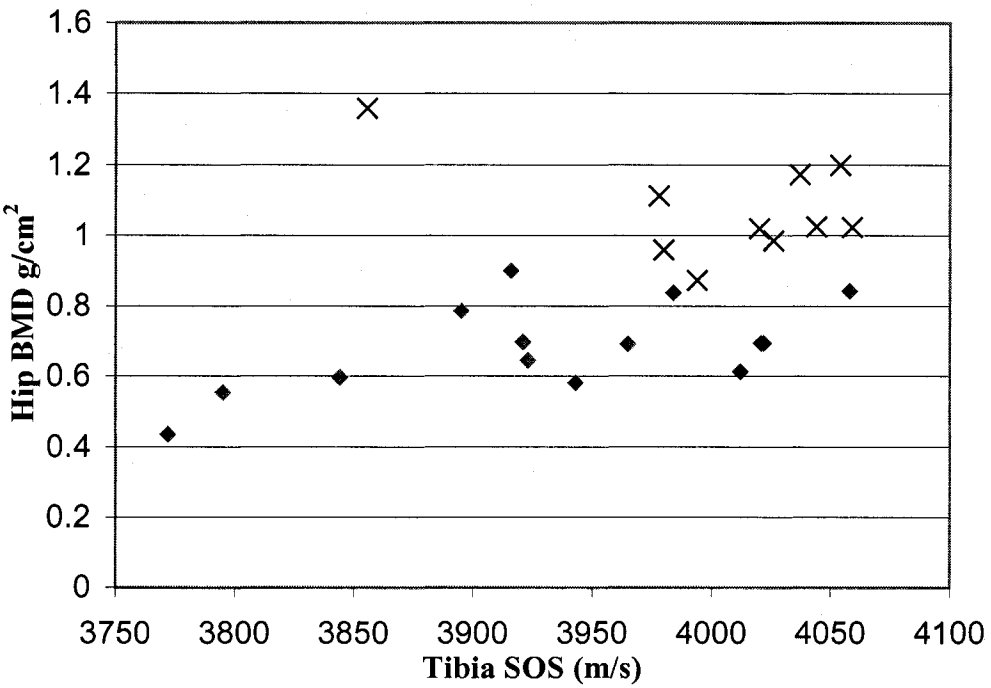


Figure 6: Hip BMD vs. Tibia SOS

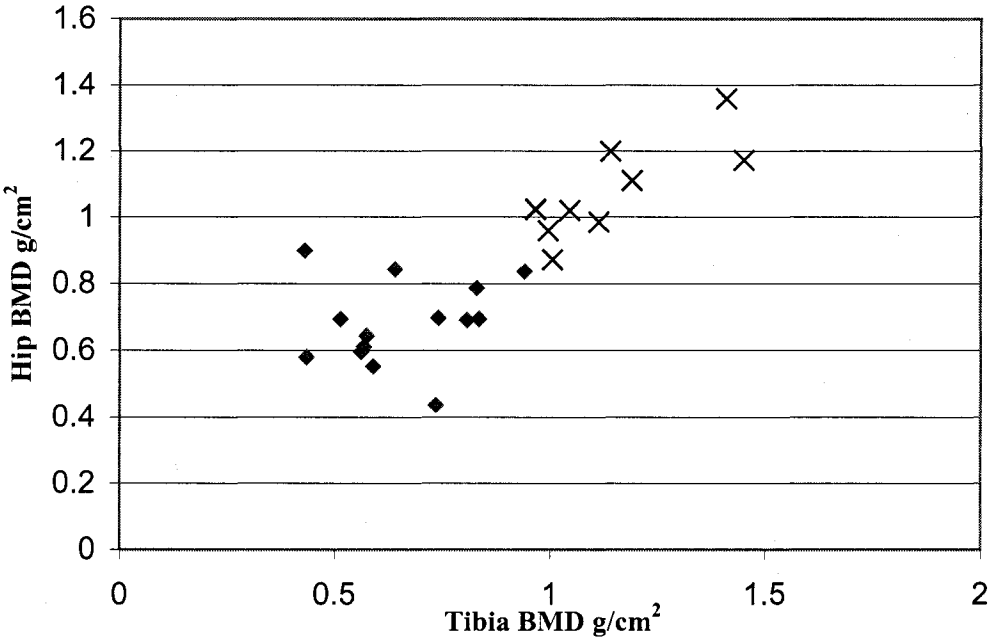


Figure 7: Hip BMD vs. Tibia BMD

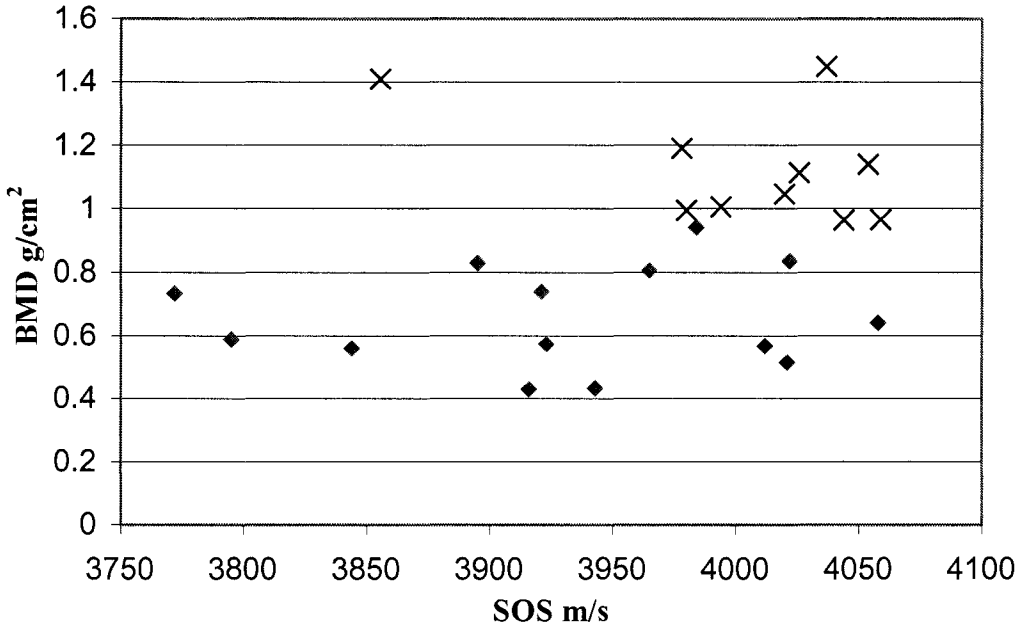


Figure 8: Tibia BMD vs Tibia SOS

4.5 Discussion

This study examined the inter-relationships between BMD at the hip and tibia and SOS at the tibia and radius in individuals with and without SCI. It is well established that individuals with SCI experience bone loss following injury, so it was not surprising that 9 of the 14 (64%) individuals with SCI studied would be considered osteoporotic at the hip according to the WHO criteria. The remaining SCI subjects were in the osteopenic range. In addition, given the age range of the individuals without SCI, it was not surprising that their BMDs were normal according to the WHO criteria.

Most of the SOS T-scores of the individuals with SCI were within the normal healthy population ranges. This was somewhat surprising given the extent of lower-limb bone loss that occurs following a spinal cord injury. Also, one of the individuals without SCI had a high hip BMD and tibia BMD (total hip T-score +2.2, tibia T-score +2.6), yet for the same individual, the tibia SOS was lower than most of the individuals with SCI (T-score –1.4). Correlation coefficients revealed that, although hip and tibia BMD are correlated, tibia SOS is not correlated with tibia BMD and is only moderately correlated with hip BMD. The weak to moderate correlations found in this study are similar to those demonstrated previously (58;58;106).

There are several possible explanations for the above findings. First, the lower-limb sites differ in location and bone type. SOS was measured at mid-tibia, whereas BMD measurements were taken at the proximal femur and proximal tibia. The proximal femur and proximal tibia sites contain both cortical and trabecular bone, whereas the mid-tibia site would be solely cortical bone. Second, the Omnisense Ultrasonometer measures the shortest path traveled by the ultrasound wave through bone and therefore reflects the properties of the cortical shell. It has been suggested that tibia SOS measurements are influenced more by the density of the outermost layer of the cortex than by the inner regions (152). SOS is also dependent upon other bone properties, such as the structural organization of the bone, which will affect the relationship between BMD and SOS. Finally, it may not be appropriate to apply the WHO T-score criteria for osteoporosis to ultrasound measurements, or sites other than the proximal femur (56). In addition, the relationship between T-score and fracture risk may be different in the SCI population. The WHO T-score criteria for BMD measurements are based on the likelihood of hip fracture in postmenopausal women, and may only apply to that population, skeletal site and measurement technique.

The use of quantitative ultrasound for assessing bone status in individuals with SCI is associated with a few practical limitations. The presence of lower limb edema can make it difficult to obtain a signal at the tibia. Two or three measurements were required in a few subjects in order to mobilize some of the fluid to increase the contact between the probe and the bone surface. In addition, radius and phalangeal SOS measurements can be difficult in individuals with contractures and scar tissue. Finally, heterotopic ossification, which can occur after a spinal cord injury, may significantly interfere with BMD data. Heterotopic ossification was not an exclusion criterion for our study, and

could potentially alter any associations between BMD and SOS measurements. However, that provides further argument for not using tibia SOS as a surrogate for tibia and hip BMD measurements in the SCI population.

Previous studies have demonstrated quantitative ultrasound to be sensitive to bone changes after SCI. In a cross-sectional study, "stiffness" at the heel (a composite measure derived from broadband ultrasound attenuation, or BUA, and SOS) was significantly lower in individuals with SCI than in a healthy reference population (34). Clearly the calcaneus consists predominantly of trabecular bone and the structural organization may be an important variable contributing to the ultrasound measurement. A recent prospective study demonstrated that ultrasound measurements of SOS and BUA were sensitive to bone changes in the acute stages of SCI. The changes in BUA over a 6-week period during the first 6 months of injury were similar in magnitude to the changes in BMD at the calcaneus and tibia (193). However, both of these studies employed ultrasound measurements at the calcaneus, and the ultrasound devices used were different from each other and from the one used in the current study.

The Omnisense multi-site ultrasound device can differentiate between pre- and post-menopausal women, and can distinguish individuals with hip or vertebral fracture from controls (68;68;83;106). However, the four measurement sites available with the multi-site device (radius, phalanx, metatarsal and tibia) may not have the same ability to predict fracture. Unlike the other three sites, tibia SOS was not able to distinguish women with vertebral fracture from women without vertebral fracture (106). Another study measured a broader range of sites and all were able to discriminate subjects with hip fracture from controls, although the tibia was not included among the sites measured (83). Radius SOS was able to discriminate Colles' fracture cases from controls, however the odds ratio was lower than for spine or hip BMD (105). SOS at the radius has also been demonstrated to be able to discriminate between elderly women with hip fracture and age-matched controls (195). Nevertheless, as with BMD measurements, there was some overlap in SOS values between the fracture and non-fracture groups. It is difficult to establish whether quantitative ultrasound is useful for osteoporosis screening in SCI when most studies investigating fracture risk have evaluated hip and vertebral fracture in non-SCI patients with osteoporosis. The ability of the multi-site ultrasound device to predict femoral and tibial fractures, the most common sites of fracture in the SCI population, has not been established.

4.6 Conclusions

The results of this study confirm that individuals with SCI have low bone mass according to the WHO criteria for osteoporosis. Practical diagnostic techniques for the evaluation of fracture risk after SCI are essential. Quantitative ultrasound is an attractive alternative to dual-energy x-ray absorptiometry because it is portable, patient-friendly, and free of ionizing radiation. However, it may not be appropriate to use speed of sound measurements as a surrogate for bone mineral density measurements in the SCI population. Future research should establish whether quantitative ultrasound

demonstrates the ability to predict fracture risk in order to validate its use as a surrogate measure of bone status.

5.0 CHAPTER 5: DOES BODY WEIGHT SUPPORTED TREADMILL TRAINING HAVE A POSITIVE IMPACT ON MUSCULOSKELETAL HEALTH IN INDIVIDUALS WITH CHRONIC, INCOMPLETE SPINAL CORD INJURY?

5.1 Abstract

Objective: To evaluate the impact of thrice-weekly body-weight supported treadmill training (BWSTT) for 144 sessions on musculoskeletal health in men and women with an incomplete spinal cord injury (SCI).
Methods: Thirteen individuals (2 females, 11 males) who had sustained a traumatic, incomplete SCI participated in thrice-weekly BWSTT for a total of 144 sessions (~12 months). The level of lesion ranged from C4 to T12, the average age of the participants was 29 years, and average time post-injury was 7.70 years (range 1.17 to 24 years). At baseline and after completion of training, bone mineral densities (BMDs) of the proximal femur, spine, and whole body were measured using whole body dual-energy x-ray absorptiometry (DXA) scans. As well, muscle CSA and bone density and geometry were measured using computed tomography scans at the mid-femur and proximal tibia. Serum osteocalcin and urinary deoxypyridinoline were measured at baseline, and after 36, 72 and 144 sessions of training, and ultrasound measurements at the radius and tibia were taken at those time points, as well as after 108 sessions. Significance was set at $p < 0.05$.
Results: The efficacy of BWSTT for improving walking ability in this group of participants has been published previously (89). Participants experienced significant increases in lean mass, from 45.9kg ± 2.3 to 47.8kg ± 2.4 (mean ±SE) before and after training ($p<0.003$). On average, lean mass in males increased from 48.1kg ± 2.2 to 50.2kg ± 2.2, and in females from 33.9kg ± 3.0 to 34.7kg ± 1.9. Total body fat mass did not change significantly after BWSTT. Corresponding increases in muscle cross sectional areas of 4.9% ± 2.1 and 8.2% ± 2.5 were observed at the thigh and lower leg sites, respectively. There were no significant changes in bone mineral density or bone geometry at any specific site, but whole body BMD exhibited a small but significant decrease ($p<0.006$). Similarly, there were no significant changes in bone biochemical markers or ultrasound measurements with BWSTT.
Conclusions: Body weight supported treadmill training is an intervention that has been shown to improve walking ability in individuals with spinal cord injury, and may also be a promising intervention for increasing lean mass. However, our data suggest that it may not increase, or prevent the loss of bone in individuals with chronic incomplete spinal cord injury.

5.2 Introduction

In the first few months after a spinal cord injury (SCI), bone mass and muscle mass are lost at a rapid rate, whereas fat mass has a tendency to increase (201). Osteoporosis in individuals with SCI predisposes them to an increased risk of fracture, particularly in the lower limbs, where the greatest bone loss occurs. Lower-limb fractures are often a result of trivial injuries or from falls that would not normally cause a fracture, demonstrating the severity of osteoporosis after SCI (93). A high percentage fat mass and reduced muscle mass may predispose individuals with SCI to an increased risk for cardiovascular disease, due to a myriad of factors such as reduced insulin sensitivity (10).

Rehabilitation techniques, such as functional electrical stimulation (FES), have been used as strategies to increase, or prevent the loss of bone and muscle mass in individuals with SCI. The ability of FES to increase lower limb muscle mass in individuals with SCI has been well documented (16;50;147;162), but the effects of FES exercise on the skeleton are not as well established. Several studies have demonstrated no effect of FES strengthening or cycle ergometry on measures of bone health (13;19;54;116;147), whereas a few studies have demonstrated increases in bone mass after FES-induced muscle strengthening (16) and FES cycle ergometry (135). FES and other potential therapies for improving lower limb bone mass in individuals with SCI need to be explored further. The data on standing or walking interventions in chronic SCI are limited, and are restricted to the effects on bone, not on muscle. A walking intervention for 12-20 weeks, incorporating an ambulation device that combined FES and a modified walker, did not result in increased hip BMD (141). Regular standing with the use of a standing frame did not increase BMD in individuals with chronic SCI, but the average duration of the intervention was only 135 days (109).

Body weight supported treadmill training (BWSTT) is a gait-retraining intervention that has been recently applied as a rehabilitation technique for individuals with incomplete SCI. Individuals with SCI who train with BWSTT have demonstrated improvements in their treadmill speed and exercise duration, and some have shown functional improvements in their ambulatory abilities (89;198). Since BWSTT involves progressive mechanical loading of the limbs while individuals walk on a treadmill, it was hypothesized that BWSTT might serve as a means for improving bone mass and body composition in individuals with chronic, incomplete SCI. Stewart and colleagues from our research group have reported that increases in muscle fibre size and a shift of the fibre types toward a less fatigueable fibre type profile occurs after only 68 sessions (~6 months) of thrice-weekly BWSTT (173). The current study reports the impact of 144 sessions (~12 months) of BWSTT on musculoskeletal health in individuals with SCI.

5.2.1 Hypotheses

1. Thrice-weekly BWSTT for 144 session will improve ambulatory abilities, on the treadmill and perhaps over ground, in individuals with chronic, incomplete SCI.

2. Skeletal health, as documented using bone biochemical markers, bone density and bone geometry, will improve as a result of 144 sessions of thrice-weekly BWSTT.
3. Muscle cross-sectional areas of the thigh and calf, as well as whole body lean mass, will increase after the BWSTT intervention.

5.3 Methods

5.3.1 Participants

The study was approved by Hamilton Health Sciences' Research Ethics Board. Participants were recruited via contact with medical staff at The Central Ontario, West Regional SCI Rehabilitation Program at Chedoke Hospital in Hamilton, and through local advertisement. 11 males and three females agreed to participate in the study, and provided informed written consent. All participants had sustained a traumatic incomplete spinal cord injury a minimum of 12 months prior to entering the study, and all but two had some motor function, as indicated by an American Spinal Injury Association (ASIA) score of C (47). Participants agreed to do BWSTT thrice weekly for 144 sessions (~12 months), had medical clearance from their physician to participate, and had mobility of the joints in their lower limbs. The age, gender, lesion levels, ASIA scores and years post-injury of all participants are presented in Table 7. Exclusion criteria were as follows: cardiac pacemaker or documented heart disease, uncontrolled cardiac dysrhythmia, chronic obstructive lung disease, uncontrolled autonomic dysreflexia, recent non-traumatic fracture, tracheostomy, bilateral hip and knee flexion contractures greater than 20°, drug addiction, age greater than 60 years, or persons greater than 40 years who failed phase 1 of a progressive incremental exercise tolerance test, and severe muscle shortening or severe skin ulcerations.

Table 7: Descriptive Characteristics of BWSTT Participants

Participant	Sex	Age	Lesion Level	ASIA score	Years Post-Injury
1	M	31	C4	C	1.2
2	M	22	T12 L1	C	1.3
3	F	32	C5/6	C	15
4	M	22	C5	C	3.5
5	M	26	T8	C	1.7
6	M	24	C5	C	24
7	M	28	C4	C	3.3
8	M	33	C4-5-6	C	10
9	M	53	C567	C	14
10	F	20	C5	C	5
11*	F	27	C5	C	11
12	M	29	C56	B	7
13	M	24	C56	C	9
14	M	32	T12(nerveT8)	B	1.5

C=cervical spine, T = thoracic spine
* did not complete the study

Four individuals originally recruited to participate were not able to commit to thrice weekly BWSTT for 12 months, and were subsequently included to provide a reference group for the degree of variation of bone mass and biochemical markers over the period of one year. After 6 months, one of these subjects was no longer able to return for testing for personal reasons, and his data will not be included in this study. Descriptive data for the reference control subjects is presented in Table 8.

Table 8: Descriptive Characteristics of Reference Controls

Subject	Sex	Age	Lesion Level	ASIA score	Years Post-Injury
1CN	M	32	T12	D	3
2CN	M	41	C56	B	24
3CN*	M	39	C8	D	13
4CN	M	40	C567	B	25

CN=control
C=cervical spine, T = thoracic spine
* did not complete the study

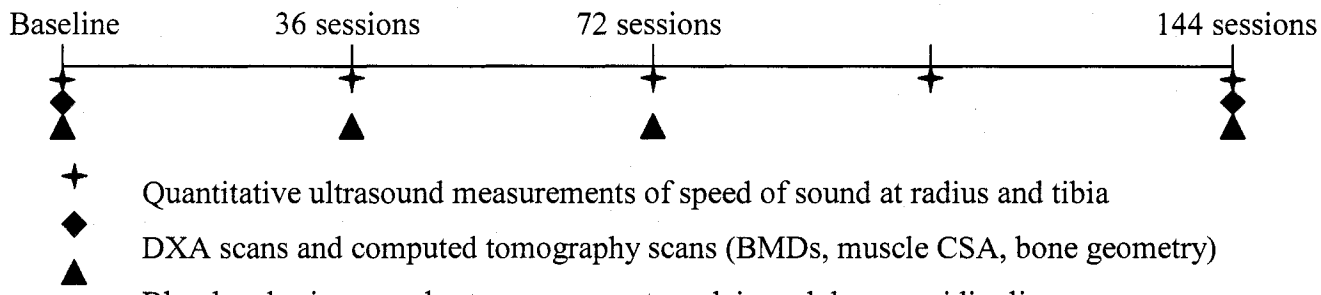
All participants were instructed to maintain current physical activity levels. One participant was involved in regular physiotherapy visits when entering the study and continued to do so throughout the duration of the study. One participant participated in regular aerobic and resistance training at a fitness centre, and had prior experience with functional electrical stimulation. This participant chose to cease this training while participating in the study due to time constraints. For all other participants, the major source of physical activity was the manual wheeling of wheelchairs. For ethical reasons, participants who were taking medications for osteoporosis were not asked to discontinue taking them. Of the 13 participants in BWSTT, 4 were taking a bisphosphonate, and 1 of the 3 reference group participants was taking a bisphosphonate at the time of recruitment.

5.3.2 Study Design and Outcome Measures

A longitudinal, prospective within-subject design was used in which the participants completed 144 sessions of body weight-supported treadmill training over a period of 12-15 months. Bone mineral density (BMD) scans and computed tomography scans were made at baseline and at the end of the study. Biochemical markers, namely serum osteocalcin and urinary deoxypyridinoline, were assessed at baseline and after 36, 72 and 144 training sessions, and quantitative ultrasound measurements were made at every 36-session time point at the radius and tibia. Briefly, proximal femur, distal femur, proximal tibia and lumbar BMDs were obtained from scans at each of those sites. From a whole body scan, whole body BMD, lean mass and fat mass were obtained. Muscle cross sectional areas (CSA) at the mid-thigh and the proximal end of the lower leg (66% of the tibia length, starting from the distal end) were obtained from computed tomography scans, as well as BMD, bone CSA, and maximum, minimum and polar moments of

inertia. Figure 9 depicts the schedule of testing throughout the study. Refer to Chapter 2 for a complete description of the methods used.

Figure 9: Schematic depicting the timeline for measurement of outcomes



Walking duration per session, number of walking bouts, heart rate during each walking session and amount of body weight support provided were recorded for each training session. Weekly attendance was recorded, and the reason for any missed sessions was noted. At baseline, and after 36, 72, 108 and 144 training sessions, a modified version of a scale developed by Wernig and collegues (198;199) was used to evaluate over ground walking abilities. The Modified Wernig Scale was provided in Chapter 2 (Table 2), and is also provided below.

Table 3: Modified Wernig Scale

Score	Classification
0	No walking capability, even with help of two therapists
1	Capable of walking < 5 steps with the help of two therapists OR along parallel bars
2	Capable of walking ≥ 5 steps with the help of two therapists OR along parallel bars
3	Capable of walking >1 length of the parallel bars, requiring assistance to turn
4	Capable of walking >1 length of the parallel bars, turning independently
5	Capable of walking along railing (< 5 steps) with the help of one therapist
6	Capable of walking along railing (> 5 steps) with the help of one therapist
7	Capable of walking with a rolling walking frame > 5 steps
8	Capable of walking with canes or crutches > 5 steps
9	Capable of walking without devices > 5steps

5.3.3 Statistical Analysis

The effects of the BWSTT intervention on the bone and muscle outcome variables were assessed using multiple analyses of variance (MANOVA) for repeated measures. Variables were analyzed in groups according to measurement technique. The densitometry data were further subdivided by measurement site, such that the hip data, lumbar spine data, whole body data, femur data and tibia data were all analyzed separately. The CT muscle data was analyzed separately from the CT bone data. If a MANOVA was significant at the $p \leq 0.05$ level, then variables were individually contrasted using a Student's T-test. DXA scan results were also normalized to T scores using normative values provided with the Hologic QDR 4500A software, in order to present the skeletal status of the participants relative to normative data. Speed of sound data for the radius and tibia were normalized to T scores using normative values provided with the Sunlight Omnisense Ultrasonometer, in order to express the participants' speed of sound measurements relative to the normal population. Raw data and statistical analyses are given in appendices H and I, respectively.

5.4 Results

5.4.1 Compliance and Methodological Considerations

Participants were considered compliers with the intervention if they were able to complete the required 144 sessions in a maximum of 15 months. After 6 months of training, one female subject was not able to maintain the attendance requirement of three sessions per week, and her data will not be included in the results of this study. Average (±SD) compliance during the BWSTT study was 78.7 (±7.5) percent, where compliance was defined as the number of sessions completed divided by the total possible sessions, presented as a percentage. The average number of sessions completed per week was 2.4±0.2. Attendance data are available in Appendix J. In one participant, serum samples could not be obtained due to poor venous access. In three participants, urine and serum samples were not obtained at the 36-session time point, as the decision to include a sample at 36 sessions occurred after the study had already begun, and these participants were already past that time point. A urine sample was not obtained in another participant at the 72-session time point due to scheduling conflicts. A signal could not be obtained at the radius for the speed of sound measurement in one participant at the 144-session time point. Lumbar spine BMD could not be obtained in three participants due to metal implants or other limitations. As mentioned previously (Chapter 2), tibia and femur BMD measurements were not obtained in five participants, as the required equipment was not available at the time of their scans.

5.4.2 Treadmill and Over Ground Walking Abilities

At the beginning of training, all participants required some assistance with at least one, if not both legs, while walking on the treadmill. The over ground walking abilities of each participant at each time point, measured with the Modified Wernig Scale, are listed in Table 9. The impact of BWSTT on ambulation in this group has been published previously (89). Of the 13 participants, four improved their walking abilities by at least 2 points, and one person improved their score by one. The amount of body weight support required during treadmill walking decreased progressively with training, and is depicted in Figure 10. All participants were able to progressively increase the speed at which they walked on the treadmill during training, and most were also able to increase the duration they walked for. Changes in treadmill training intensity (BWS, speed, duration) are listed in Appendix K.

Table 9: Scores on the Modified Wernig Scale After BWSTT

ID	Baseline	36 Sessions	72 Sessions	108 Sessions	144 Sessions
1	8	8	7	8	9
2	0	4	4	7	7
3	4	4	4	6	6
4	0	0	0	0	0
5	0	4	0	0	4
6	7	7	7	7	7
7	0	0	0	0	0

8	0	0	0	0	0
9	0	2	2	2	2
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	0

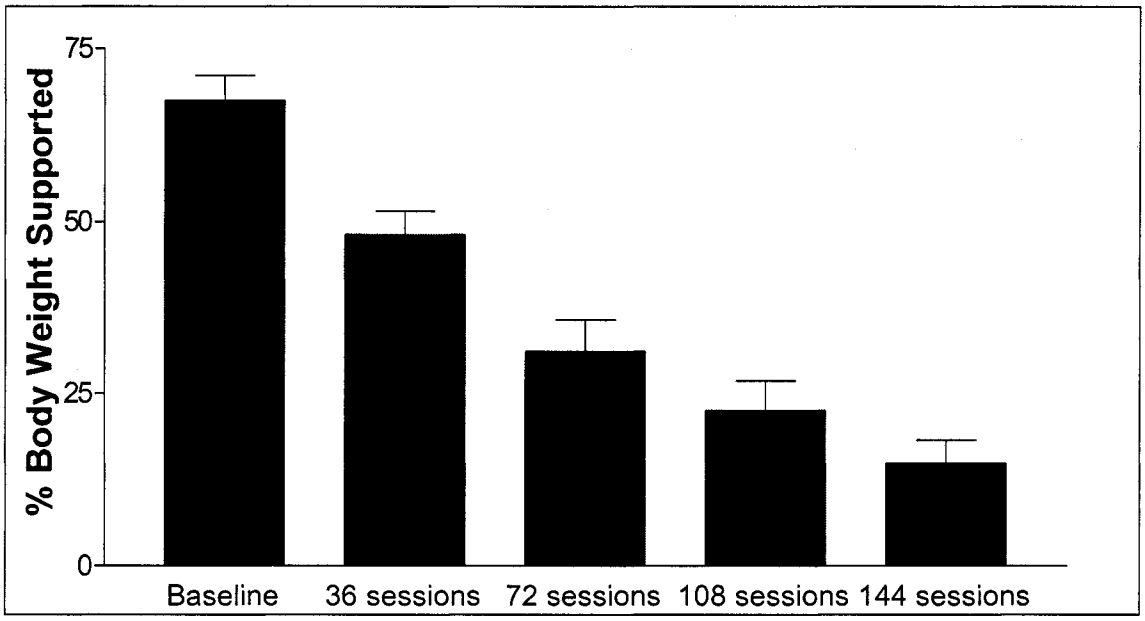


Figure 10: Average Body Weight Support Provided (%) at Each Time Point

5.4.3 Quantitative Ultrasound

Measurements of speed of sound (SOS) at the *distal third radius* were within the normal population ranges for all participants at baseline, when compared to the manufacturer's reference population. There were no significant changes in radius SOS at any time point during the intervention. At the *mid-tibia,* two participants had tibia SOS T-scores between –1 and –2.5, and all other participants were within the normal population ranges for tibia SOS. There were no significant changes in tibia SOS at any time point during the intervention. Average SOS data are presented in Table 10.

Table 10: Mean Speed of Sound Values at the Radius and Tibia in Males and Females During the BWSTT Intervention

	Radius SOS (m/s) Mean ±SEM			Tibia SOS (m/s) Mean ±SEM		
	Baseline	72 Sessions	144 Sessions	Baseline	72 Sessions	144 Sessions
Males (n=11)	4167±25	4174±32	4179±26	3934±30	3949±29	3962±24
Females (n=2)	4292±107	4224±145	4227±201	3966±45	3949±37	4043±6

5.4.4 Bone Biochemical Markers

Serum osteocalcin and urinary deoxypyridinoline concentrations were compared to reference ranges provided by the manufacturer of the assays used to analyze the biochemical markers. The reference ranges were provided in Table 3 (Chapter 2) and are also provided below. Levels of osteocalcin were at the high end of the normal range at baseline and throughout the study, and levels of deoxypyridinoline were approximately 2-3 times higher than the normal range at baseline and throughout the study. Levels of both biochemical markers were not significantly different from baseline at any time point during BWSTT. Average levels of osteocalcin and deoxypyridinoline at each time point are listed in Table 11.

Table 3: Reference Ranges for Urinary Deoxypyridinoline and Serum Osteocalcin

	Female Reference Range	Male Reference Range
Osteocalcin	3.7 – 10.0 ng/mL	3.4 – 9.1 ng/mL
Deoxypyridinoline	3.0 – 7.4 nmol DPD/mmol Cr	2.3 – 5.4 nmol/DPD/mmol Cr

Table 11: Mean (±SEM) Levels of Serum Osteocalcin and Urinary Deoxypyridinoline at Baseline, and after 36, 72 and 144 sessions of BWSTT

	Baseline	36 sessions	72 sessions	144 sessions
Osteocalcin (ng/ml)	13.8±5.0	9.7±1.5	14.8±6.8	12.5±4.2
Deoxypyridinoline (nmol DPD/mmol Creatinine)	9.9±1.4	9.3±0.8	10.2±1.2	10.8±0.9

5.4.5 Bone Mineral Density and Body Composition

Applying the WHO criteria for osteoporosis to the baseline hip BMD data, 8 participants would be considered osteoporotic at the right proximal femur, 3 would be osteopenic, and 2 would be considered to have normal BMD. The corresponding proportions for the left proximal femur would be 7 with osteoporosis, 4 with osteopenia and 2 with normal BMD. Of the subset of 10 participants in whom lumbar spine BMD measurements were possible, 4 participants would be considered osteopenic at the lumbar spine, and all others had BMD values within the normal range. There were no significant changes in BMDs at the proximal femur, lumbar spine, proximal tibia or distal femur with 144 sessions of BWSTT. Average proximal femur BMD data are presented in Table 12. Proximal tibia and distal femur data are presented in Table 13. The inter-individual variability in the BMD response to BWSTT was large. For example, changes in hip BMD ranged from a 5.6% increase to a 15.8% decrease after 144 sessions of BWSTT. The differential responses to BWSTT did not appear to be related to the amount of BWS required, although no statistical analyses were performed due to the small sample size. In most individuals, the change in BMD was within the range of measurement error. However, when BMDs were examined on an individual basis, changes in distal femur BMD were positive in all subjects except for one, whose change was –11%. Coincidentally, this individual was less than 2 years post-injury, whereas the remaining

individuals in the sub-group with distal femur BMD measurements were greater than 2 years post-injury. Similarly, 3 of 4 individuals who experienced reductions in average proximal femur BMD that were greater than 3 percent were less than 2 years post-injury at the start of the study. The only other individual who was less than 2 years post-injury experienced an increase in proximal femur BMD of approximately 5 percent.

Table 12: Mean Proximal Femur Bone Mineral Density (BMD) in grams /cm^2 Before and After BWSTT

	Right Proximal Femur BMD Mean ±SEM		Left Proximal Femur BMD Mean ±SEM	
	Baseline	144 Sessions	Baseline	144 Sessions
Males (n=11)	0.728±0.059	0.714±0.056	0.732±0.056	0.731±0.057
Females (n=2)	0.615±0.009	0.608±0.04	0.687±0.037	0.668±0.037

Table 13: Mean Distal Femur and Proximal Tibia Bone Mineral Densities (BMD) in grams/cm^2 Before and After BWSTT

	Distal Femur BMD Mean ±SEM		Proximal Tibia BMD Mean ±SEM	
	Baseline	144 Sessions	Baseline	144 Sessions
Males (n=7)	0.650±0.048	0.656±0.050	0.685±0.041	0.693±0.041
Females (n=1)	0.539	0.576	0.631	0.647

The impact of BWSTT on body composition variables was significant ($p \leq 0.05$), so the effect of BWSTT on the individual variables was investigated. Average whole body BMD and body composition data are presented in Table 14. There was a statistically significant reduction in whole body BMD ($p=0.006$). Whole body lean mass exhibited a significant increase after 144 sessions of BWSTT ($p=0.003$), with no significant change in whole body fat mass (Figure 11). The significant increases in lean mass prompted a regional analysis in order to determine where the changes in lean mass occurred. The whole body scan was divided into two sections, where the trunk and upper limbs were considered to represent the upper body and the lower limbs were considered to represent the lower body. Significant increases in lower body lean mass occurred after BWSTT ($p \leq 0.05$), and the increases in upper body lean mass approached significance ($p=0.06$).

Table 14: Body Composition Data Before and After BWSTT

	Before BWSTT Mean ±SEM	After BWSTT Mean ±SEM	% Change
Bone Mineral Density	1.118±0.028	1.094±0.029*	-2.1±0.6
Bone Mineral Content	2261±101	2232±97	-1.19±0.7
Lean Mass	45.9±2.4	47.8±2.5*	4.4±1.2
Fat Mass	23.6±3.0	24.0±2.9	5.1±4.0

Total Body Mass	71.7±4.3	74.1±4.4	3.6±1.7
Leg Lean Mass	13.5±1.1	14.7±1.1*	11.2±4.5
Upper Body Lean Mass	28.8±1.4	29.6±1.5	2.7±1.2

* significant at $p \leq 0.05$

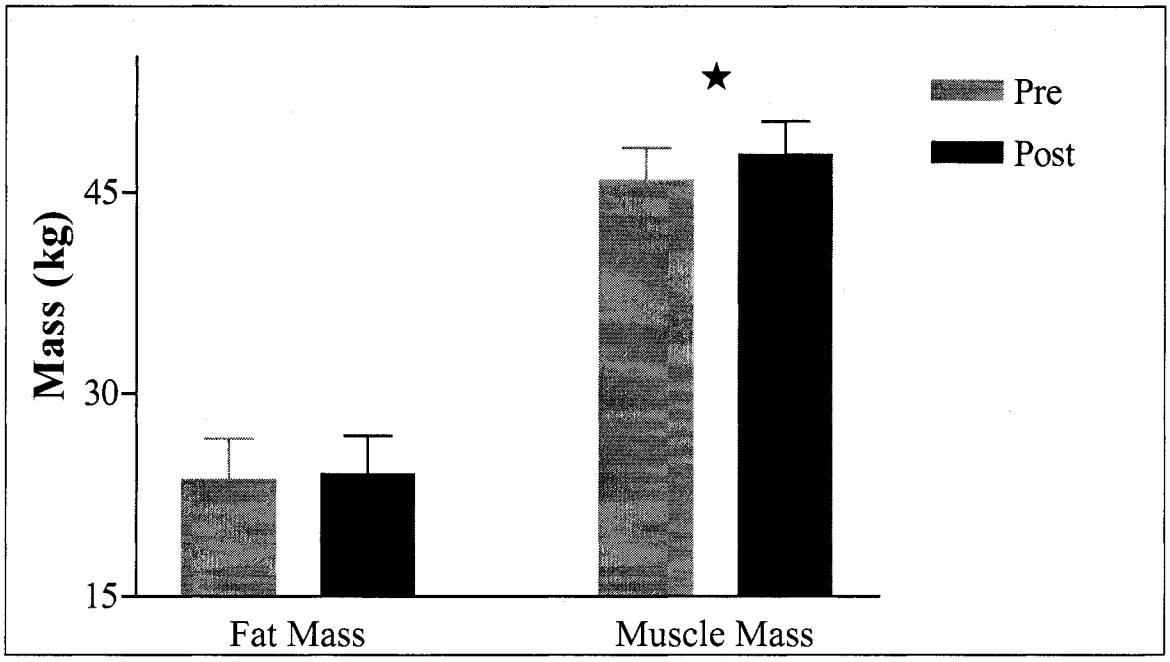


Figure 11: Average Whole Body Lean Mass and Fat Mass Before and After 144 Sessions of BWSTT

5.4.6 Muscle Cross-sectional Area (CSA)

Significant increases in thigh muscle CSA and lower leg muscle CSA (mm^2) occurred following 144 sessions of BWSTT ($p \leq 0.05$). The average increases in muscle CSA at the mid-thigh and lower leg (66% tibia length) sites were 4.9±2.1 percent and 8.2±2.5 percent, respectively. Average muscle CSA data are presented in Table 15.

Table 15: Average Thigh and Lower Leg Muscle CSAs Before and After BWSTT

	Before BWSTT Mean ±SEM	After BWSTT Mean ±SEM	% Change
Mid-Thigh Muscle CSA (mm^2)	9189±1156	9628±1229*	4.9±2.1
Lower Leg Muscle CSA (mm^2)	4849±563	5147±560*	8.2±2.5

*significant at $p \leq 0.05$

5.4.7 Bone Density and Geometry Measured with Computed Tomography

According to repeated measures multivariate analysis containing all bone geometry variables as well as volumetric bone mineral density (BMD) in g/cm^3, there were no significant changes in bone geometry or bone density at the mid-femur and 66% tibia sites with 144 sessions of BWSTT. Average values for all bone variables measured from CT scans are presented in Table 16.

Table 16: Mean Bone Density and Bone Geometry at Mid-Thigh and Lower Leg Before and After BWSTT

	Mid-Femur		Lower Leg	
	Before BWSTT Mean ±SEM	After BWSTT Mean ±SEM	Before BWSTT Mean ±SEM	After BWSTT Mean ±SEM
CSA, mm^2	434.5±24.4	429.0±23.0	387.0±18.5	381.3±17.5
BMD, g/cm^3	770.4±24.7	758.1±23.6	745.0±24.3	727.8±19.8
BMC, g	1673.9±109.5	1626±100.9	1437.3±78.2	1384.2±69.7
I_{max}	31240±2892	31155±2879	37041±3166	36312±2879
I_{min}	23100±2445	23069±2452	16840±1546	16712±1478
I_{pol}	54341±5212	54102±5193	53880±4517	53024±4140
Cortical CSA, mm^2	361.8±27.2	353.0±24.9	297.0±18.7	291.0±167
Cortical BMD, g/cm^3	847.9±13.4	840.9±12.0	851.3±15.6	834.2±10.5

I = moment of inertia, max = maximum, min = minimum, pol = polar

5.4.8 Reference Control Group

No statistical comparisons were made between the reference control and exercise groups due to the small number of participants (n=3) in the former. Bone biochemical markers did not appear to change over time in the reference group. Measurements of SOS at the radius and tibia were in the normal range, similar to the exercise group. Proximal femur BMD was reduced in one control participant (right –2.7%, left –10%), but not the other two. Two of the three control participants experienced reductions in proximal tibia

BMD, and all three experienced reductions in distal femur BMD, ranging from –0.9 to –8.6 percent, whereas only one participant in the exercise group experienced an appreciable change in distal femur BMD (-11%). The three participants in the reference group did not demonstrate reductions in whole body BMD. All individuals in the reference group experienced reductions in thigh and lower limb muscle CSA, with changes ranging from –2.3 to –16.8 percent.

5.5 Discussion

The goals of this study were to evaluate the impact of 144 sessions of progressive weight bearing exercise, in the form of body weight supported treadmill training (BWSTT) 3 times per week over 12-15 months, on bone and muscle in individuals with incomplete SCI. Previous studies of shorter duration demonstrated that BWSTT could improve walking abilities in incomplete SCI (43;44;197;198). This study represents the first prospective, longitudinal study evaluating the effects of BWSTT in individuals with chronic incomplete SCI. In addition to traditional outcome measures, such as walking abilities, we incorporated a more thorough analysis of the effects of BWSTT on musculoskeletal health. Since the dramatic losses of muscle and bone are attributed, at least in part, to the reduction in voluntary muscle activity and mechanical loading that occurs after SCI, it was hypothesized that the re-introduction of regular walking exercise may provide enough of a stimulus to partially reverse muscle atrophy and bone loss in individuals with SCI. Our results indicate that BWSTT can improve treadmill walking ability in individuals with incomplete SCI, but not all individuals will achieve over ground walking capability. BWSTT can increase lean mass of individuals with chronic incomplete SCI, particularly in the lower limbs. BWSTT did not appear to have a significant positive impact on the skeleton.

5.5.1 Effects of BWSTT on Walking Abilities

BWSTT has a positive impact on ambulation. Individuals who participated in regular walking on the treadmill with body weight support were able to improve their walking abilities, measured as treadmill speed, duration of walking and body weight support required, and these changes have been described previously (89). There are several potential mechanisms for improvements in walking abilities with BWSTT. Earlier work using BWSTT examined the effects of short-term training, and suggested that BWSTT could dramatically improve the walking abilities of wheelchair bound individuals (43;44;198). These conclusions were based on the theory that a central pattern generator exists in the spinal cord and that the stimuli provided by treadmill stepping could activate it, allowing individuals with SCI to learn to walk without usual supraspinal influences. Several studies have demonstrated that with locomotor training, such as BWSTT, the human spinal cord can produce electromyographic activity resembling that during locomotion (43;44;84;151;202). In support for the theory that improved ambulation after BWSTT is due to an activation of the central pattern generator, Wernig et al incorporated measurements of voluntary muscle activity and demonstrated that an improved capacity for independent stepping was not necessarily associated with an increase in voluntary muscle activity. Most studies concur that functional improvements in walking with locomotor training can only be achieved in individuals with incomplete lesions, suggesting that other factors are important for the attainment of over ground walking abilities.

Another potential mechanism for improved walking abilities after BWSTT is a musculoskeletal adaptation. In our study, not all participants were able to transfer improvements on the treadmill to over ground walking. Evaluating the improvements on

a case-by-case basis, it is evident that the level of residual motor function may dictate whether walking abilities learned on the treadmill can be transferred to over ground walking, with or without assistance. The individuals who experienced the largest improvements in walking abilities and achieved some level of over ground walking had the most residual motor function, defined as an ability to stand independently, or even take a few steps using assistive devices. Individuals who had little motor function made much smaller improvements on the treadmill, and could not walk over ground after BWSTT training, with or without assistive devices.

These observations are consistent with the theory that BWSTT training results in a muscle adaptation, such that it acts to improve muscle mass, and potentially muscle strength and coordination, so that improvements in walking abilities are achieved via maximizing the potential of the residual motor function. The regular walking exercise could be considered an exercise stimulus that resulted in increased muscle strength and muscle mass. In fact, the largest changes in muscle cross-sectional areas were noted in the individuals who made the greatest improvements in over ground walking. As well, participants experienced increases in muscle fibre size and a shift of the fibre types toward a less fatigueable fibre type profile after only 68 training sessions (173). Other studies support the theory that muscle adaptation and residual motor function are important for gains in ambulation after SCI. Dietz et al demonstrated that in patients with cauda lesions, BWSTT had effects on the muscle-tendon systems that could be separated from effects on spinal locomotor centres (45). Another study revealed that ambulatory SCI subjects had partial preservation of the long ascending and descending spinal pathways below the level of the neurologic lesion (151). Longitudinal, prospective studies of BWSTT and its effect on walking abilities and muscle activation patterns are necessary to deduce whether improvements are due to improved muscle function and coordination, the activation of a central pattern generator, or both.

There are distinctive differences between previous research in BWSTT and the current study. We chose to emulate a traditional outpatient rehabilitation setting, where participants came to the centre three times per week, and our inclusion criteria were less restrictive. We included participants who had minimal motor function, and most of our participants had cervical injuries. In the often-cited work by Wernig et al, participants were trained once or twice per day, for five days per week in a residential rehabilitation hospital. As well, participants were required to have some voluntary activity in the lower limb muscles and a reasonable potential to use canes or other assistive devices (198). It is possible that the training intensity and selection of patients who would be most likely to benefit could explain why Wernig et al observed such dramatic changes in walking abilities, whereas we did not. Given that our participants could not maintain perfect attendance at a frequency of three times per week, it is likely that a prescription of five times per week may be impractical in an outpatient rehabilitation setting.

5.5.2 Effects of BWSTT on the Skeleton

Thrice-weekly BWSTT did not appear to have a demonstrable effect on skeletal health. The absence of change in SOS measurements at the tibia was not unexpected because most participants did not have low SOS values compared to the reference population provided by the manufacturer of the ultrasonometer. Concentrations of the bone biochemical markers osteocalcin and deoxypyridinoline measured throughout the study did not change, consistent with the absence of overall change in bone mineral density or bone geometry at any site. We did note a statistically significant decrease in whole body BMD, but in the absence of change at standard sites used to assess fracture risk (i.e. proximal femur) this decrease does not necessarily indicate an increase in risk, especially given the small magnitude of the change. Nevertheless, no reduction in whole body BMD was noted in the 3 individuals in the reference control group.

With respect to skeletal effects, our results are consistent with the results of Needham-Shropshire and colleagues (141), who incorporated functional electrical stimulation and a modified walker into a walking intervention for individuals with chronic SCI, and found no significant changes in bone mineral density. Their intervention lasted 12-20 weeks, whereas this study is the first to examine a walking intervention in individuals with chronic SCI for the duration of one year or more. Individuals with chronic SCI who participated in regular standing for 135 days did not experience a change in bone mass (109), whereas individuals who performed standing in the acute stages after SCI appeared to have attenuated bone loss (77). Another study of individuals with acute SCI demonstrated that walking on a treadmill with weight support or standing resulted in attenuated bone loss compared to the loss experienced in a control group (41). Taken together, these results suggest that weight-bearing interventions may need to be initiated in the acute stages after SCI in order to realize any positive impact on the skeleton.

Functional electrical stimulation (FES), either during cycle ergometry or during contractions against resistance, has also been used in an attempt to improve bone mass after SCI, with mixed results. Many studies have demonstrated that FES exercise does not increase lower limb BMD in individuals with chronic SCI (13;19;116;147). Two FES studies that have shown increases in BMD in individuals with chronic SCI were of longer duration than most studies, and one incorporated FES exercise five days per week (16;135). The participants in these two FES studies had chronic complete SCI, and had lower baseline BMDs than those with incomplete SCI in our study. The longer study had individuals with SCI participating in FES cycling thrice weekly for 12 months, and then once weekly for an additional 6 months. Although they found a significant increase in proximal tibia BMD after 12 months, after the additional 6 months the increase had disappeared (135). Two studies of FES exercise in acute SCI had conflicting results; one demonstrated an attenuation of bone loss (82), while another did not (54).

Although the current study did not demonstrate any beneficial skeletal effects of BWSTT, it is possible that the potential for recovery of lost bone after SCI is minimal, or

that the level of strain imposed by BWSTT is not sufficient to stimulate increases in bone mass. According to Frost's mechanostat theory (64), the level of strain imposed on the bone would have to exceed a certain threshold minimum effective strain in order to induce increased bone formation and bone modeling. The intensity of BWSTT is largely determined by an individual's capabilities, so predetermined training intensities that are consistent across subjects cannot be incorporated into the intervention. It is quite possible that the applied strain rate and strain magnitude associated with BWSTT was not sufficient to exceed the modeling threshold, especially since most participants still required some body weight support at the end of the study, and most of the walking speeds were below what would be considered a normal walking speed. Muscle mass was increased, so it would be expected that loading due to muscle contraction was also increased, and perhaps with enough muscle growth a corresponding increase in bone might occur. However, the lack of significant change in osteocalcin or deoxypyridinoline levels throughout the study does not support an increase in bone formation or turnover with BWSTT. The frequency of exercise may not have been sufficient: participants were asked to participate in BWSTT three times per week, and the average attendance was 2.4±0.2 times per week. In accordance with recent research, shorter, more frequent exercise bouts (easy to administer to animals but not always practical in humans) may be the best strategy for increasing bone mineral accretion (157).

When one considers the influence of exercise on the skeleton in individuals without SCI, it is perhaps not surprising that BWSTT did not have a significant impact on the skeleton. As mentioned in Chapter 1, the potential for mechanical loading to enhance bone strength is greatest in the growing skeleton. Among adults exercise may help to prevent age-related bone loss, but does not appear to increase bone mass to a great extent, if at all (178). A recent systematic review revealed that exercise can have a positive impact on the skeleton in pre- and post-menopausal women, however the effect appears to be more a prevention of bone loss rather than a means of achieving bone gain (192). The youngest individual in our study was 20 years of age, so it is not likely that any of the participants were still experiencing skeletal growth and modeling to a great extent. Therefore, as in adults without SCI, perhaps the potential for BWSTT to increase bone mass and bone geometry is minimal in adults with SCI. It is possible that regular BWSTT could prevent further loss from occurring in adults with SCI, especially with increasing age.

The fact that the re-introduction of mechanical loading in individuals with SCI may not be able to restore lost bone is intriguing. The bone cells that have been implicated as the primary sensors of mechanical loading are the osteocytes, and mechanical loading can indirectly activate bone cells via fluid flow through the canalicular spaces (182). It is possible that the condition of disuse results in changes at the cellular level that alter the ability of bone cells to detect change. For example, it has been demonstrated that osteocytes deprived of bone loading became hypoxic after 24 hours of disuse, indicating that mechanical loading may be essential to maintain nutrient supply and waste removal for this cell population (49). A brief (< 4 minutes) loading protocol prevented osteocyte hypoxia from occurring. The under-stimulation of osteocytes during situations of chronic

disuse may result in osteocyte apoptosis and/or the removal of osteoclast-inhibiting signals (22). If chronic disuse results in a permanent deterioration of the osteocytes within the lacuno-canalicular network, the ability of the re-introduction of mechanical loading to increase bone formation may be limited. As well, if considerable micro-architectural deterioration was present in individuals with SCI, such as the perforation of trabecular plates and struts, it may be impossible to rebuild them through increased formation. It has been demonstrated that individuals with SCI have fewer trabeculae than individuals without SCI, and the remaining trabeculae are spaced further apart than in controls (133). The potential to re-build deteriorated trabecular structure after it has been lost is unknown.

There may also be other factors that determine whether BWSTT can positively impact bone mass, such as age, age at injury, duration post injury, level of injury etc. For example, four individuals experienced reductions in average proximal femur BMD greater than 3 percent, and three of these individuals were less than 2 years post-injury at the start of the study. These individuals might still have been experiencing accelerated loss as a result of the SCI, and BWSTT did not completely prevent that loss. Conversely, the only other individual who was less than 2 years post-injury experienced an increase in proximal femur BMD of approximately 5 percent. It is likely that there are a number of factors that may influence bone changes after SCI. If a larger number of participants could be incorporated in BWSTT studies, it would be possible to evaluate the factors that might influence the skeletal response to BWSTT.

5.5.3 Effects of BWSTT on Muscle

After 144 sessions of BWSTT, participants experienced significant increases in lean mass, as measured by both dual-energy absorptiometry and quantitative computed tomography. The noted increases in thigh muscle CSA of approximately five percent are smaller than those reported for FES cycle ergometry or FES-induced high force loading (50;169), but the differences may be attributable to the intensity of the loading. The amount of muscle activity that can be achieved with FES is likely larger than could be achieved during BWSTT. The changes achieved in this study are more comparable to those achieved in a study of elderly subjects participating in resistance training for one year, where an average increase of 5.5% in the knee extensors was observed (129). Of particular importance, the increases in muscle CSA have even more value when compared to the decreases in muscle CSA, ranging from 2.3% to 16.8%, seen in the three individuals in the reference control group. Not only is BWSTT effective for preventing further muscle atrophy, but it also appears to improve muscle mass in individuals with SCI.

BWSTT appears to provide enough of an exercise stimulus to progressively overload the muscles of the lower limbs and cause muscle hypertrophy. Significant muscle hypertrophy is often more likely associated with resistance training than with an endurance activity such as treadmill walking (120). However, it seems that the gradual reductions in body weight support and increases in speed that occurred as part of the

BWSTT protocol provided enough of a progressive training stimulus to overload the muscles of the individuals with incomplete SCI who participated in our study. Unfortunately, we were not able to measure muscle mass and muscle CSA changes throughout the study, which would have allowed an evaluation of the time course of the changes in muscle in response to training. However, a sub-group of these participants underwent muscle biopsies, and that data is presented elsewhere (173). Thrice-weekly BWSTT for ~6 months BWSTT resulted in significant increases in mean muscle fibre area of 25.5±6.3%. After SCI, the phenotype of muscle fibres in muscles below the level of lesion shifts to a higher proportion of type IIx fibres, which are less fatigue-resistant, and there is a reduction in muscle oxidative potential (165). After 68 sessions of BWSTT, there was an increase in the proportion of type IIa fibres and a reduction in type IIax/IIx fibres. These data provide further support for the findings that BWSTT results in muscle growth and positive changes in muscle morphology in the atrophied muscles of individuals with incomplete SCI.

5.5.4 Clinical Relevance

The positive effects of BWSTT on muscle and walking ability in individuals with chronic incomplete SCI could impact on other important aspects of health. Individuals with SCI have a reduced metabolic rate and are predisposed to carbohydrate and lipid abnormalities, due to their level of inactivity (10;102;163). There is also a tendency for individuals with SCI to have increased fat mass relative to controls without SCI (108;163). A benefit of more muscle mass may be improved glucose tolerance and increased metabolic rate, leading to less fat deposition. Referring again to the study conducted by Stewart et al in our research group, 68 sessions of BWSTT had a beneficial effect on blood lipid profiles, with significant reductions in total and low-density lipoprotein cholesterol, as well as decreases in total cholesterol/high-density lipoprotein cholesterol ratios and improved glucose metabolism (173). Increased lower limb muscle mass may also reduce seating pressures, and along with improved peripheral blood flow, may reduce the prevalence of decubitus ulcers (5). Anecdotally, participants indicated that regular BWSTT resulted in decreased lower limb edema, reduced levels of spasticity, and a feeling of warmth in the feet. One participant could even cease using support stockings.

5.5.5 Limitations

Several limitations of the current study should be acknowledged. Most individuals recruited to take part were participants in the intervention group, and those that served as a "reference control group" self-selected into that group, as they felt they could not commit to the longitudinal training intervention. A randomized, controlled design was not employed due to the small number of subjects recruited and the high potential for drop out among subjects who would be randomized to the control group, as we have experienced in previous exercise studies in this population. A randomized controlled trial is the ideal way to evaluate the effectiveness of an intervention, but in the chronic SCI population, it is not realistic. Unless a large number of participants are recruited, it is difficult to establish adequate matching between control and intervention groups in the

SCI population due to inter-individual variability in characteristics such as age, gender, level of lesion, ASIA score and time post-injury. Although the number of participants included in this study was not large, the logistical difficulties associated with conducting a yearlong longitudinal study of thrice-weekly training in the chronic SCI population are intimidating. The current study is the first to accomplish this, as well as being the first to incorporate measures of muscle and skeletal health as outcomes.

In studies of BWSTT, it is difficult to delineate the amount of work done by the participants versus the amount of effort assumed by the therapists working the legs. This has implications for the observed changes in treadmill speed and amount of body weight support required: changes in these variables may be partially a result of the therapists assuming proportionally more of the workload than the participant, so the actual improvement of performance is less. However, 5 of the participants were able to improve their over-ground walking abilities and were able to walk without assistance on the treadmill after training with BWSTT, indicating that the participants were exhibiting improved performance due to training. Although the gains that we could have achieved may have been greater with an increased training frequency or intensity, we aimed to emulate an environment that was representative of what would occur in traditional rehabilitation settings. Finally, the positioning device for knee BMD measurements was only available for a sub-group of the participants because the technique was not being used at the site where the scans were performed at the time when the study began. As this is the most clinically relevant site to measure BMD in the SCI population, it would have been advantageous to monitor change in all participants.

5.5.6 Future Directions

The effect of BWSTT on muscle mass and ambulation are encouraging, and warrant further investigation. All participants in the study were at least one-year post injury, and it is important to evaluate the ability of BWSTT to attenuate muscle atrophy and potentially prevent bone loss in the acute stages after SCI. Future studies of BWSTT should aim to incorporate multiple centres in order to include a greater number of participants. The current study had only two female participants. Future research should aim to include more females, as it is not appropriate to assume that the response to BWSTT would be the same in males and females. As well, in order to tease out whether improvements in walking ability are due to improved muscle function versus activation of a central pattern generator, improved methods of measuring muscle activity during training and voluntary muscle activation should be incorporated. Furthermore, the selection criteria for inclusion in BWSTT studies should be firmly established, so that appropriate comparisons can be made between studies. It may not be ethical to exclude participants because they are not as likely to make improvements in walking abilities, since we have demonstrated that even in the absence of dramatic changes in walking abilities, BWSTT can increase lean mass in individuals with incomplete SCI. If this intervention is to become standard therapy in the SCI population, the potential risk to the skeleton associated with BWSTT, both in measures of BMD, and in the risk of fracture associated with weight-bearing loading, should be fully clarified. Other outcome

measures that should be considered are those of importance to the participants, such as measures of edema or objective measures of spasticity.

5.5.7 Summary

Thrice-weekly BWSTT had a significant impact on whole body and lower limb lean mass and lower limb muscle cross-sectional area. Individuals participating in regular BWSTT improved their walking abilities on the treadmill, and a few individuals were also able to improve their over ground walking abilities. BWSTT did not appear to have a significant positive effect on bone mineral density of the lower limbs. Multi-centre, randomized controlled trials are required to ascertain the effectiveness of BWSTT as a gait-retraining intervention, and to further elucidate its effects on important health outcomes, such as body composition and skeletal health.

6.0 CHAPTER 6: DOES BODY WEIGHT SUPPORTED TREADMILL TRAINING HAVE A POSITIVE IMPACT ON MUSCULOSKELETAL HEALTH IN INDIVIDUALS WITH ACUTE SPINAL CORD INJURY?

6.1 Abstract

Objective: To evaluate the impact of twice-weekly body-weight supported treadmill training (BWSTT) for 48 sessions on musculoskeletal health in men and women with an acute spinal cord injury (SCI).

Methods: Five individuals (2 males, 3 females) who had sustained a traumatic SCI within 3-6 months of the start of training were recruited to participate in twice-weekly BWSTT for a total of 48 sessions (~6 months). The level of lesion ranged from C4 to T12, the average age of the participants was 29.6 years old, and average time post-injury was 114 days (range 66 to 170 days). At baseline and after completion of training, bone mineral densities (BMDs) of the proximal femur, spine, and whole body were measured using whole body dual-energy x-ray absorptiometry (DXA) scans. As well, muscle CSA and bone density and geometry were measured using computed tomography scans at the mid-femur and proximal tibia. Serum osteocalcin and urinary deoxypyridinoline were measured at baseline, and after 24 and 48 sessions of training. Significance was set at $p < 0.05$.

Results: All participants were able to improve their walking abilities on the treadmill, manifested as reductions in BWS required and increases in treadmill speed and duration. One participant was able to progress to independent walking over ground with a walker. All participants experienced increases in whole body lean mass, as well as increased muscle cross-sectional areas at the thigh and lower leg. Fat mass was also increased. Reductions in bone density, measured with both densitometry and computed tomography were evident in all participants at almost all sites after 48 sessions of training. No consistent changes were observed in bone geometry variables. BWSTT did not appear to alter the expected pattern of change in bone biochemical markers over time, although a reduction in levels of deoxypyridinoline was observed. Of note, the individual who made the most progress in ambulatory abilities demonstrated the smallest reduction in BMD at lower limb sites, and the individual who completed the fewest BWSTT sessions and was considered a non-complier demonstrated the largest reductions in BMD.

Conclusions: Body weight supported treadmill training is an intervention that has been shown to improve walking ability in individuals with spinal cord injury, and may also be a promising intervention for increasing lean mass. The data do not provide sufficient support for increases in bone mass or prevention of bone loss with BWSTT in individuals with acute SCI.

6.2 Introduction

Osteoporosis and muscle atrophy are frequently cited complications occurring after a spinal cord injury (SCI). A large proportion of the losses in bone and muscle occur in the first few years following the injury, with losses in muscle being the most rapid. In one study, average muscle cross-sectional areas (CSAs) were 18% to 46% lower in individuals who had sustained SCI only 6 weeks earlier when compared to control subjects (28). Prospective follow-up of these patients up to 24 weeks post-SCI revealed further declines in average thigh and lower limb muscle CSAs of 12-24 % (28). Another prospective study that employed dual-energy x-ray absorptiometry (DXA) to measure fat-free mass documented a 15% loss of lower limb lean mass in the first year after SCI (201). At the skeletal level, the rate of bone loss after SCI has been reported to be rapid and linear in the acute stages, establishing a new, lower steady state bone mass level 1-2 years after the event (73). Although the majority of bone loss occurs in the first few years after the injury, significant bone loss has been reported many years after an SCI, indicating that bone loss may not plateau as previous studies had reported (73;160;174).

Exercise interventions have been implemented in the acute stages following SCI as a potential strategy to ameliorate the musculoskeletal changes that occur. Functional electrical stimulation is the most commonly used means of exercising the lower limbs in individuals with SCI, and has been demonstrated to have a positive impact on muscle in both the acute and chronic stages after SCI (5;16;50;147;162). The effects of FES on the skeleton in the acute stages after SCI are less well established, and conflicting studies point to the need for further study before any conclusions can be made (54;82). Perhaps weight-bearing loading is a necessary stimulus for the prevention of bone loss in the acute stages after SCI. In a cross-sectional study, individuals with complete SCI who had performed standing during the acute phase post-injury, either with long leg braces, a standing frame or a standing wheelchair, had better preserved BMD at the femoral shaft than those who did no standing, and those who used long leg braces had higher total proximal femur BMD compared with patients using other standing devices (77).

Body weight supported treadmill training (BWSTT) is an intervention that allows individuals with SCI to walk on a treadmill with some of their body weight supported, and with therapists aiding the movement of the lower limbs if necessary. Individuals with SCI who train with BWSTT have demonstrated improvements in their treadmill speed and exercise duration, and some have shown functional improvements in their ambulatory abilities (89;198). Since BWSTT involves mechanical loading of the limbs while individuals walk on a treadmill, it was anticipated that BWSTT might serve as a means for improving musculoskeletal health in individuals with chronic, incomplete SCI. Previous work using early weight bearing after acute SCI by standing or treadmill walking resulted in no loss or only moderate loss in trabecular bone, compared to immobilized subjects, who lost 7-9% of trabecular bone at the tibia (41), but no studies have evaluated the effects of treadmill walking on muscle and bone together in acute SCI. The purpose of the present study was to evaluate the effects of twice-weekly BWSTT on bone and muscle in the acute stages after SCI.

6.2.1 Hypotheses

1. Individuals with acute SCI who participate in twice-weekly BWSTT for 6-8 months will improve their walking abilities, either on the treadmill or over ground.
2. The BWSTT intervention will prevent substantial skeletal changes (as measured by bone biochemical markers, bone density and bone geometry) from occurring in individuals with acute SCI.
3. Further muscle atrophy will be prevented in individuals with acute SCI participating in twice-weekly BWSTT.

6.3 Methods

6.3.1 Participants

Approval for the current study was received from the Research Ethics Board of Hamilton Health Sciences. Participants were recruited via contact with medical staff at The Central Ontario, West Regional SCI Rehabilitation Program at Chedoke Hospital in Hamilton. Two males and three females agreed to participate in the study, and provided informed written consent. All participants had sustained a traumatic spinal cord injury from 66 to 170 days prior to starting the intervention. Participants agreed to do BWSTT twice weekly for 6 months (48 sessions), had medical clearance from their physician to participate, and had mobility of the joints in their lower limbs. The age, gender, lesion levels, ASIA scores and days post-injury of all participants are presented in Table 17. Exclusion criteria were as follows: cardiac pacemaker or documented heart disease; uncontrolled cardiac dysrhythmia; chronic obstructive lung disease; uncontrolled autonomic dysreflexia; recent non-traumatic fracture; tracheostomy; bilateral hip and knee flexion contractures greater than 20°; drug addiction; age greater than 60 years; persons older than 40 years who failed phase 1 of a progressive incremental exercise tolerance test; severe muscle shortening or severe skin ulcerations. All participants continued conventional in-patient rehabilitation until they were discharged home, and continued with out-patient rehabilitation for the duration of the study. None of the participants were taking bisphosphonates during the study.

Table 17: Demographic Data of Acute BWSTT Participants

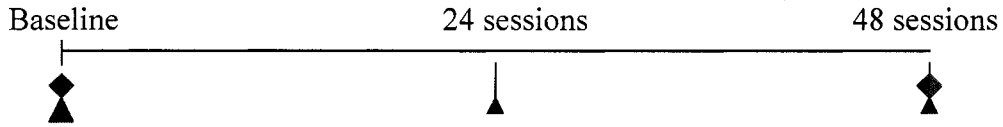
ID	Sex	Age (years)	Height (cm)	Pre-Injury Weight (kg)	Lesion Level*	ASIA score	Days Post-Injury at Intervention Start Date
1	M	26	173	90.7	C6	B	66
2	M	26	188	81.6	C3	B	82
3	F	40	152	52.2	C8	B	94
4	F	37	169	90.7	C5	B	159
5	F	19	180	72.6	C6	C	170

*C (lesion level)=cervical spine

6.3.2 Study Design and Outcome Measures

A prospective pre-post design was used in which the participants completed 48 sessions of twice-weekly body weight-supported treadmill training. Refer to Chapter 2 for a complete description of the methods used. In brief, the biochemical markers osteocalcin and deoxypyridinoline were assessed at baseline and after 24 and 48 training sessions. Bone mineral density scans and computed tomography scans were made at baseline and at the end of the study. Proximal femur, distal femur, proximal tibia and lumbar BMDs were obtained from scans at each of those sites. From a whole body scan, whole body BMD, lean mass and fat mass were obtained. Muscle cross sectional areas (CSA) at the mid-thigh and the proximal end of the lower leg (66% of the tibia length, starting from the distal end) were obtained from computed tomography scans, as well as BMD, bone CSA, and maximum, minimum and polar moments of inertia. Figure 12 depicts the schedule of testing throughout the study.

Figure 12: Schematic depicting the timeline for measurement of outcomes



◆ DXA scans and computed tomography scans (BMDs, muscle CSA, bone geometry)

▲ Blood and urine samples to measure osteocalcin and deoxypyridinoline

Walking duration per session, number of walking bouts, heart rate and amount of body weight support provided were recorded after each training session. Weekly attendance was recorded, and the reason for any missed sessions was noted. At baseline, and after 24 and 48 training sessions, a modified version of a scale developed by Wernig and colleagues (198;199) was used to evaluate over ground walking abilities. The Modified Wernig Scale was provided in Table 2 (Chapter 2), and is depicted below.

Table 2: Modified Wernig Scale

Score	Classification
0	No walking capability, even with help of two therapists
1	Capable of walking < 5 steps with the help of two therapists OR along parallel bars
2	Capable of walking ≥ 5 steps with the help of two therapists OR along parallel bars
3	Capable of walking >1 length of the parallel bars, requiring assistance to turn
4	Capable of walking >1 length of the parallel bars, turning independently
5	Capable of walking along railing (< 5 steps) with the help of one therapist
6	Capable of walking along railing (> 5 steps) with the help of one therapist

7	Capable of walking with a rolling walking frame > 5 steps
8	Capable of walking with canes or crutches > 5 steps
9	Capable of walking without devices > 5steps

6.3.3 Statistical Analysis

The small number of participants prohibited any formal statistical analyses of the outcome measures, so the data will be presented as a case series. DXA scan results were normalized to T scores using age- and sex-specific normative values provided with the Hologic QDR 4500A software, in order to present the skeletal status of the participants relative to normative data. Since changes in muscle cross-sectional area occur quite rapidly after SCI, age-, height-, pre-injury weight- and gender-matched non-SCI control subjects were recruited to provide estimates of pre-injury muscle cross-sectional areas for comparison, as measured by computed tomography. The demographic data of the control subjects is presented in Table 18. Controls were matched to participants as follows: same gender, same age within 4 years, same height ± 6 cm, and same weight ± 9.1 kg. Whether or not the changes in muscle CSA, fat CSA and bone geometry variables that occurred were meaningful was evaluated according to criteria outlined in Appendix 1. In brief, the reproducibility of the variables measured using CT was determined and expressed as root mean squared standard deviations (RMSSD) (76). If the observed change for a given variable was greater than the RMSSD for that variable multiplied by three, it can be stated with 99% confidence that the change that occurred was greater than that expected resulting from measurement error. All raw data are presented in appendix M.

Table 18: Demographic Data of the Height-, Weight-, Age- and Gender-matched Non-SCI Controls

ID of Match	Gender	Age (years)	Height (cm)	Weight (kg)
1	M	27	179	99.8
2	M	27	190	83.9
3	F	41	152	49.4
4	F	43	165	84.4
5	F	21	180	72.6

6.4 Results

6.4.1 Compliance and Methodological Considerations

Participants were considered compliers with the intervention if they were able to complete the required 48 sessions in a maximum of 8 months. One individual, participant 4, was not able to fulfill this requirement but since this was considered a feasibility study her data are still included here. For all outcome measures, the last time point for participant 4 did not represent her values after 48 training sessions, rather they were taken approximately 8 months after she entered the study. Average (±SD) compliance for all five subjects during the BWSTT study was 78.0 (±18.4) percent (the number of sessions completed divided by the total possible sessions X 100). The average number of sessions completed per week was 1.6 ±0.4. If subject 4 is not considered, the average (±SD) compliance was 85.4 (± 8.4) percent, and the average number of sessions completed per week was 1.7 (±0.2). Attendance data are listed in Appendix L.

6.4.2 Treadmill and Over Ground Walking Abilities

At the beginning of training, all participants required some assistance with both legs while walking on the treadmill. The over ground walking abilities of each participant at each time point, measured with the Modified Wernig Scale, are listed in Table 19. Of the 5 participants, only participant 5 improved her over ground walking and the improvement was large, progressing from no walking initially to being able to walk greater than 5 steps with a rolling frame. The amount of body weight support required during treadmill walking decreased with training, although substantial changes were only noted in participant 5. All participants were able to progressively increase the speed at which they walked on the treadmill during training, and most were also able to increase the duration they walked for. Changes in treadmill training intensity (BWS, speed, duration) are listed in Table 20.

Table 19: Scores on the Modified Wernig Scale After BWSTT

ID	Baseline	24 sessions	48 sessions
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	N/a
5	0	7	7

Table 20: Changes in % BWS, Treadmill Speed and Walking Duration with BWSTT

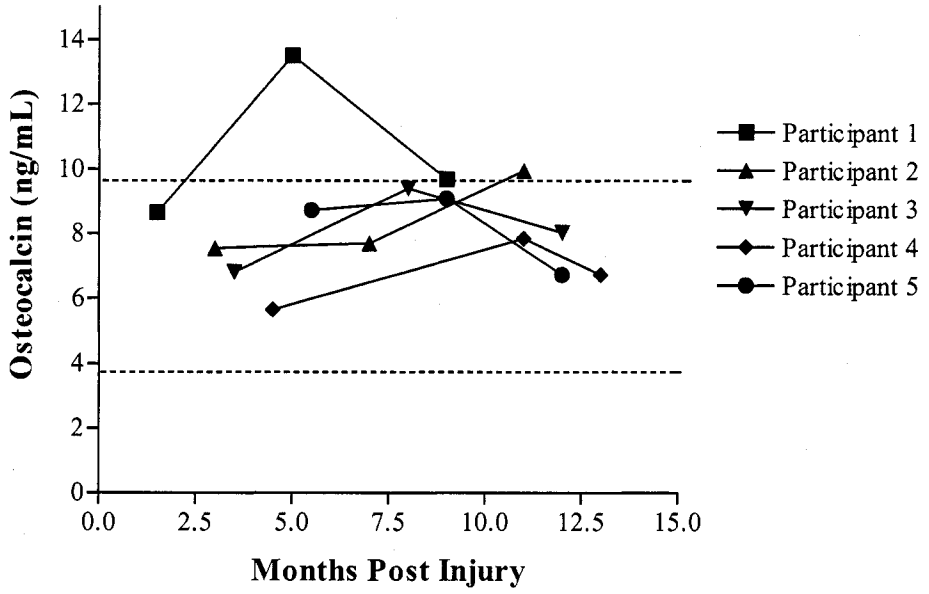
	% Body Weight Support			Treadmill Speed (km/hour)			Walking Duration per Bout (minutes)		
ID	Baseline (B)	24 sessions	48 sessions	B	24 sessions	48 sessions	B	24 sessions	48 sessions
1	94	55	45	0.5	1.6	2	5	10	10
2	87	52	57	0.5	1.7	1.7	5	10	10
3	91	77	66	0.7	1.7	1.8	5	12	15
4	97	55	N/a	0.5	1.7	N/a	5	7	N/a
5	54	0	0	0.6	1.0	0.7	5	10	10

6.4.3 Bone Biochemical Markers

Concentrations of osteocalcin and deoxypyridinoline were compared to reference ranges provided by the manufacturer of the assays used to analyze the biochemical markers. Reference ranges provided by the manufacturer were given in Table 3 (Chapter 2) and are also provided below. Levels of osteocalcin were at the high end of the normal range at baseline and throughout the study. When compared to the mid-point of the normal range provided by the manufacturer of the assay kits, levels of deoxypyridinoline were approximately 6.5 to 21 times higher than the normal range at baseline. After 24 and 48 sessions of training, DPD levels were reduced compared to baseline, but on average were still 2.4 to 10 times higher than the normal ranges. Each of the participants began the BWSTT intervention at different time points post-injury, and because OC and DPD levels vary depending on the time-point post-injury, the OC level and DPD level for each participant relative to their time post-injury at the time of the sample are presented in Figures 13 and 14 respectively. It is evident from Figure 14 that the DPD levels were elevated at baseline, which corresponded with the first 2-6 months post-injury, after which they began to decline in all participants.

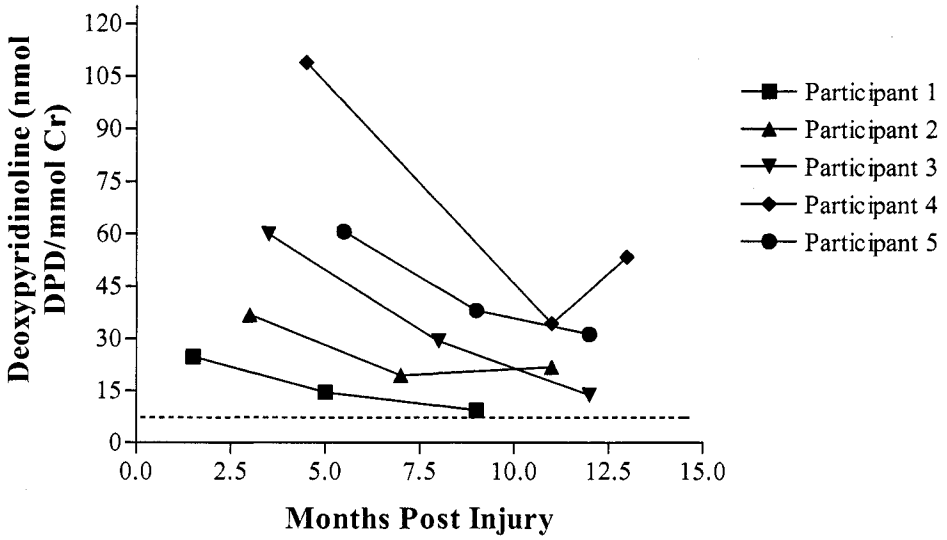
Table 3: Reference Ranges for Urinary Deoxypyridinoline and Serum Osteocalcin

	Female Reference Range	Male Reference Range
Deoxypyridinoline	3.0 – 7.4 nmol DPD/mmol Cr	2.3 – 5.4 nmol/DPD/mmol Cr
Osteocalcin	3.7 – 10.0 ng/mL	3.4 – 9.1 ng/mL



-------- Represents the range for normal healthy adults, 3.7 to 10 ng/ml and 3.4 to 9.1 ng/ml for females and males, respectively

Figure 13: Osteocalcin Levels at Baseline, and after 24 and 48 Sessions of BWSTT, Relative to Time Post-Injury



-------- Represents the high end of the normal range for females, 7.4 nmolDPD/mmolCr, where the high end of the normal range for males is 5.4 nmolDPD/mmolCr.

Figure 14: Deoxypyridinoline Levels at Baseline, and after 24 and 48 Sessions of BWSTT, Relative to Time Post-Injury

6.4.4 Bone Mineral Density and Body Composition

Applying the WHO criteria for osteoporosis to the proximal femur BMD data, one participant would have been considered osteopenic at baseline, and at the end of the study two more people became osteopenic and one had progressed to osteoporosis. All participants experienced reductions in total proximal femur BMD, ranging from 4.3% to 22.6%. Similar reductions in proximal tibia and distal femur BMD were noted after BWSTT, although one individual experienced an increase in proximal tibia BMD of 14.6%. Of note, the individual experiencing the smallest reductions in BMD at almost all lower limb sites was the same person who made the greatest improvements in ambulatory abilities, in that she was able to go from no ambulatory abilities to walking with a rolling frame. As well, the largest decreases in BMD at all lower limb sites occurred in the individual who was considered a non-complier with the BWSTT intervention. For the lower limb sites and the lumbar spine, BMD values pre- and post-training, as well as percentage change for each, are listed in Tables 21 and 22, respectively.

Table 21: Lumbar Spine and Total Proximal Femur BMDs After BWSTT

	Lumbar Spine BMD (g/cm^2)			Total Proximal Femur BMD (g/cm^2)		
ID	Baseline	After BWSTT	% Change	Baseline	After BWSTT	% Change
1	1.02	1.022	0.2	1.245	1.154	-7.3
2	1.063	1.023	-3.8	0.935	0.747	-20.1
3	0.972	0.936	-3.7	0.929	0.835	-10.1
4	1.225	1.134	-7.4	0.980	0.758	-22.6
5	1.186	1.174	-1.0	1.036	0.991	-4.3

Table 22: BMD at the Proximal Tibia and Distal Femur After BWSTT

	Proximal Tibia BMD (g/cm^2)			Distal Femur BMD (g/cm^2)		
ID	Baseline	After BWSTT	% Change	Baseline	After BWSTT	% Change
1	0.829	0.95	14.6	1.209	1.093	-9.6
2	0.933	0.879	-5.8	1.032	0.944	-8.5
3	0.952	0.855	-10.2	0.901	0.844	-6.3
4	1.105	0.894	-19.1	1.141	0.836	-26.7
5	0.985	0.973	-1.2	1.211	1.056	-12.8

Total body bone mineral content (BMC) and BMD from the whole body scans were reduced in 3 of the 4 participants. Whole body BMD and BMC could not be obtained from participant 3 after BWSTT. Participant 5 demonstrated little change in whole body BMD and BMC. On average, total body lean mass and fat mass were increased after 48 sessions of BWSTT, however participant 5 experienced a reduction in whole body lean mass. The average change in lean mass was an increase of 3.8%,

whereas the average increase in fat mass was 31%. Figures 15 and 16 depict the individual changes in these variables.

Table 23: Total Body BMC and BMD After BWSTT

	Total Body BMC (g)			Total Body BMD (g/cm^3)		
	Baseline	After BWSTT	% Change	Baseline	After BWSTT	% Change
1	2706.6	2536.3	-6.3	1.215	1.191	-2.0
2	2918.44	2684.7	-8.0	1.204	1.138	-5.5
3	1890.36	n/a		1.137	n/a	
4	2580.86	2278.2	-11.7	1.223	1.14	-6.8
5	2931.95	2967.7	1.2	1.193	1.202	0.8

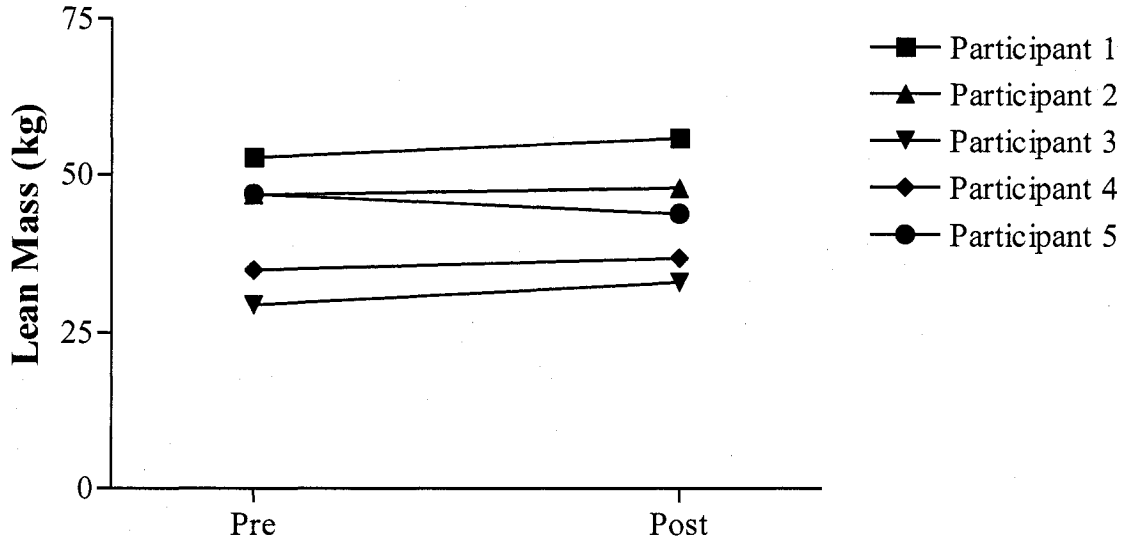


Figure 15: Individual Changes in Total Body Lean Mass Before and After BWSTT

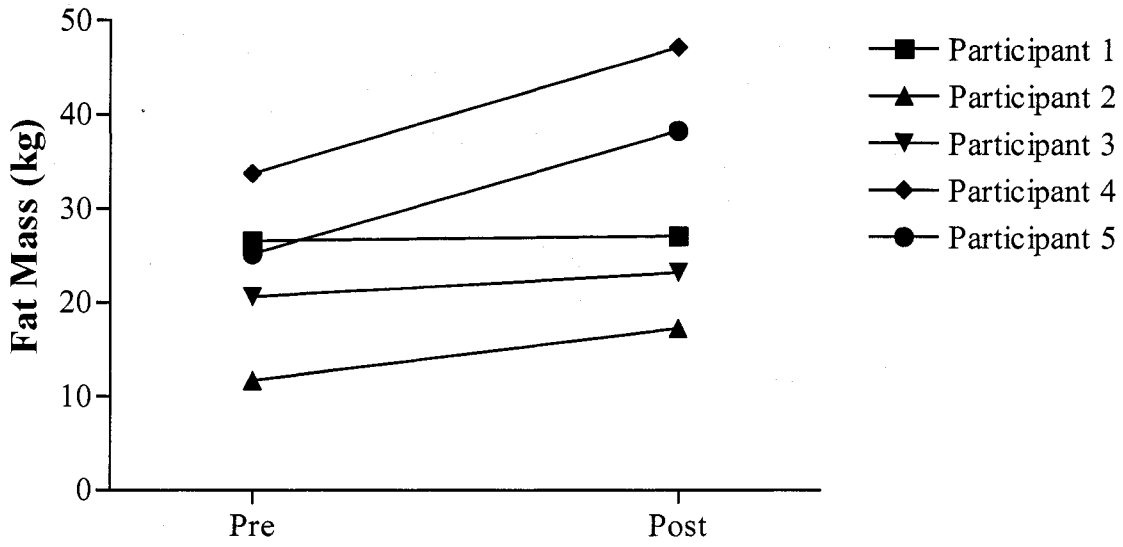


Figure 16: Individual Changes in Total Body Fat Mass Before and After BWSTT

6.4.5 Muscle Cross-sectional Area (CSA)

Muscle cross-sectional areas of the thigh and calf increased after 48 sessions of BWSTT. Each individual's muscle CSA for thigh and calf, along with percent changes, are presented in Table 24. All participants experienced an increase in thigh and calf muscle CSA after BWSTT, and the magnitude of the increase ranged from 3.8% to 56.9%. Individual changes in fat CSA for the thigh and calf are presented in Table 25. Fat CSA also increased in all participants at the thigh and calf sites, with the magnitude of the increases ranging from 10.3% to 54.4%.

As demonstrated in Table 24, the increase in thigh muscle CSA was greater than measurement error for two of the five individuals (participants 3 and 4). In participant five the change was greater than the RMSSD multiplied by two, such that with 95% confidence we could conclude that it was greater than measurement error. For calf CSA, the increase in muscle CSA was greater than three time the RMSSD in four of the five participants. The increase was greater than measurement error in four of five participants for thigh fat CSA, and for all participants for calf fat CSA.

Table 24: Individual Changes in Muscle CSA After BWSTT in Acute SCI

	Thigh Muscle CSA				Calf Muscle CSA			
ID	Baseline	After BWSTT	Change	% Change	Baseline	After BWSTT	Change	% Change
1	13979	14553	574	4.1	7001	9077	2076[b]	29.7
2	9478	9891	413	4.4	3396	3524	128	3.8
3	5438	8531	3093[a]	56.9	3922	4295	373[b]	9.5
4	5402	6970	1568[a]	29.0	2587	3974	1387[b]	53.6
5	9567	10328	760	7.9	3884	4772	888[b]	22.9

[a] Greater than three times the RMSSD of 852
[b] Greater than three times the RMSSD of 287

Table 25: Individual Changes in Fat CSA After BWSTT in Acute SCI

	Thigh Fat CSA				Calf Fat CSA			
ID	Baseline	After BWSTT	Change	% Change	Baseline	After BWSTT	Change	% Change
1	13794	16209	2415	17.5	2944	3693	749[d]	25.4
2	7033	10859	3826[c]	54.4	1452	1989	538[d]	37.0
3	13707	15124	1417	10.3	3422	4625	1203[d]	35.1
4	18573	21482	2909[c]	15.7	5269	7419	2150[d]	40.8
5	14098	18353	4255[c]	30.2	2930	3618	688[d]	23.5

[c] Greater than three times the RMSSD of 2807
[d] Greater than three times the RMSSD of 145

Because muscle is lost so rapidly following SCI, age-, height-, pre-injury weight- and gender-matched control subjects were recruited to provide an estimate of pre-injury muscle CSA, to be used for comparison. Figure 17 depicts the average muscle and fat CSAs for the thigh and calf, both at baseline and after 48 sessions of BWSTT, relative to the levels in control subjects. At baseline, the participants with SCI had thigh and calf muscle CSAs that were on average 60% and 65% of control values, respectively. Fat CSAs for the thigh and calf in the SCI participants were 113% and 100% of controls, respectively, at baseline. After 48 sessions of BWSTT, the participants with SCI had muscle CSAs that were on average 72% and 79% of controls at the thigh and calf, respectively. The corresponding fat CSAs were 142% and 133%.

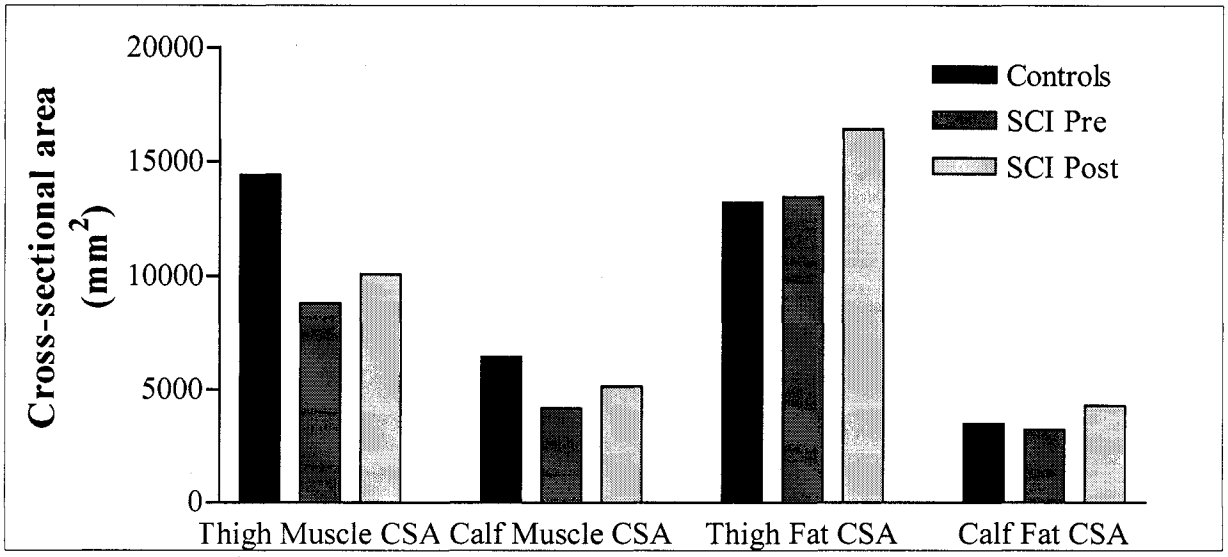


Figure 17: Muscle Cross-sectional Areas at the Thigh and Calf in Individuals with Acute SCI Before and After BWSTT, Compared to Non-SCI Individuals

6.4.6 Bone Density and Geometry Measured with Computed Tomography

Using the criteria of three times the RMSSD to identify meaningful change in the bone density and geometry variables between baseline and post-training measurements, it was evident that there were definite reductions in bone mineral density at both the mid-femur and 66% tibia sites in several subjects (Tables 26 and 27). With respect to all other variables, a few additional changes were noted to be larger than three times the RMSSD, but they were specific to an individual, site (tibia versus femur) and limb (left versus right). For example, participant 4 experienced reductions in bone area of 15% and 22% at the left and right mid-femur, and approximately 10% at the tibia in both the right and left legs. All reductions in bone area but the left mid-femur for participant 4 were greater than three times the RMSSD. In participant 4 reductions in the polar moment of inertia at one site (right mid-femur) and the maximum moment of inertia at another site (right tibia) were noted. An increase in bone area, as well as maximum, minimum and polar moments

of inertia occurred at the right mid-femur site in participant 5, as well as increased area and minimum moment of inertia at the left tibia. However, reductions in maximum and polar moments of inertia were noted for participant 5 for the right tibia site. Finally, increases in maximum, minimum and polar moments of inertia were also noted at the right mid-femur site in participant 1. When the left and right leg values were averaged for all variables, the only changes that were greater than three times the RMSSD were the bone density values, as mentioned above (Tables 26 and 27), the reduced bone areas for participant 4, and the increase in the polar moment of inertia at mid-femur in participant 5.

Table 26: Bone Density (mg/cm^3) at the Mid-Femur Before and After BWSTT

	Right Femur				Left Femur			
	BMD Pre	BMD Post	Change	% Change	BMD Pre	BMD Post	Change	% Change
1	814.4	744.7	-69.7[e]	-8.6	819.1	749.5	-69.6[e]	-8.5
2	782.7	783.0	0.3	0.0	768.7	769.0	0.3	0.0
3	816.0	798.0	-18.0[e]	-2.2	827.0	788.0	-39.0[e]	-4.7
4	786.0	698.0	-88.0[e]	-11.2	778.0	636.1	-141.9[e]	-18.2
5	773.0	754.1	-18.9[e]	-2.4	781.0	786.3	5.3	0.7

[e] Greater than three times the RMSSD of 10.2

Table 27: Bone Density (mg/cm^3) at the 66% Tibia Site Before and After BWSTT

	Right 66% Tibia				Left 66% Tibia			
	BMD Pre	BMD Post	Change	% Change	BMD Pre	BMD Post	Change	% Change
1	771.5	667.4	-104.1[f]	-13.5	781.2	704.7	-76.4[f]	-9.8
2	793.1	793.0	-0.1	0.0	774.8	775.0	0.2	0.0
3	870.0	866.0	-4.0	-0.5	871.0	793.0	-78.0[f]	-9.0
4	785.0	701.6	-83.4[f]	-10.6	803.0	681.1	-121.9[f]	-15.2
5	751.0	748.1	-2.9	-0.4	758.0	730.7	-27.3[f]	-3.6

[f] Greater than three times the RMSSD of 13.6

6.5 Discussion

The goals of this study were to evaluate the impact of 48 sessions of weight bearing exercise, in the form of body weight supported treadmill training (BWSTT) twice per week over 6-8 months, on bone and muscle in individuals with acute SCI. Previous studies of shorter duration demonstrated that BWSTT could improve walking abilities in both chronic and acute incomplete SCI (43;44;197;198). This study represents the first prospective, longitudinal study evaluating the effects of BWSTT on bone, muscle and walking abilities in individuals with acute SCI. Muscle atrophy occurring in the acute stages after SCI is rapid and dramatic, and substantial bone loss also occurs, albeit more slowly. These musculoskeletal changes have been attributed, at least in part, to the reduction in voluntary muscle activity and mechanical loading that occurs after SCI. It

was hypothesized that the re-introduction of mechanical loading, using BWSTT, might partially reverse muscle atrophy and prevent further muscle and bone loss from occurring in individuals with acute SCI. Our results indicate that BWSTT can improve treadmill walking abilities in individuals with acute SCI, however, the gains in over ground walking abilities may depend on residual motor function. It is important to note that BWSTT can increase lean mass of individuals with acute SCI, particularly in the lower limbs, but it cannot completely prevent bone loss.

6.5.1 Effects of BWSTT on Walking Abilities

All participants were able to improve parameters of walking on the treadmill, defined as amount of body weight support required, treadmill speed and training duration. One participant made a striking improvement in treadmill walking abilities and progressed to over ground walking with a rolling frame. Given that this participant was the only one with a motor incomplete lesion (ASIA C), it was expected that she would have the greatest potential for improvement. However, this participant entered the BWSTT study later than the other participants (170 days post-injury) and had completed conventional in-patient rehabilitation. The likelihood of spontaneous recovery decreases substantially 6 months after injury (15). It should be noted that she was still attending outpatient rehabilitation for 4 sessions per week. Previous research has demonstrated that only individuals with motor incomplete lesions (both acute and chronic) are able to recover some locomotor function after BWSTT (43;44;151;197;198). Based on the ASIA impairment classifications of the other participants, it would not be expected that they would be able to achieve any degree of independent ambulation.

It has been suggested that BWSTT is more effective than conventional rehabilitation in the acute stages after SCI for retraining locomotion (198). Early research using BWSTT was based on the theory that a central pattern generator exists in the spinal cord and that the stimuli provided by treadmill stepping could activate it, allowing individuals with SCI to learn to walk without usual supraspinal influences. Several studies have demonstrated that with locomotor training, such as BWSTT, the human spinal cord can elicit electromyographic activity in muscles resembling that observed during locomotion (43;44;84;151;202). However, most studies concur that functional improvements in walking with locomotor training can only be achieved in individuals with incomplete lesions, suggesting that other factors are important for the attainment of over ground walking abilities.

The ability of BWSTT to retrain gait may depend on the amount of residual motor function a person possesses. BWSTT can be considered a form of exercise training, where the potential to achieve over ground walking depends on the extent to which muscle strength, balance and coordination can be improved. The participant who was able to achieve over ground walking had some residual motor function in the lower limbs, whereas the other participants did not. Motor skills can be trained with weight support initially, and as muscle strength, balance and other components of locomotion improve, the amount of BWS can be reduced. The inability to achieve over ground locomotion with conventional rehabilitation in many individuals with incomplete injuries

may be due in part to a deficit in lower limb strength, which makes them unable to bear weight, much less ambulate. By strengthening the lower limb musculature, the potential for achieving over ground walking skills may be increased. In individuals with motor complete lesions, their lack of residual motor function restricts the ability to train the lower limb musculature to bear weight, so the extent to which walking abilities can improve is limited. Dietz et al demonstrated that in patients with cauda lesions, BWSTT had effects on the muscle-tendon systems that could be separated from effects on spinal locomotor centres, indicating that part of the improvement in walking abilities could be a result of improved muscle function (45). Evidence for a muscle adaptation to BWSTT has been demonstrated in individuals with chronic spinal cord injury in our rehabilitation centre (refer to Chapter 5). As well, the current study demonstrated that BWSTT also improves lower limb lean mass after acute spinal cord injury (see Effects of BWSTT on Muscle). These adaptations may have contributed to the walking improvements noted among our subjects.

6.5.2 Effects of BWSTT on the Skeleton

The results of the current study indicate that BWSTT cannot prevent bone loss from occurring after acute SCI. The dramatic increase in the bone resorption marker deoxypyridinoline and the small fluctuations in the bone formation marker osteocalcin both demonstrated similar patterns to those normally occurring after acute SCI in the absence of any intervention (156). Similarly, bone density loss, as measured by DXA was evident at all lower limb sites in the participants. However, an important observation was that the participant who made the largest gains in ambulatory capacity experienced the smallest changes in bone mineral density at almost all sites, whereas the participant whose attendance was so infrequent that she did not fulfill the criteria to be considered a complier lost the most bone, and had the highest levels of the bone resorption marker deoxypyridinoline and the lowest levels of osteocalcin throughout the study. Although these changes may provide support for the beneficial effects of BWSTT on the skeleton, it must also be considered that these observations may have occurred in the absence of an intervention, and it is difficult to assess the ability of BWSTT to prevent bone loss in the absence of controls matched for age, gender, as well as injury level and completeness. Another unique observation is that four of the five participants experienced reductions of lumbar spine BMD, confirming that bone loss after SCI may not be limited to the lower limbs. Lumbar spine BMD has been documented to be increased, decreased or unchanged after SCI (18;39;72;117;160;176). However, the changes in lumbar spine BMD were much smaller compared to the decrement in BMD that occurred at the lower limb sites, reflecting the importance of mechanical loading for maintenance of bone mass in the bones of the lower limbs.

The results of this study are in contrast to another study of weight-bearing after acute SCI. Walking on a treadmill with weight support or standing resulted in attenuated bone loss in individuals with acute SCI compared to the loss experienced in a control group (41). Although the results of that study are promising, they also elucidate the difficulties inherent in exercise studies after SCI. Although randomized controlled trials

are ideal, it is extremely difficult to match exercise and control groups on all confounding variables. For example, in the study mentioned above by de Bruin et al (1999), four of the nine individuals in the weight-bearing group had ASIA classifications of C or D, whereas the control group had one ASIA C, two ASIA B and an ASIA A. The ASIA C and D individuals may have been more likely to have better preserved bone mass in the absence of intervention. A few studies have examined the ability of FES exercise to prevent bone loss after acute SCI. Hangartner and colleagues (82) demonstrated an attenuation of bone loss in the acute stages of SCI with FES exercise, whereas Eser and colleagues did not (54). A few important differences between these studies make comparison difficult. Although they both used computed tomography, the former evaluated changes in proximal and distal tibia bone density, in both trabecular and cortical compartments, whereas the latter examined BMD changes at the tibial diaphysis. In addition, Hangartner et al compared the losses in the intervention group to the expected losses calculated by the authors using regression lines, whereas Eser et al compared an intervention group to a control group (54;82).

The mechanostat theory, proposed by Frost (64), provides a theoretical basis for the influence of mechanical loading on skeletal health. Mechanical loading of bone produces microstrain at the bone tissue level. When the level of microstrain on the bone is within a physiological loading zone, the amount of bone being resorbed and formed during bone turnover remains relatively equal, so that bone mass is maintained. If the level of microstrain on bone falls below a certain threshold, disuse-mode remodeling takes place, whereby the amount of bone resorbed is greater than the amount of bone formed. Bone is removed until the level of microstrain on the bone again falls within the physiological loading zone. Therefore, one could hypothesize that after SCI the dramatic reduction in microstrain at the bone tissue level results in a switch to disuse-mode remodeling, and that the amount of bone removed will be related to the change in microstrain on the bone when comparing loading conditions after the SCI to loading conditions before the SCI.

We hoped that by reintroducing a mechanical loading stimulus via BWSTT, the microstrain on the bone would be increased and ameliorate the removal of bone. The current study did not demonstrate notable changes in the pattern of biochemical markers after SCI. Prevention of bone loss might have occurred in the participant who achieved over ground walking, since she experienced the smallest reductions in BMD, compared to the other participants who did not achieve over ground walking. It is possible that the level of strain imposed by BWSTT was not sufficient to shift the level of microstrain at the bone tissue level into the physiological loading zone, especially since most participants still required some body weight support at the end of the study, and most of the walking speeds were below what would be considered a normal walking speed. Also, the intervention was for less than one hour twice per week, meaning that even if the loading resulted in microstrain within the physiological loading zone, the participants' bones only experienced that level of microstrain for a relatively small period of time. Recent research has suggested that shorter, more frequent exercise bouts may be the best means for preventing bone loss or increasing bone mass (157). Finally, a component of

the microstrain on bone comes from the strain imposed by muscle contraction pulling on the bone. Even at baseline, participants had already experienced substantial muscle atrophy when compared to age-, height-, gender- and weight-matched control subjects. If that component of microstrain has already been lost, attempts to re-create the same level of bone loading may not be feasible.

6.5.3 Effects of BWSTT on Muscle

The observation that BWSTT results in increases in thigh and calf muscle cross-sectional areas in individuals with acute SCI is exciting. Although we hypothesized that BWSTT would have a positive impact on muscle, we anticipated that the magnitude of change would be a prevention of muscle atrophy. The finding that BWSTT had a positive influence on muscle was substantiated by the observation that whole body lean mass also increased in our participants. In addition, as noted in Chapter 5, similar results were observed after BWSTT in individuals with chronic SCI.

A case study documenting the changes in calf muscle CSA with 8 weeks of cast immobilization and subsequent remobilization and rehabilitation demonstrated that the highest rate of atrophy occurs during the first 2 weeks of immobilization, where all three calf muscles lost over 5% CSA per week (187). The amount of muscle strength lost after immobilization was disproportionately greater than the amount of CSA lost. However, 10 weeks of rehabilitation, including endurance and strength training, was able to largely reverse the muscle atrophy that occurred. This case study highlights the plasticity of skeletal muscle. Functional electrical stimulation (FES) is the only other exercise intervention that has been examined as a means of preventing muscle atrophy after acute SCI. It has been demonstrated that FES cycle ergometry can prevent muscle atrophy in acute SCI, and even result in hypertrophy, when compared to a control group (5). However, it is important to note that FES cycle ergometry was more effective than FES-induced isometric contractions at preserving, and increasing muscle after acute SCI. In contrast, another study demonstrated a reversal of muscle atrophy after either dynamic or isometric FES-induced contractions in individuals within a year of SCI, in that quadriceps femoris muscle CSA had returned to levels measured at 6 weeks post-injury (50). These results highlight the need to further elucidate the optimal means for reversing muscle atrophy after SCI.

Similar to what was observed in individuals with chronic SCI, BWSTT can progressively overload the muscles of individuals with acute SCI and induce muscle hypertrophy. Treadmill walking would not normally be considered a method for inducing muscle hypertrophy. However, in individuals with acute SCI who have experienced a dramatic reduction in muscle activity the walking stimulus may represent a considerable challenge to the atrophied muscle. With only 5 participants, it is difficult to elucidate why some participants experienced changes in muscle of a greater magnitude than others, but it may be related to residual motor function, injury level, and exercise intensity achieved. The extent to which progressive overloading of the muscle can take place is largely dependent on the individual's capabilities; increases in treadmill speed and reductions in

body weight support are altered according to participant tolerance. Individuals with minimal motor function who did not recover any voluntary muscle activation limited the therapists' ability to increase the exercise intensity. However, despite these limitations, all participants demonstrated positive changes in muscle CSA, which exceeded our expectations of simply preventing further atrophy. These results indicate that the potential to increase muscle CSA with BWSTT exists even in individuals with motor complete lesions. Since the highest degree of atrophy occurs in the first few weeks of immobilization, strategies to prevent atrophy should be implemented as soon as possible after an SCI.

6.5.4 Clinical Relevance

Preserving muscle in individuals with acute SCI may have a positive impact on several health related outcomes, such as glucose tolerance, risk for cardiovascular disease, and quality of life. Individuals with SCI have a reduced metabolic rate and are predisposed to carbohydrate and lipid abnormalities, in direct relation to their level of inactivity (10;102;163). There is also a tendency for individuals with SCI to have increased fat mass relative to non-SCI individuals (108;163). A benefit of more muscle mass may be improved glucose tolerance and increased metabolic rate, leading to less fat deposition. A recent study by our research group demonstrated improved glucose regulation after BWSTT in individuals with incomplete SCI (148). BWSTT has been demonstrated to have a beneficial effect on blood lipid profiles in individuals with chronic SCI, with significant reductions in total and low-density lipoprotein cholesterol, as well as decreases in total cholesterol/high-density lipoprotein cholesterol ratios (173). Increased lower limb muscle mass may also reduce seating pressures, and along with improved peripheral blood flow, may reduce the prevalence of decubitus ulcers (5).

6.5.5 Limitations

Several limitations of the current study should be acknowledged. All participants who were recruited participated in the intervention, so there was no control group. A randomized, controlled design was not employed since only a small number of subjects could be recruited. Unless a large number of participants are recruited, it is difficult to establish adequate matching between control and intervention groups in the SCI population due to inter-individual variability in characteristics such as age, gender, level of lesion, ASIA score and time post-injury. Indeed, adequate matching may never be possible in this heterogeneous group. As well, since BWSTT has been demonstrated previously to have a positive impact on walking abilities and muscle mass, we felt it would be unethical to deny participants the intervention.

In studies of BWSTT, it is difficult to delineate the amount of work done by the participants versus the amount of effort assumed by the therapists working the legs. This has implications for the observed changes in treadmill speed and amount of body weight support required; changes in these variables may be partially a result of the therapists assuming proportionally more of the workload than the participant, so the actual improvement of performance is less. However, one of the participants was able to

improve over-ground walking abilities and was able to walk without assistance on the treadmill after training with BWSTT, indicating that performance improved due to training. Although the gains that we could have achieved may have been greater with an increased training frequency or intensity, we aimed to emulate an environment that was representative of what would likely occur in traditional rehabilitation settings. As well, since 4 of the 5 participants were still in in-patient rehabilitation at the start of the study, increasing the frequency of the BWSTT intervention would have interfered with the participants' conventional rehabilitation schedule, which would not be appropriate given that the efficacy of BWSTT in acute SCI is not fully established.

6.5.6 Future Directions

The effects of BWSTT on muscle mass and ambulation are encouraging, and warrant further study. Future studies of BWSTT should aim to incorporate multiple centres in order to include a greater number of participants, so that a randomized controlled design can be employed. The current study had only two male participants, so future research should aim to include more males, since they comprise a greater proportion of the SCI population. As well, in order to evaluate whether improved ambulation is related to improved muscle function versus activation of the central pattern generator, measurements of muscle activity during training and voluntary muscle activation should be incorporated. The criteria used in the selection of participants for whom BWSTT training would be beneficial should be firmly established, so that appropriate comparisons can be made between studies. Exclusion of participants because they are less likely to make improvements in ambulation may not be ethical, since we have demonstrated that even in the absence of dramatic changes in walking abilities, BWSTT can increase muscle mass and CSA in individuals with acute SCI. Outcome measures that may be relevant to the participants, such as lower limb edema or objective measures of spasticity, should also be considered

6.5.7 Conclusions

In summary, twice-weekly BWSTT for approximately 6 months partially reversed lower limb muscle atrophy in individuals with acute SCI. Individuals participating in regular BWSTT demonstrated improved ambulation on the treadmill, and an individual with a motor incomplete lesion was able to achieve over ground ambulation after BWSTT. BWSTT did not appear to have any effect on bone mineral density of the lower limbs. Multi-centre trials are required to confirm the effectiveness of BWSTT for improving ambulation, and to further clarify its effects on important health outcomes, such as body composition and skeletal health.

7.0 CHAPTER 7: GENERAL DISCUSSION AND CONCLUSIONS

7.1 Overview

The bulk of the research comprising this thesis focused on the influence of body weight supported treadmill training (BWSTT) on bone and muscle in individuals with spinal cord injury (SCI). Supporting research included a study evaluating the influence of metal rods on bone mineral and body composition measurements using dual-energy x-ray absorptiometry (DXA), and a cross-sectional comparison study of bone mineral density (BMD) and speed of sound (SOS) measurements (using quantitative ultrasound) in individuals with SCI and a group of individuals without SCI. It was revealed that the presence of metal rods weighing 100 or 150 grams significantly increased reported total body mass and bone mineral content. The proximity of the metal to the x-ray source did not influence the errors induced by the metal, and the metal effects were reproducible. The cross-sectional comparison of BMD and SOS measurements revealed that lower limb bone mass is reduced in spinal cord-injured individuals, but SOS at the mid-tibia is not. Hip BMD and tibia SOS were moderately correlated in the study sample.

In both acute and chronic SCI, BWSTT resulted in significant increases in lean mass, manifest as both increases in whole body lean mass (measured by DXA) and increases in thigh and lower limb muscle cross-sectional areas (measured by computed tomography). In individuals with chronic SCI, 144 sessions of thrice-weekly BWSTT resulted in a small albeit statistically significant reduction in whole body BMD, but otherwise, BWSTT did not have a significant impact on indices of skeletal health, namely lower limb BMD (measured by DXA), bone biochemical markers, SOS, or bone density and geometry measured by computed tomography. In individuals with acute SCI, twice-weekly BWSTT was not sufficient to completely prevent bone loss, as measured by DXA, bone biochemical markers, or computed tomography. The following discussion reviews the findings, examines methodological limitations and highlights potential suggestions for future research.

7.2 Effects of Metal Implants on Whole Body DXA Measurements of Bone Mineral Content and Body Composition

7.2.1 Major Findings and Discussion

Individuals with SCI often have metal rods implanted to stabilize the spine at the site of the injury. As well, in cases of fractured limbs, in both individuals with SCI and individuals without SCI, metal rods are often implanted to stabilize a fracture, and are not always removed. Since DXA is a commonly used clinical tool for the assessment of skeletal health, and is often used in research settings for measurement of body composition, knowledge of the effects of metal implants on these outcome measurements is useful. Several questions were examined in this methodological study. First, metal implants are often of different shapes and sizes, so the influence of increasing amounts of metal was investigated. It was revealed that metal had the largest impact on total body BMC, inducing errors of 1.5 to 3%. The presence of metal also had a significant impact on total body mass, and in some cases, soft tissue. Second, the proximity of the metal to the x-ray source did not have a significant influence on the body composition errors

induced by the metal. Finally, the effects of metal implants on measurements of bone mineral content and body composition will be reproducible during repeat scans of an individual patient. Therefore, serial whole-body DXA scans do not need to be adjusted for the metal effect. It is only important to take caution in interpretation of whole-body scans containing metal when taking one-time measurements of BMC where an error of 1.5% to 3% would be meaningful.

7.2.2 Limitations and Future Directions

A few methodological limitations should be acknowledged. The study was conducted in young, healthy adults without SCI, and it is possible that the impact of metal implants may be different in individuals with different body compositions. The metal rods used in this study were fabricated with 300LVM stainless steel, and although this type of metal is used in implants, metal implants composed of other materials may have dissimilar effects. However, given the small magnitude of the errors induced by the metal, and the fact that the errors are reproducible in repeat scans, it is likely that bone mineral and body composition data obtained from whole-body scans that include metal implants can be considered representative of an individual's true values, and that repeat scans do not need to be adjusted. Future research could evaluate the impact of other types of metal, and include study participants with a broader range of age and physical characteristics.

7.3 Speed of Sound in Bone at the Tibia: Is it related to lower limb bone mineral density in spinal cord injured individuals?

7.3.1 Major Findings and Discussion

Few studies have evaluated the clinical utility of quantitative ultrasound as a diagnostic tool in individuals with spinal cord injury. The cross-sectional study conducted as part of this thesis revealed that although most individuals with SCI were either osteopenic or osteoporotic at lower limb sites, mid-tibia SOS measurements were within the population normal ranges. This disparity between outcome measurements could have several plausible explanations. The lower limb sites where the measurements were performed differed in location and bone type. SOS measurements may reflect a bone property other than BMD, such as bone material properties. Finally, it may not be appropriate to apply the criteria used to diagnose osteopenia and osteoporosis to the SCI population and/or to quantitative ultrasound measurements, as they were selected based on the likelihood of proximal femur fracture in postmenopausal women (56).

This is the first study to evaluate SOS measurements in the SCI population using the Sunlight Omnisense Ultrasonometer. Previous research incorporating quantitative ultrasound measurements in individuals with SCI measured ultrasound transmission at the calcaneus, where the technology incorporated by the ultrasonometers varies from that used in the Omnisense. A cross-sectional study demonstrated that ultrasound measurements at the calcaneus were lower in individuals with SCI than in a healthy

reference population (34). In individuals with acute SCI, it was demonstrated that calcaneus ultrasound measurements were sensitive to change, and were similar in magnitude to BMD changes at the same site, as well as at the tibia (193). The current study does not necessarily suggest that the Omnisense is not a useful clinical tool, as it has been demonstrated to discriminate between individuals who have experienced a fracture (radius, spine or hip) and controls. However it appears that SOS measurements at the mid-tibia site in particular are less useful than SOS measurements at sites such as the distal radius (105;195).

7.3.2 Limitations and Future Directions

This study is limited by its cross-sectional nature. As well, a high proportion of the SCI individuals included in this study had incomplete injuries. It would be useful to evaluate a larger number of individuals with complete injuries, and to include a larger number of females. It would be of use to examine longitudinal changes in ultrasound measurements in the SCI population, to determine if they are sensitive to change, and also to evaluate whether they can predict fracture. Comparisons between quantitative ultrasound devices, with respect to their association with BMD, their sensitivity to change and their ability to predict fracture in the SCI population, would be useful if this technique is to become a widely used clinical tool. The presence of lower limb edema in the case of tibia SOS measurements and contractures or scar tissue in the case of radius and phalangeal SOS measurements pose practical limitations in the use of ultrasound in individuals with SCI. As well, measurements of skeletal status at a predominantly cortical site such as the tibia may not be as useful as measurements at a fracture-prone site, such as the distal femur, in individuals with SCI.

7.4 Does Body Weight Supported Treadmill Training Have A Positive Impact On Musculoskeletal Health In Individuals With Spinal Cord Injury?

7.4.1 Major Findings and Discussion

Two studies were conducted to answer the above question. The focus of both studies was to evaluate whether the re-introduction of weight-bearing loading would have a positive impact on indices of skeletal health in individuals with chronic and acute SCI. In addition, the ability of BWSTT to reverse the muscle atrophy that occurs following an SCI was investigated. It was also hypothesized that BWSTT would improve ambulatory abilities in individuals with chronic SCI and that it would enhance the recovery of ambulatory abilities in individuals with acute SCI. The main findings were that individuals with chronic and acute SCI participating in BWSTT could improve walking abilities on the treadmill, and some individuals could improve over ground walking performance. In addition, the BWSTT was a sufficient stimulus for muscle hypertrophy, particularly in the lower limbs. Although there was a small, statistically significant reduction in whole body BMD in individuals with chronic SCI, there was not a statistically significant impact of BWSTT on BMD at clinically relevant sites such as the proximal or distal femur, or the proximal tibia.

Improved ambulation following BWSTT in individuals with incomplete SCI has been demonstrated previously (153;198;199). Despite distinctive differences between the current study and previous BWSTT research in individuals with SCI, our data are consistent with previous findings that BWSTT is a promising intervention for the re-training of gait in individuals with incomplete SCI. Previous research demonstrated more dramatic improvements in ambulatory abilities in study subjects, and that may be related to factors such as the participant selection criteria or the frequency of training. As the current study attempted to emulate an outpatient rehabilitation setting, and was more liberal with respect to inclusion criteria, it is likely that these findings are more representative of the potential for BWSTT to improve ambulatory abilities in individuals with incomplete SCI outside of a long-term residential rehabilitation setting.

These studies are the first prospective, longitudinal studies to evaluate the impact of BWSTT on ambulation and musculoskeletal health. One other study conducted in individuals with acute SCI demonstrated that early loading with BWSTT or standing results in a reduction in the amount of bone lost (41). No study to date has evaluated the ability of BWSTT to induce muscle hypertrophy. The impact of BWSTT on muscle morphology was also investigated in a sub-group of the participants with incomplete SCI, and is published elsewhere (173). In brief, 68 sessions of BWSTT resulted in significant increases in mean muscle fibre area of 25.5±6.3%, and there was an increase in the proportion of type IIa fibres and a reduction in the type IIax/IIx fibres.

Functional electrical stimulation (FES) is another form of exercise employed in the SCI population, and results from previous research are conflicting as to whether it has a positive impact on bone mass (13;16;19;54;116;135;147). However, the potential of FES to induce muscle hypertrophy in individuals with both acute and chronic SCI has been well documented (5;37;50;91;147;162). The magnitude of change in lean mass and/or muscle cross-sectional areas observed with FES is often larger than the changes occurring in the BWSTT studies presented here, which may be related to the intensity of muscle activity that can be achieved with each of these training modalities. However, the smaller magnitude of the change in muscle that occurred in the BWSTT participants does not diminish its impact. In the chronic SCI study, the three participants included as reference controls experienced losses of muscle mass, indicating that even in individuals with chronic SCI, muscle atrophy can be prevented with BWSTT. Of note, the changes in muscle CSA in individuals with acute SCI appeared to be larger in magnitude than the changes in individuals with chronic SCI, particularly for the lower leg CSA measurement. These findings are somewhat unexpected given that the chronic study was longer in duration. The individuals with acute SCI typically had a lower baseline muscle CSA. However, the three individuals in the chronic study who made the largest gains in lower leg muscle CSA were also the only three who were less than 2 years post-injury. It is possible that the potential to reverse muscle atrophy is greatest in the acute period following the injury.

Although the loss of bone mass following an SCI is often attributed, at least in part, to the removal of regular weight-bearing loading, the re-introduction of weight bearing with BWSTT was not sufficient to have a demonstrable effect on the skeleton in individuals with SCI. It is difficult to make conclusions about the impact of BWSTT on the skeleton in the study of individuals with acute SCI, as there was no control group. It is possible that some preservation of bone mass occurred. For example, as was discussed in more detail in Chapter 6, the individual who made the greatest gains in ambulatory abilities lost the least bone, and the individual who completed the fewest sessions lost the most bone. However, it is equally plausible that similar changes in bone would have been observed in the absence of intervention. As well, the pattern of change in bone biochemical markers is similar to what has been observed previously in the acute SCI population, indicating bone remodeling activity was not influenced by the BWSTT (156;185).

Using Frost's mechanostat theory (64) as the theoretical basis for bone's response to mechanical loading, as has been done throughout this thesis, it is possible that the applied strain rate and magnitude imposed on the bone during BWSTT did not exceed the threshold minimum effective strain in order to induce increased bone formation and bone modeling. The BWSTT training intensity was determined by an individual's capabilities. Most participants still required some body weight support at the end of the study, and most of the walking speeds were below what would be considered a normal walking speed. BWSTT induced muscle hypertrophy, so loading due to muscle contraction may have increased, and perhaps with time a corresponding increase in bone mass would be observed. The frequency of exercise may not have been sufficient; participants were asked to participate in BWSTT two or three times per week (depending on the study), and the average attendance was often less than what was scheduled due to unanticipated problems such as illness or transportation difficulties. In accordance with recent research, shorter, more frequent exercise bouts may be the best strategy for increasing bone mineral accretion (157).

Another potential explanation for the absence of change in indices of skeletal health with BWSTT is that there are other factors that influence bone loss after SCI in addition to the reduction in mechanical loading, such as blood flow or bone innervation, and BWSTT may not influence these factors. A recent review compared the skeletal changes that occur under conditions of mechanical unloading, namely spaceflight, bedrest and spinal cord injury, and although bone loss and biochemical changes occur under all of these conditions, it is apparent that the magnitude of change is greatest after SCI (75). Alterations in parathyroid hormone and vitamin D also occur after SCI, however these systemic changes are unlikely to be the sole mechanism for the localized lower limb bone loss that occurs after SCI. Finally, it is possible that once bone is lost in adults, it may be difficult to recover, particularly if substantial micro-architectural deterioration and/or permanent reductions in the mechanosensory abilities of bone cells have occurred. It is possible that the abilities of bone cells to sense the mechanical loading imposed by regular BWSTT is reduced in individuals with SCI. Only 24 hours of disuse was required

for osteocytes to become hypoxic (49), indicating that the ability of bone cells to detect loading may be impaired, even in acute SCI. As well, substantial micro-architectural deterioration has been reported after SCI (133). The potential to re-establish trabecular structure if it has been broken down is not known.

7.4.2 Limitations

Several limitations to each of the BWSTT studies were discussed in their respective chapters, and are reviewed here briefly. A randomized, controlled design was not employed in either study due to the small number of subjects recruited and the high potential for drop out among subjects who would be randomized to the control group, as we have experienced in previous exercise studies in this population. As well, unless a large number of participants are recruited, it is difficult to establish adequate matching between control and intervention groups in the SCI population due to inter-individual variability in characteristics such as age, gender, level of lesion, ASIA score and time post-injury. It is difficult to quantify the amount of work done by the participants versus the amount of effort assumed by the therapists during BWSTT, which has implications for the observed changes in treadmill speed and amount of body weight support required. However, 5 of the participants in the chronic study and 1 participant in the acute study were able to improve their over-ground walking abilities, and 4 of these individuals were able to walk on the treadmill without assistance from therapists, indicating that walking improvements were due to training rather than an increased amount of work performed by the therapists.

It may have been possible to achieve greater gains in ambulation, as well as in musculoskeletal health with an increase in the frequency of training, but the logistical difficulties of maintaining a high frequency of training in an outpatient setting in this population would make it an impractical goal. The inability of most subjects to adhere to the required number of sessions per week, and the fact that one subject in each study had to be considered non-compliers due to their inability to complete the required number of sessions in a reasonable amount of time infers that training more often than 3 times per week is an unattainable goal for many individuals with SCI. Despite these limitations, this work represents an incredible effort by our exercise rehabilitation team. There have been no prospective longitudinal studies of BWSTT to date that have investigated its impact on so many important health outcomes. Implementing the BWSTT intervention for one year, and six months, in individuals with chronic and acute SCI respectively, is another aspect of this work that is unique in the literature, as no other studies have attempted such long intervention durations.

7.4.3 Future Directions

A randomized controlled design, albeit extremely difficult to achieve in the SCI population, would confirm the impact of BWSTT on ambulatory abilities and musculoskeletal health in both acute and chronic populations. Although a randomized controlled trial is the ideal way to evaluate the effectiveness of an intervention, in the SCI population it may not be possible to ascertain adequate matching unless a very large number of participants are recruited. Future studies of BWSTT should aim to incorporate

multiple centres in order to include a greater number of participants. Despite the fact that a greater proportion of individuals with SCI are male, future BWSTT research should include more females. As well, in order to tease out whether improvements in walking ability are due to improved muscle function versus activation of a central pattern generator, improved methods of measuring muscle activity during training and voluntary muscle activation should be incorporated.

The selection criteria for inclusion in BWSTT studies may have a substantial impact on the outcome measures. In order to determine, and recommend the most appropriate candidates for BWSTT the selection criteria should be firmly established. As well, the selection criteria should not be based solely on which subset of the SCI population is likely to make improvements in ambulatory abilities. The research presented here indicates that muscle hypertrophy can occur with BWSTT even in individuals with SCI who have a limited likelihood of achieving over ground walking. It may be unethical to exclude participants from future BWSTT simply because they are less likely to improve their ability to walk. If BWSTT is to become standard therapy in the SCI population, the potential risks associated with BWSTT should be fully clarified, such as the risk for injury, increased pressure sores, or an increased risk of fracture. It is of importance to clarify the effects of BWSTT, as well as its cessation, on psychological measures such as health-related quality of life and depression. Other outcome measures that should be considered are those that are of importance to the participants, namely measures of edema or objective measures of spasticity.

With respect to musculoskeletal health, it would be advantageous to measure the cortical and trabecular bone compartments separately. Since trabecular bone is more metabolically active than cortical bone, it is possible that changes that may occur in this bone compartment with BWSTT cannot be detected with the techniques used in this study. Peripheral computed tomography measurements of the lower limb could achieve this objective, and allow for measurements of lower limb muscle cross-sectional areas. The changes in bone biochemical markers after acute SCI should be confirmed in larger subject pools, and extended beyond the 6-month post-injury time point. As well, it may be beneficial to identify whether the changes in biochemical markers after SCI are related to the severity of injury, and whether they are influenced to a great extent by early mobilization.

7.5 Conclusions

This thesis explored the impact of a novel rehabilitation technique, body weight supported treadmill training, on walking abilities and musculoskeletal health in individuals with SCI. Although the two longitudinal studies of BWSTT comprise the bulk of the thesis, the methodological studies are also important. They are essential for the interpretation of the results of this thesis and will aid in conducting future research assessing musculoskeletal health in SCI. The methodological studies revealed that the presence of metal results in reproducible errors in estimations of bone mineral content and body composition as detected by DXA. Because the effect is reproducible within a

given subject, there is no need for adjustment of data due to the metal effect. As well, mid-tibia speed of sound measurements obtained with quantitative ultrasound might not adequately reveal the changes that occur in the bones of the lower limbs after SCI.

The two longitudinal prospective BWSTT studies presented here add to the growing body of literature that BWSTT can improve ambulation in individuals with chronic and acute SCI. Individuals participating in regular BWSTT improved their walking abilities on the treadmill, and a few individuals were also able to improve their over ground walking abilities. The more obvious effects of SCI on walking abilities often overshadow the secondary complications associated with SCI, including the muscle atrophy and bone loss that occurs. Further research in both acute and chronic SCI populations is required to confirm whether BWSTT can prevent bone loss from occurring. A unique and noteworthy finding in this thesis is that BWSTT can partially reverse muscle atrophy in individuals with acute and chronic SCI. This may have implications for the incidence of cardiovascular disease, diabetes and decubitus ulcers in the SCI population. As well, these findings suggest that BWSTT therapy should not be limited to individuals who are likely to improve their walking abilities, as it could potentially have a positive impact on the secondary complications associated with SCI across a broader range of individuals with SCI. Improved muscle function may also be an important factor in the recovery of ambulation in individuals with incomplete SCI. The ultimate conclusion of this thesis is that individuals with SCI stand to benefit from BWSTT, and the benefits are not limited to improved ambulation.

7.6 Reference List

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7.7 Appendices

Appendix A: Reproducibility of Muscle and Bone Variables Obtained Using Quantitative Computed Tomography

Methods

Five participants were recruited to have repeat computed tomography scans done in order to evaluate the reproducibility of the bone and muscle measurements at the mid-femur and 66% tibia sites. As in the 6-month training study in acute SCI, a General Electric CTI Scanner (GE, Milwaukee, Wisconsin, USA) was used to scan all participants, and a scout scan was performed in order to determine the lengths of the tibia and femur. Five millimetre slices were taken at both the mid-femur and at the point of maximal lower limb muscle cross-sectional area, defined to be 65% of the length of the tibia, starting from the distal end and measuring upwards. The system parameters used were as follows: slice thickness 5 mm, pixel matrix 512 x 512, and exposure factors of 120 kV, 200mA and 2 s, standard reconstruction algorithm. After the first scan, the participants were asked to get up from the scan table, turn around in a circle and then lie back down on the scan table for a repeat scan.

CT scans were analyzed using a validated software program (BonAlyse 1.3, BonAlyse Oy, Jyvaskyla, Finland), according to the software manufacturer's instructions. Muscle cross-sectional area, bone cross-sectional area (CSA, mm^2), volumetric bone mineral density (vBMD, mg/cm^3) without bone marrow, as well as maximum (I_{max}), minimum (I_{min}) and polar (I_{polar}) cross-sectional moments of inertia were analyzed in the mid-femur and mid-tibia slices. The thresholds –270 to –101 Hounsfield Units (HU) were used to identify fat and the thresholds –101 to 270 HU were used to identify muscle. The thresholds for the outer and inner border of the bone were 280 mg/cm^3 and 70 mg/cm^3, respectively.

Statistical Analyses

The statistical method used to evaluate reproducibility has been described previously (1). The root mean squared coefficient of variation was calculated for all measured variables, using the equations below:

$$RMSSD = \sqrt{\frac{\sum_{j=1}^{m}(SDj)^2}{m}}$$

where m = the number of subjects, j = subject
SDj = within subject standard deviation
RMSSD = root mean squared standard deviation
RMSCV = root mean squared coefficient of variation

$$RMSCV = \frac{(RMSSD)}{\frac{\sum_{j=1}^{m}\bar{X}_j}{m}}$$

Results

The RMSCVs for all of the variables analyzed from the computed tomography scans are listed in the Table below. All raw data is listed in Appendix B.

	Muscle Area	Fat Area	Bone Area	Bone Density	I_{max}	I_{min}	I_{polar}
RMSSD for variables at mid-femur	284.1	935.6	5.08	3.39	453.7	464.8	547.4
RMSCV (%) for variables at mid-femur	1.57	6.7	1.27	0.49	1.80	2.57	1.26
RMSSD for variables at 66% tibia	95.6	47.9	2.74	4.53	645.4	187.5	780.8
RMSCV (%) for variables at 66% tibia	1.17	1.2	0.82	0.67	2.18	1.42	1.82

Discussion and Conclusions

Based on these results, it can be said that the muscle and bone variables analyzed from the computed tomography scans can be measured with reproducibility, as assessed via RMSCV, of under 2% for the area and density variables and under 2.6% for the moment of inertia variables. The reproducibility of the fat CSA is less certain, as the thigh fat CSA was 6.7%. However, the raw data suggests that the increase in RMSCV for thigh fat CSA when compared to the other variables was primarily due to the right and left scans from one subject. The percent differences for the right and left scans for that subject were 15.8% and 9.6%, respectively, whereas for the other subjects the percent differences were comparable to the other variables. However, caution is warranted in reporting changes in fat CSA. The bone and muscle variables obtained from computed tomography scans can be measured with acceptable reproducibility. In instances, such as the study in acute SCI subjects (Chapter 6), where there will be a small number of subjects and no statistical analysis will be performed, the criteria for a meaningful change in the above variables, detected with 99% confidence, will be the RMSSD for the particular variable multiplied by three.

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Appendix B: Raw Data for Appendix A

ID	SID	FEMUR BONE AREA 1	2	SD^2	means	DENSITY 1	2	SD^2	means	IMAX 1	2	SD^2	means
EH	R	356	356	0	356	856	861	13	859	14153	13874	38921	14014
MK	R	569	574	11	572	837	840	5	839	39755	40090	56113	39923
BS	R	419	415	10	417	843	841	2	842	27908	27522	74498	27715
JS	R	519	515	8	517	776	787	61	782	35752	35429	52165	35591
LL	R	443	422	232	433	788	789	1	789	23777	23532	30160	23655
ME	R	636	637	0	636	830	832	2	831	47804	47944	9775	47874
EH	L	369	369	0	369	877	877	0	877	15782	15953	14621	15868
MK	L	582	583	1	583	848	842	18	845	38904	39292	75272	39098
BS	L	414	419	10	417	848	848	0	848	28515	29078	158485	28797
JS	L	509	508	1	508	793	792	1	793	30346	29974	69192	30160
LL	L	435	428	27	432	789	791	3	790	22017	23718	#######	22868
ME	L	660	664	10	662	829	821	34	825	46496	47438	443502	46967
		SD		**5.07924**		**SD**		**3.39**		**SD**		**453.66**	
					400.59				**691.88**				**25224**
		CVSD		**1.26795**		**CVSD**		**0.49**		**CVSD**		**1.7985**	
		SD*2		**10.1585**	**492**	**SD*2**		**6.78**		**SD*2**		**907.33**	
		SD*3		**15.2377**		**SD*3**		**10.17**		**SD*3**		**1361**	

ID	SID	IMIN 1	2	SD^2	means	IPOL 1	2	SD^2	means
EH	R	11757	11949	18432	11853	25911	25823	3872	25867
MK	R	29493	30543	551250	30018	69247	70633	######	69940
BS	R	15795	15669	7938	15732	43704	43191	######	43448
JS	R	21648	21280	67712	21464	57401	56708	######	57055
LL	R	20104	18750	916761	19427	43881	42282	######	43082
ME	R	34103	34147	977	34125	81907	82091	16935	81999
EH	L	12847	12579	35912	12713	28628	28532	4608	28580
MK	L	33045	32823	24642	32934	71949	72115	13778	72032
BS	L	15681	16074	77225	15878	44196	45152	######	44674
JS	L	23087	23298	22261	23193	53433	53272	12961	53353
LL	L	19938	18620	867967	19279	41955	42338	73590	42147
ME	L	39624	39577	1086	39600	86119	87015	######	86567
		SD		**464.773**		**SD**		**547.3**	
					18111				**43336**
		CVSD		**2.5662**		**CVSD**		**1.263**	
		SD*2		**929.545**		**SD*2**		**1095**	
		SD*3		**1394.32**		**SD*3**		**1642**	

ID	SID	TIBIA BONE AREA				DENSITY			
		1	2	SD^2	means	1	2	SD^2	means
EH	R	291	289	2	290	867	869	2	868
MK	R	486	493	23	489	823	824	1	824
BS	R	390	387	6	388	793	790	5	792
JS	R	389	390	1	389	764	772	32	768
LL	R	357	360	6	358	770	767	4	769
ME	R	547	544	4	546	831	836	11	833
EH	L	288	293	10	290	878	873	13	876
MK	L	487	492	15	489	831	817	98	824
BS	L	405	407	2	406	807	808	1	808
JS	L	375	374	1	375	776	782	18	779
LL	L	344	350	18	347	754	744	57	749
ME	L	529	531	2	530	827	823	6	825
		SD	**2.74396**			**SD**	**4.53**		
					335.12				**678.24**
		CVSD	**0.8188**			**CVSD**	**0.668**		
		SD*2	**5.48792**			**SD*2**	**9.06**		
		SD*3	**8.23188**			**SD*3**	**13.59**		

ID	SID	AREA WEIGHT IMAX				IMIN				IPOL			
		1	2	SD^2	means	1	2	SD^2	means	1	2	SD^2	means
EH	R	14984	15162	15842	15073	6697	6656	841	6677	21681	21818	9385	21750
MK	R	49277	51729	3006152	50503	22822	23437	######	23130	72099	75166	#######	73633
BS	R	34753	34147	183618	34450	14181	14172	41	14177	48933	48319	188498	48626
JS	R	34854	35132	38642	34993	14969	14989	200	14979	49823	50121	44402	49972
LL	R	27013	26725	41465	26869	11991	12239	30560	12115	39004	38963	830	38984
ME	R	55449	54723	263739	55086	28000	28261	33915	28130	83449	82983	108502	83216
EH	L	15799	14857	443682	15328	6861	6711	11250	6786	22660	21568	596232	22114
MK	L	53654	54210	154568	53932	23258	23712	######	23485	76912	77922	510050	77417
BS	L	36292	36595	45905	36444	15674	15589	3613	15632	51966	52184	23762	52075
JS	L	33256	32942	49298	33099	13521	13660	9661	13591	46777	46602	15313	46690
LL	L	27986	29168	697643	28577	10699	10980	39374	10839	38685	40147	#######	39416
ME	L	54415	54755	57633	54585	25189	25157	520	25173	79604	79911	47201	79758
		SD	**645.38**			**SD**	**187.6**			**SD**	**780.81**		
					29648				**13225**				**42873**
		CVSD	**2.17681**			**CVSD**	**1.418**			**CVSD**	**1.8212**		
		SD*2	**1290.76**			**SD*2**	**375.1**			**SD*2**	**1561.6**		
		SD*3	**1936.14**			**SD*3**	**562.7**			**SD*3**	**2342.4**		

Subject	SIDE	THIGH MUSCLE Scan1			Scan 2				Mean	% Diff Scan
		1	2	Mean	1	2	Mean	SD^2	1&2	1 vs 2
EH	R	10099	10094	10097	10074	10074	10074	258	10085	-0.2
MK	R	18769	18770	18769	18628	18634	18631	9515	18700	-0.7
BS	R	13850	13846	13848	13777	13774	13775	2639	13811	-0.5
LL	R	9991	9989	9990	9488	9504	9496	121796	9743	-4.9
ME	R	20400	20376	20388	20264	20249	20257	8587	20322	-0.6
EH	L	9416	9424	9420	9427	9424	9426	17	9423	0.1
MK	L	19339	19344	19341	19290	19284	19287	1466	19314	-0.3
BS	L	13542	13541	13541	13437	13437	13437	5387	13489	-0.8
LL	L	10313	10324	10318	9337	9324	9330	488171	9824	-9.6
ME	L	19698	19690	19694	19564	19575	19569	7731	19632	-0.6
							SD	**284.07**		
							sum of means/8		**18043**	
							CVSD	**1.57441**		
							SD*3	**852.211**		

Subject	SIDE	THIGH FAT Scan1			Scan 2				Mean	% Diff Scan
		1	2	Mean	1	2	Mean	SD^2	1&2	1 vs 2
EH	R	8269	8258	8263	8144	8148	8146	6868	8205	-1.4
MK	R	5543	5508	5525	5502	5536	5519	21	5522	-0.1
BS	R	7860	7877	7868	7807	7812	7810	1729	7839	-0.7
LL	R	20394	20360	20377	17144	17169	17157	5185005	18767	-15.8
ME	R	15553	15563	15558	15728	15870	15799	29089	15679	1.6
EH	L	8014	8015	8014	8197	8186	8191	15744	8103	2.2
MK	L	5045	5037	5041	5000	4982	4991	1258	5016	-1.0
BS	L	7945	7927	7936	8017	7979	7998	1969	7967	0.8
LL	L	19348	19288	19318	17451	17493	17472	1704135	18395	-9.6
ME	L	16426	16427	16427	16612	15564	16088	57274	16257	-2.1
							SD	**935.621**		
							sum of means/8		**13969**	
							CVSD	**6.69799**		
							SD*3	**2806.86**		

Subject	SIDE	CALF MUSCLE Scan1			Scan 2				Mean	% Diff Scan
		1	2	Mean	1	2	Mean	SD^2	1&2	1 vs 2
EH	R	4715	4707	4711	4489	4484	4487	25133	4599	-4.8
MK	R	6850	6856	6853	6901	6902	6901	1174	6877	0.7
BS	R	6540	6551	6546	6429	6440	6434	6211	6490	-1.7
LL	R	6510	6512	6511	6611	6610	6611	4995	6561	1.5
ME	R	7950	7941	7946	7965	7948	7957	57	7951	0.1
EH	L	4241	4241	4241	4258	4257	4257	133	4249	0.4
MK	L	6898	6891	6895	6827	6843	6835	1773	6865	-0.9
BS	L	6510	6501	6506	6557	6565	6561	1515	6533	0.8
LL	L	6980	6988	6984	7222	7206	7214	26404	7099	3.3
ME	L	8139	8145	8142	8037	8034	8035	5735	8089	-1.3
							SD	**95.6097**		
							sum of means/8		**8164.1**	
							CVSD	**1.1711**		
							SD*3	**286.829**		

Subject	SIDE	CALF FAT Scan1			Scan 2				Mean	% Diff Scan
		1	2	Mean	1	2	Mean	SD^2	1&2	1 vs 2
EH	R	1952	1962	1957	2014	2008	2011	1453	1984	2.8
MK	R	1993	2016	2005	1986	2005	1996	41	2000	-0.5
BS	R	3128	3133	3131	3122	3098	3110	216	3120	-0.7
LL	R	5949	5967	5958	5953	5960	5956	1	5957	0.0
ME	R	3014	2973	2993	2904	2883	2894	4970	2943	-3.3
EH	L	2108	2080	2094	2102	2113	2107	88	2101	0.6
MK	L	1960	1974	1967	1929	1935	1932	611	1949	-1.8
BS	L	3196	3195	3195	3234	3221	3228	517	3212	1.0
LL	L	6196	6179	6187	6060	6027	6043	10411	6115	-2.3
ME	L	2496	2484	2490	2496	2498	2497	27	2493	0.3
							SD	**47.8742**		
							sum of means/8		**3984.4**	
							CVSD	**1.20154**		
							SD*3	**143.623**		

Appendix C: Validation Protocol for the Conversion of Hounsfield Units to Density

In order to measure bone mineral density using computed tomography (CT), the relationship between the attenuation coefficient μ (Hounsfield Units) and density must be determined. Known concentrations of K_2HPO_4 were used as simulated bone standards, and scanned, along other materials of known density, to determine if a linear relationship exists between Hounsfield Units (HU) and density.

Methods

A CT scanner (General Electric CTI Scanner, Milwaukee, Wisconsin) was used to measure the HU of the various materials. The materials are listed in Table 1, along with their known densities. The system parameters used were as follows: slice thickness 5 mm, pixel matrix 512 x 512, and exposure factors of 120 kV, 200mA and 2 s, with a standard reconstruction algorithm. CT scans were analyzed using a validated software program (BonAlyse 1.3, BonAlyse Oy, Jyvaskyla, Finland). The CT scans for each material were imported into the analysis program. A region of interest was placed in the centre of the material on the CT scan, and the average HU for that material was recorded. The HU for each material are listed in the table below.

Hounsfield Units Measured After Scanning Materials of Known Density

Material	Density (g/ml) or (g/cm^3)	Hounsfield Units
Methanol	-210	-226
Nitromethane	130	57
Tetrahydrofuran	-110	-164
Sterile Water	0	-11
Air	-1000	-961
Wood Block (Maple)	-217	-278
Wood Block (Pine)	-525	-537
Wood Block (Light Pine)	-626	-633
Wood Block (Balsa)	-884	-856
K_2HPO_4	250	488
K_2HPO_4	490	910
K_2HPO_4	720	1334

The known densities were plotted against the measured HU in Figure 1. Density was placed on the y-axis as the derived equation will be used to predict density from HU. A linear relationship can be derived between HU and density (see figure below). The relationship between HU and density can be explained by the equation:

Density = (HU x 0.7402) – 111.07

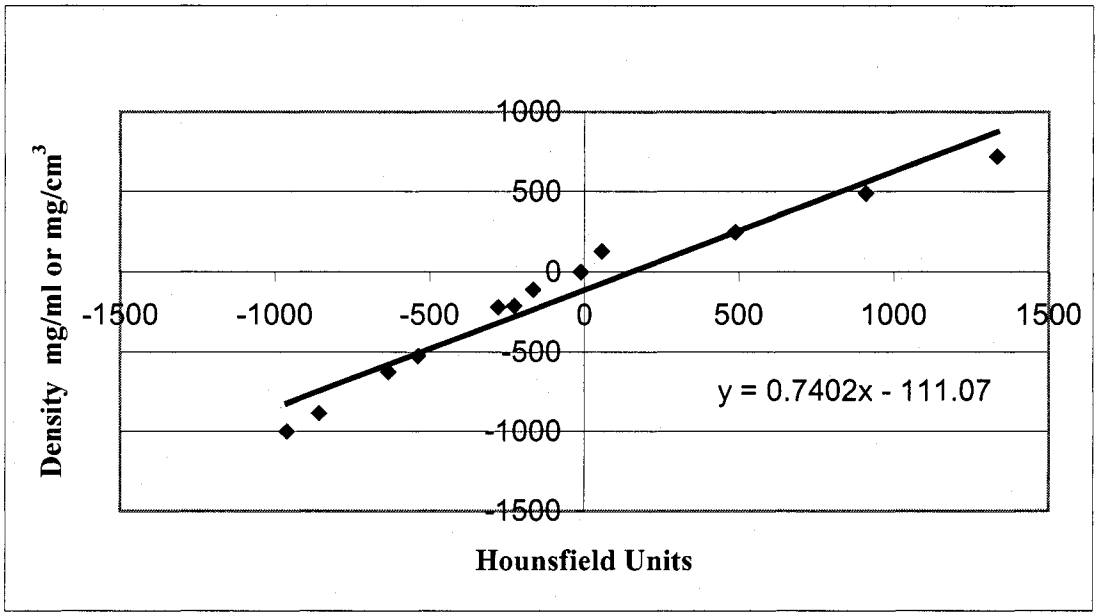

Figure 1: Relationship between HU and density of various materials in CT measurements

Appendix D: Raw Data for Chapter 3

Participant Info

ID	Age	M/F	Study
LM	24	F	REPRO
ME	24	F	REPRO
CM	26	F	REPRO
LS	22	M	REPRO
BT	25	M	REPRO
SH	23	M	REPRO

	ID LM	ME	CM	LS	BT	SH
MASS						
NO METAL	55382.2	49723.8	47293.3	92937.3	84437.6	77223
BACK 1	55635.5	50069.3	47578.3	93405	84562.3	76759.2
FRONT	55495	50113.9	47430.4	92953.2	84928.7	77295.4
BACK 2	55620.5	50193.7	47608.4	93103.4	85113.2	77507
BMC						
NO METAL	1906.5	2186.4	1816.4	3179.2	2948.4	3313.6
BACK 1	1983.3	2281.1	1876.2	3236.6	3021.7	3335.2
FRONT	1963.6	2256.6	1874.8	3195.5	2976.9	3379.5
BACK 2	1967.5	2265.7	1905.5	3267.1	3024.4	3383.5
SOFT TISSUE						
NO METAL	53475.8	47537.5	45476.8	89758.1	81489.2	73909.4
BACK 1	53652.1	47788.1	45702.1	90168.4	81540.7	73424.1
FRONT	53531.5	47857.3	45555.6	89757.6	81951.8	73915.8
BACK 2	53652.7	47928	45703.9	89836.3	82088.8	74123.5
LEAN						
NO METAL	37967.2	39244.6	32468.5	67310.5	61703.9	64010.8
BACK 1	38310.9	39520.7	32313.4	67242.1	62399.6	62777.9
FRONT	38352.2	39488.4	32262.3	67051.2	62071.7	63726.3
BACK 2	38473.4	39675.1	32605.2	67104.1	62661.5	63633
FAT						
NO METAL	15508.6	8292.9	13008.3	22447.6	19785.3	9898.6
BACK 1	15341.2	8267.4	13388.7	22926.3	19141.1	10646.2
FRONT	15179.3	8368.9	13293.3	22706.4	19880.1	10189.5
BACK 2	15179.3	8252.9	13098.7	22732.2	19427.3	10490.5

Participant Info

ID	Age	M/F	Study
MI	26	M	3XMETAL
MA	25	M	3XMETAL
JO	22	M	3XMETAL
TI	27	M	3XMETAL
AM	20	F	3XMETAL
AL	23	F	3XMETAL
MH	25	F	3XMETAL

	ID						
	MI	MA	JO	TI	AM	AL	MH
MASS							
NO METAL	75906	63850	73856.1	80670	59293	76965	55466
LARGE	76105	64280	74049.6	81310	60075	76635	55824
MEDIUM	76277	64517	74171.4	81444	59854	77022	55810.5
SMALL	76085	64152	73762.7	81070	59698	76729	55713.3
BMC							
NO METAL	2705.3	2153.4	2823.7	3171.2	2348	2677.8	1940.3
LARGE	2773.9	2220	2882.8	3260.3	2465	2653.1	1995.2
MEDIUM	2730.5	2239.3	2842.2	3179.4	2439	2658.2	1969.3
SMALL	2745.2	2199.8	2818.3	3165.3	2423	2653.4	1945.8
SOFT TISSUE							
NO METAL	73201	61697	71032.5	77498	56946	74287	53525.7
LARGE	73331	62059	71166.8	78050	57610	73982	53828.7
MEDIUM	73546	62278	71329.2	78265	57416	74364	53841.2
SMALL	73339	61952	70944.5	77905	57275	74075	53767.5
LEAN							
NO METAL	57744	53804	59509.6	65712	38979	51876	36747.4
LARGE	57502	53768	59612.1	66077	39261	51607	37307.1
MEDIUM	58112	53860	60086.3	66210	39562	51876	37141.2
SMALL	57934	53539	59539.9	65594	39658	51714	37449
FAT							
NO METAL	57744	53804	59509.6	65712	38979	51876	36747.4
LARGE	57502	53768	59612.1	66077	39261	51607	37307.1
MEDIUM	58112	53860	60086.3	66210	39562	51876	37141.2
SMALL	57934	53539	59539.9	65594	39658	51714	37449

Appendix E: Statistical Analyses for Chapter 3

t-Test: Paired Two Sample for Means
BACK 1 BW

	Variable 1	*Variable 2*
Mean	67832.87	68002
Variance	3.8E+08	4E+08
Observations	6	6
Pearson Correlation	0.999861	
Hypothesized Mean Difference	0	
df	5	
t Stat	-1.2538	
P(T<=t) one-tail	0.132672	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.265343	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
BACK 2 BW

	Variable 1	*Variable 2*
Mean	67832.87	68191
Variance	3.8E+08	3.8E+08
Observations	6	6
Pearson Correlation	0.999955	
Hypothesized Mean Difference	0	
df	5	
t Stat	-4.73213	
P(T<=t) one-tail	0.002593	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.005186	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
BACK 1 BMC

	Variable 1	*Variable 2*
Mean	2558.417	2622.4
Variance	444348.3	425303
Observations	6	6
Pearson Correlation	0.999538	
Hypothesized Mean Difference	0	
df	5	
t Stat	-6.33889	
P(T<=t) one-tail	0.000721	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.001442	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
BACK 2 BMC

	Variable 1	*Variable 2*
Mean	2558.417	2635.62
Variance	444348.3	445108
Observations	6	6
Pearson Correlation	0.99987	
Hypothesized Mean Difference	0	
df	5	
t Stat	-17.5928	
P(T<=t) one-tail	5.44E-06	
t Critical one-tail	2.015049	
P(T<=t) two-tail	1.09E-05	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
back 1 fat

	Variable 1	*Variable 2*
Mean	14823.55	14952
Variance	30682810	3E+07
Observations	6	6
Pearson Correlation	0.995976	
Hypothesized Mean Difference	0	
df	5	
t Stat	-0.62152	
P(T<=t) one-tail	0.280746	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.561492	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
BACK 2 FAT

	Variable 1	*Variable 2*
Mean	14823.55	14863.5
Variance	30682810	3E+07
Observations	6	6
Pearson Correlation	0.997906	
Hypothesized Mean Difference	0	
df	5	
t Stat	-0.26758	
P(T<=t) one-tail	0.399854	
t Critical one-tail	2.909992	
P(T<=t) two-tail	0.799707	
t Critical two-tail	3.532223	

t-Test: Paired Two Sample for Means
BACK 1 LEAN

	Variable 1	*Variable 2*
Mean	50450.92	50427
Variance	2.4E+08	2E+08
Observations	6	6
Pearson Correlation	0.999127	
Hypothesized Mean Difference	0	
df	5	
t Stat	0.086252	
P(T<=t) one-tail	0.467307	
t Critical one-tail	2.909992	
P(T<=t) two-tail	0.934613	
t Critical two-tail	3.532223	

t-Test: Paired Two Sample for Means
BACK 2 LEAN

	Variable 1	*Variable 2*
Mean	50450.92	50692.1
Variance	2.4E+08	2.4E+08
Observations	6	6
Pearson Correlation	0.999531	
Hypothesized Mean Difference	0	
df	5	
t Stat	-1.19884	
P(T<=t) one-tail	0.142153	
t Critical one-tail	2.909992	
P(T<=t) two-tail	0.284305	
t Critical two-tail	3.532223	

t-Test: Paired Two Sample for Means
BACK1 ST

	Variable 1	*Variable 2*
Mean	65274.47	65379
Variance	3.57E+08	4E+08
Observations	6	6
Pearson Correlation	0.999866	
Hypothesized Mean Difference	0	
df	5	
t Stat	-0.82387	
P(T<=t) one-tail	0.223759	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.447517	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
FRONT ST

	Variable 1	*Variable 2*
Mean	65274.47	65428.3
Variance	3.57E+08	3.6E+08
Observations	6	6
Pearson Correlation	0.999948	
Hypothesized Mean Difference	0	
df	5	
t Stat	-1.96547	
P(T<=t) one-tail	0.05327	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.10654	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
BMC FRONT VS BACK2

	Variable 1	*Variable 2*
Mean	2607.817	2635.6
Variance	430554.2	445108
Observations	6	6
Pearson Correlation	0.99927	
Hypothesized Mean Difference	0	
df	5	
t Stat	-2.46935	
P(T<=t) one-tail	0.028288	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.056575	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
BW FRONT VS BACK 2

	Variable 1	*Variable 2*
Mean	68036.1	68191
Variance	3.79E+08	3.8E+08
Observations	6	6
Pearson Correlation	0.999998	
Hypothesized Mean Difference	0	
df	5	
t Stat	-8.03612	
P(T<=t) one-tail	0.000241	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.000483	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
BMC FRONT VS BACK1

	Variable 1	*Variable 2*
Mean	2607.817	2622.4
Variance	430554.2	425303
Observations	6	6
Pearson Correlation	0.998761	
Hypothesized Mean Difference	0	
df	5	
t Stat	-1.08509	
P(T<=t) one-tail	0.163704	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.327407	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
BW FRONT VS BACK1

	Variable 1	*Variable 2*
Mean	68036.1	68001.6
Variance	3.79E+08	3.8E+08
Observations	6	6
Pearson Correlation	0.999827	
Hypothesized Mean Difference	0	
df	5	
t Stat	0.232187	
P(T<=t) one-tail	0.412799	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.825597	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
ST NO METAL VS BACK1

	Variable 1	*Variable 2*
Mean	65274.47	65379
Variance	3.57E+08	4E+08
Observations	6	6
Pearson Correlation	0.999866	
Hypothesized Mean Difference	0	
df	5	
t Stat	-0.82387	
P(T<=t) one-tail	0.223759	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.447517	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
ST NO METAL VS FRONT

	Variable 1	*Variable 2*
Mean	65274.47	65428.3
Variance	3.57E+08	3.6E+08
Observations	6	6
Pearson Correlation	0.999948	
Hypothesized Mean Difference	0	
df	5	
t Stat	-1.96547	
P(T<=t) one-tail	0.05327	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.10654	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
ST FRONT VS BACK1

	Variable 1	*Variable 2*
Mean	65428.27	65379
Variance	3.56E+08	4E+08
Observations	6	6
Pearson Correlation	0.999832	
Hypothesized Mean Difference	0	
df	5	
t Stat	0.344906	
P(T<=t) one-tail	0.3721	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.7442	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
ST NO METAL VS BACK2

	Variable 1	*Variable 2*
Mean	65274.47	65555.5
Variance	3.57E+08	3.6E+08
Observations	6	6
Pearson Correlation	0.999952	
Hypothesized Mean Difference	0	
df	5	
t Stat	-3.70293	
P(T<=t) one-tail	0.006978	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.013957	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
FRONT BMC

	Variable 1	*Variable 2*
Mean	2558.417	2607.82
Variance	444348.3	430554
Observations	6	6
Pearson Correlation	0.999581	
Hypothesized Mean Difference	0	
df	5	
t Stat	-5.54904	
P(T<=t) one-tail	0.001306	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.002611	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
FRONT BW

	Variable 1	*Variable 2*
Mean	67832.87	68036.1
Variance	3.8E+08	3.79E+08
Observations	6	6
Pearson Correlation	0.999952	
Hypothesized Mean Difference	0	
df	5	
t Stat	-2.60535	
P(T<=t) one-tail	0.023969	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.047939	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
FRONT FAT

	Variable 1	*Variable 2*
Mean	14823.55	14936.3
Variance	30682810	3.1E+07
Observations	6	6
Pearson Correlation	0.999088	
Hypothesized Mean Difference	0	
df	5	
t Stat	-1.16693	
P(T<=t) one-tail	0.14793	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.295859	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
FRONT LEAN

	Variable 1	*Variable 2*
Mean	50450.92	50492.02
Variance	2.4E+08	2.37E+08
Observations	6	6
Pearson Correlation	0.999801	
Hypothesized Mean Difference	0	
df	5	
t Stat	-0.31113	
P(T<=t) one-tail	0.384127	
t Critical one-tail	2.909992	
P(T<=t) two-tail	0.768254	
t Critical two-tail	3.532223	

t-Test: Paired Two Sample for Means
BACK2 ST

	Variable 1	*Variable 2*
Mean	65274.47	65555.5
Variance	3.57E+08	3.6E+08
Observations	6	6
Pearson Correlation	0.999952	
Hypothesized Mean Difference	0	
df	5	
t Stat	-3.70293	
P(T<=t) one-tail	0.006978	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.013957	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
ST FRONT VS BACK2

	Variable 1	*Variable 2*
Mean	65428.27	65555.53
Variance	3.56E+08	3.57E+08
Observations	6	6
Pearson Correlation	0.999996	
Hypothesized Mean Difference	0	
df	5	
t Stat	-6.20949	
P(T<=t) one-tail	0.000791	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.001582	
t Critical two-tail	2.570578	

t-Test: Paired Two Sample for Means
LARGE BW

	Variable 1	*Variable 2*
Mean	69429.51	69754
Variance	95586400	9E+07
Observations	7	7
Pearson Correlation	0.99941	
Hypoth Mean Difference	0	
df	6	
t Stat	-2.3783	
P(T<=t) one-tail	0.027448	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.054895	
t Critical two-tail	2.446914	

t-Test: Paired Two Sample for Means
MED BW

	Variable 1	*Variable 2*
Mean	69429.5143	69871
Variance	95586400.2	1E+08
Observations	7	7
Pearson Correlation	0.99969209	
Hypoth. Mean diff	0	
df	6	
t Stat	-4.8032733	
P(T<=t) one-tail	0.00149536	
t Critical one-tail	1.94318091	
P(T<=t) two-tail	0.00299072	
t Critical two-tail	2.44691364	

t-Test: Paired Two Sample for Means
LARGE BMC

	Variable 1	*Variable 2*
Mean	2545.629	2607
Variance	178511.5	2E+05
Observations	7	7
Pearson Correlation	0.994709	
Hypoth Mean Diff	0	
df	6	
t Stat	-3.73597	
P(T<=t) one-tail	0.004834	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.009668	
t Critical two-tail	2.446914	

t-Test: Paired Two Sample for Means
MED BMC

	Variable 1	*Variable 2*
Mean	2545.62857	2580
Variance	178511.539	2E+05
Observations	7	7
Pearson Correlation	0.99650401	
Hypothe Mean Diff	0	
df	6	
t Stat	-2.2246255	
P(T<=t) one-tail	0.03388259	
t Critical one-tail	1.94318091	
P(T<=t) two-tail	0.06776518	
t Critical two-tail	2.44691364	

t-Test: Paired Two Sample for Means
LARGE FAT

	Variable 1	*Variable 2*
Mean	14830.93	14985
Variance	23305548	2E+07
Observations	7	7
Pearson Correlation	0.998793	
Hypothesized Mean Difference	0	
df	6	
t Stat	-1.61701	
P(T<=t) one-tail	0.078502	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.157004	

t-Test: Paired Two Sample for Means
MED FAT

	Variable 1	*Variable 2*
Mean	14830.9286	14885
Variance	23305547.7	2E+07
Observations	7	7
Pearson Correlation	0.99870687	
HypotMean Diff	0	
df	6	
t Stat	-0.5289348	
P(T<=t) one-tail	0.30791884	
t Critical one-tail	1.94318091	
P(T<=t) two-tail	0.61583768	

t-Test: Paired Two Sample for Means
ST LARGE

	Variable 1	*Variable 2*
Mean	66883.9	67147
Variance	88119069	9E+07
Observations	7	7
Pearson Correlation	0.999509	
Hypothesized Mean Difference	0	
df	6	
t Stat	-2.17552	
P(T<=t) one-tail	0.036254	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.072508	
t Critical two-tail	2.446914	

t-Test: Paired Two Sample for Means
ST MED

	Variable 1	*Variable 2*
Mean	66883.9	67291.24
Variance	88119069	88238957
Observations	7	7
Pearson Correlation	0.999719	
Hypothesized Mean Difference	0	
df	6	
t Stat	-4.84191	
P(T<=t) one-tail	0.001438	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.002875	
t Critical two-tail	2.446914	

t-Test: Paired Two Sample for Means
SMALL BMC

	Variable 1	*Variable 2*
Mean	2545.629	2564.4
Variance	178511.5	166963
Observations	7	7
Pearson Correlation	0.996878	
Hypothesized Mean Difference	0	
df	6	
t Stat	-1.39194	
P(T<=t) one-tail	0.106673	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.213347	
t Critical two-tail	2.446914	

t-Test: Paired Two Sample for Means
SMALL BW

	Variable 1	*Variable 2*
Mean	69429.51	69601.19
Variance	95586400	93722529
Observations	7	7
Pearson Correlation	0.999726	
Hypothesized Mean Difference	0	
df	6	
t Stat	-1.83943	
P(T<=t) one-tail	0.057735	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.11547	
t Critical two-tail	2.446914	

t-Test: Paired Two Sample for Means
SMALL FAT

	Variable 1	*Variable 2*
Mean	14830.93	14833
Variance	23305548	2E+07
Observations	7	7
Pearson Correlation	0.997917	
Hypothesized Mean Difference	0	
df	6	
t Stat	-0.01338	
P(T<=t) one-tail	0.494879	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.989757	
t Critical two-tail	2.446914	

t-Test: Paired Two Sample for Means
LARGE FAT

	Variable 1	*Variable 2*
Mean	14830.93	14984.77
Variance	23305548	22442491
Observations	7	7
Pearson Correlation	0.998793	
Hypothesized Mean Difference	0	
df	6	
t Stat	-1.61701	
P(T<=t) one-tail	0.078502	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.157004	
t Critical two-tail	2.446914	

t-Test: Paired Two Sample for Means
LARGE LEAN

	Variable 1	*Variable 2*
Mean	52052.97	52162
Variance	1.14E+08	1E+08
Observations	7	7
Pearson Correlation	0.99963	
Hypothesized Mean Difference	0	
df	6	
t Stat	-0.92384	
P(T<=t) one-tail	0.195604	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.391209	
t Critical two-tail	2.446914	

t-Test: Paired Two Sample for Means
MED FAT

	Variable 1	*Variable 2*
Mean	14830.93	14885
Variance	23305548	2E+07
Observations	7	7
Pearson Correlation	0.998707	
Hypothesized Mean Difference	0	
df	6	
t Stat	-0.52893	
P(T<=t) one-tail	0.307919	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.615838	
t Critical two-tail	2.446914	

t-Test: Paired Two Sample for Means
MED LEAN

	Variable 1	*Variable 2*
Mean	52052.9714	52407
Variance	113806738	1E+08
Observations	7	7
Pearson Correlation	0.99975294	
Hypot Mean Diffe	0	
df	6	
t Stat	-3.9469415	
P(T<=t) one-tail	0.00378206	
t Critical one-tail	1.94318091	
P(T<=t) two-tail	0.00756413	
t Critical two-tail	2.44691364	

t-Test: Paired Two Sample for Means
SMALL LEAN

	Variable 1	*Variable 2*
Mean	52052.9714	52204
Variance	113806738	1E+08
Observations	7	7
Pearson Correlation	0.9997396	
Hypoth Mean Diff	0	
df	6	
t Stat	-1.0081097	
P(T<=t) one-tail	0.17615656	
t Critical one-tail	1.94318091	
P(T<=t) two-tail	0.35231311	
t Critical two-tail	2.44691364	

t-Test: Paired Two Sample for Means
ST SMALL

	Variable 1	*Variable 2*
Mean	66883.9	67037
Variance	88119069.1	9E+07
Observations	7	7
Pearson Correlation	0.9997446	
Hypoth Mean Diff	0	
df	6	
t Stat	-1.7937046	
P(T<=t) one-tail	0.06151011	
t Critical one-tail	1.94318091	
P(T<=t) two-tail	0.12302022	
t Critical two-tail	2.44691364	

Intraclass Correlation Coefficient - **Body weight**

```
Two-Way Mixed Effect Model (Consistency Definition):
People Effect Random, Measure Effect Fixed
 Single Measure Intraclass Correlation =    .9999*
    95.00% C.I.:            Lower =    .9996          Upper =   1.0000
 F = 39105.74   DF = (     5,   15.0)   Sig. = .0000  (Test Value =
.0000 )
 Average Measure Intraclass Correlation =   1.0000**
    95.00% C.I.:            Lower =    .9999          Upper =   1.0000
 F = 39105.74   DF = (     5,   15.0)   Sig. = .0000  (Test Value =
.0000 )*: Notice that the same estimator is used whether the
interaction effectis present or not.**: This estimate is computed if
the interaction effect is absent,  otherwise ICC is not estimable.
Reliability Coefficients N of Cases =6.0  N of Items =4 Alpha = 1.0000
```

Intraclass Correlation Coefficient - **BMC**

```
Two-Way Mixed Effect Model (Consistency Definition):
People Effect Random, Measure Effect Fixed
 Single Measure Intraclass Correlation =    .9993*
    95.00% C.I.:            Lower =    .9974          Upper =    .9999
 F = 5581.784   DF = (     5,   15.0)   Sig. = .0000  (Test Value =
.0000 )
 Average Measure Intraclass Correlation =    .9998**
    95.00% C.I.:            Lower =    .9994          Upper =   1.0000
 F = 5581.784   DF = (     5,   15.0)   Sig. = .0000  (Test Value =
.0000 )
*: Notice that the same estimator is used whether the interaction
effectis present or not.**: This estimate is computed if the
interaction effect is absent, otherwise ICC is not estimable.
Reliability Coefficients
N of Cases =      6.0        N of Items =  4 Alpha =    .9998
```

Intraclass Correlation Coefficient - **SOFT TISSUE**

```
Two-Way Mixed Effect Model (Consistency Definition):
People Effect Random, Measure Effect Fixed
 Single Measure Intraclass Correlation =    .9999*
    95.00% C.I.:            Lower =    .9996          Upper =   1.0000
 F = 38964.06   DF = (     5,   15.0)   Sig. = .0000  (Test Value =
.0000 )
 Average Measure Intraclass Correlation =   1.0000**
    95.00% C.I.:            Lower =    .9999          Upper =   1.0000
 F = 38964.06   DF = (     5,   15.0)   Sig. = .0000  (Test Value =
.0000 )*: Notice that the same estimator is used whether the
interaction effectis present or not.**: This estimate is computed if
the interaction effect is absent,
    otherwise ICC is not estimable.
Reliability Coefficients
N of Cases =      6.0                  N of Items =  4
Alpha =   1.0000
```

Intraclass Correlation Coefficient - **lean**

```
Two-Way Mixed Effect Model (Consistency Definition):
People Effect Random, Measure Effect Fixed
 Single Measure Intraclass Correlation =    .9996*
    95.00% C.I.:            Lower =    .9985          Upper =    .9999
 F = 9782.809   DF = (     5,   15.0)   Sig. = .0000  (Test Value =
.0000 )
 Average Measure Intraclass Correlation =    .9999**
    95.00% C.I.:            Lower =    .9996          Upper =   1.0000
 F = 9782.809   DF = (     5,   15.0)   Sig. = .0000  (Test Value =
.0000 )
*: Notice that the same estimator is used whether the interaction
effectis present or not.**: This estimate is computed if the
interaction effect is absent, otherwise ICC is not estimable.
Reliability Coefficients
N of Cases =      6.0 N of Items =  4 Alpha =    .9999
```

Intraclass Correlation Coefficient - **fat**

```
Two-Way Mixed Effect Model (Consistency Definition):
People Effect Random, Measure Effect Fixed
 Single Measure Intraclass Correlation =    .9980*
    95.00% C.I.:            Lower =    .9929          Upper =    .9997
 F = 2007.045   DF = (     5,   15.0)   Sig. = .0000  (Test Value =
.0000 )
 Average Measure Intraclass Correlation =    .9995**
    95.00% C.I.:            Lower =    .9982          Upper =    .9999
 F = 2007.045   DF = (     5,   15.0)   Sig. = .0000  (Test Value =
.0000 )
*: Notice that the same estimator is used whether the interaction
effect
   is present or not.
**: This estimate is computed if the interaction effect is absent,
    otherwise ICC is not estimable.
Reliability Coefficients
N of Cases =      6.0                  N of Items =  4
Alpha =    .9995
```

Appendix F: Raw data for Chapter 4

Appendix F: Raw data for Knee DXA Study

Participant Info: Non SCI

M/F	age
f	42
f	24
m	22
m	24
f	24
f	24
m	25
m	25
m	28
m	32

Participant Info: SCI

M/F	age	Yrs. Post	ASIA	Incomplete or Complete	level
M	24	24.00	C	I	C5
M	28	3.33	C	I	C4
M	33	10.17	C	I	C4-5-6
M	53	14.00	C	I	C567
F	20	4.83	C	I	C5
F	21	11.33	C	I	C5
M	29	7.08	B	I	C56
M	24	9.00	C	I	C56
M	32	1.58	C	I	T12(nerveT8)
M	32	3.00	D	I	T12
M	41	24.00	B	I	C56
M	39	13.00	D	I	C8
M	40	25.00	B	I	C567
F	46	14.00	A	C	T45

DATA: Non SCI

HIP BMD	RAD SOS	TIB BMD	TIB SOS	TIB BMD
1.023	4320	0.966	4059	0.966
0.873	4164	1.006	3994	1.006
1.359	4016	1.41	3855.5	1.41
1.173	4243	1.45	4037	1.45
1.025	4293	0.965	4044	0.965
1.02	4208	1.045	4020	1.045
1.2	4199	1.14	4054	1.14
0.985	4131	1.113	4026	1.113
1.112	4192	1.191	3978	1.191
0.959	4321	0.995	3980	0.995

DATA: SCI

HIP BMD	RAD SOS	TIB BMD	TIB SOS	TIB BMD
0.786	4184	0.830	3895	0.830
0.9	4361	0.43	3916	0.43
0.553	4186	0.59	3795	0.59
0.843	4164	0.641	4058	0.641
0.697	4185	0.741	3921	0.741
0.644	4117	0.574	3923	0.574
0.437	4219	0.735	3772	0.735
0.694	4088	0.835	4022	0.835
0.613	4056	0.568	4012	0.568
0.837	4113	0.942	3984	0.942
0.581	4322	0.434	3943	0.434
0.691	4362	0.807	3965	0.807
0.597	4114	0.561	3844	0.561
0.694	4553	0.514	4021	0.514

Appendix G: Statistical Analyses for Chapter 4

Tib BMD and Tib SOS

		VAR00001	VAR00002
VAR00001	Pearson Correlation	1	.349
	Sig. (2-tailed)	.	.094
	N	24	24
VAR00002	Pearson Correlation	.349	1
	Sig. (2-tailed)	.094	.
	N	24	24

Tib BMD and Hip BMD

		VAR00001	VAR00003
VAR00001	Pearson Correlation	1	.796
	Sig. (2-tailed)	.	.000
	N	24	24
VAR00003	Pearson Correlation	.796	1
	Sig. (2-tailed)	.000	.
	N	24	24

** Correlation is significant at the 0.01 level (2-tailed).

Hip BMD and Tib SOS

		TIBSOS	HIPBMD
TIBSOS	Pearson Correlation	1	.460
	Sig. (2-tailed)	.	.024
	N	24	24
HIPBMD	Pearson Correlation	.460	1
	Sig. (2-tailed)	.024	.
	N	24	24

* Correlation is significant at the 0.05 level (2-tailed).

Tib SOS and Rad SOS

		VAR00004	VAR00002
VAR00004	Pearson Correlation	1	.197
	Sig. (2-tailed)	.	.356
	N	24	24
VAR00002	Pearson Correlation	.197	1
	Sig. (2-tailed)	.356	.
	N	24	24

Tib BMD and Rad SOS

		VAR00001	VAR00004
VAR00001	Pearson Correlation	1	-.234
	Sig. (2-tailed)	.	.271
	N	24	24
VAR00004	Pearson Correlation	-.234	1
	Sig. (2-tailed)	.271	.
	N	24	24

Hip BMD and Rad SOS

		HIPBMD	RADSOS
HIPBMD	Pearson Correlation	1	-.083
	Sig. (2-tailed)	.	.699
	N	24	24
RADSOS	Pearson Correlation	-.083	1
	Sig. (2-tailed)	.699	.
	N	24	24

Appendix H: Raw data for Chapter 5

ID	RADIUS SOS					TIBIA SOS					PHALANX SOS				
	PRE	3 mos.	6 mos.	9 mos.	12 mos.	PRE	3 mos.	6 mos.	9 mos.	12 mos.	PRE	3 mos.	6 mos.	9 mos.	12 mos.
1	4111	4064	4112	4210	4226	4081	4047	4046	4015	3970	4149	4038	4168	4104	4131
2	4108	4081	4100	4186		3973	3916	3921	3846	3984	4383	4259	4226	4344	4328
3	4399	4385	4369	4419	4428	4010	4005	3985	3955	4037		4410	4059	4527	4158
4	4169	4144	4145	4125	4134	3926	4046	4054	3991	3983	4447	4399	4183	4364	4431
5	4189	4200	4238	4299	4171	3919	3945	3906	3992	4042	3992	4282	4133	4437	4124
6	4184	4137	4098	4094	4138	3895	3847	3906	3888	3888	4032		4054	3999	4139
7	4361	4377	4407	4224	4385	3916	3914	3815	3903	3941	3966	4018	4124	4041	4032
8	4186	4249	4210	4130	4120	3795	3918	3916	3986	3850					
9	4164	4120	4112	4125	4173	4058	4028	4065	4014	4045	4454	4238	4522	4501	4462
11	4185	4186	4079	4275	4026	3921	3853	3912	4000	4049					
12	4219	4254	4301	4256	4229	3772	3754	3790	3856	3819					
13	4088	4113	4120	4106	4157	4022	3928	4035	3966	4059	3946	4102	4000	4079	4044
14	4056	4077	4066	4068	4059	4012	4103	3988	4023	4005	4373	4214	4408	4459	4322
AVG	4186	4184	4181	4194	4187	3946	3946	3949	3957	3975	4194	4218	4188	4286	4217
SEM	27	30	32	28	34	26	27	25	17	22	65	40	47	59	45

CON	RADIUS SOS					TIBIA SOS					PHALANX SOS				
ID	PRE	3 mos.	6 mos.	9 mos.	12 mos.	PRE	3 mos.	6 mos.	9 mos.	12 mos.	PRE	3 mos.	6 mos.	9 mos.	12 mos.
1CN	4113	4062	4096	4121	4093	3984	3979	4039	3977	3966	4330	4388	4330	4384	4440
2CN	4322	4296		4327	4287	3943	4018	4019	4020	3984	4392	4604		4499	4379
4CN	4114	4185	4178	4183	4157	3844	3902	3831	3920	3890	3836	3777	3861	3869	3825
AVG	4183	4181	4137	4210	4179	3924	3966	3963	3972	3947	4186	4256	4096	4251	4215
MAX	70	68	41	61	57	42	34	66	29	29	176	248	235	194	196

ID	Body Mass			Lean Mass			Fat Mass	
	PRE	POST	% chg	PRE	POST	% chg	PRE	POST
1	87343	94934	8.7	56480	59168.7	4.8	28057	33034.2
2	52952	62204	17.5	43269	49369.5	14.1	7406.1	10495.1
3	48331	49734	2.9	30924.8	32872.8	6.3	15639.3	15081.3
4	96587.7	100496	4.0	60140.6	63208.8	5.1	33721.2	34630
5	60789	61310	0.9	49907.5	50262.8	0.7	8209.8	8379.2
6	94740	98381	3.8	53830.2	56369.2	4.7	38377.5	39505.5
7	62952	66426	5.5	50047.5	51627.3	3.2	10878.8	12582.4
8	86243	82916	-3.9	52671.8	51447.6	-2.3	31836.6	29714
9	60631	63076	4.0	43321	44699.8	3.2	14964.2	16004.2
11	75724	70803	-6.5	36874.6	36604.4	-0.7	36891.9	32302
12	65945	64344	-2.4	37920.8	38542.5	1.6	26053.3	23829.7
13	74522	80365	7.8	41698.3	44052.9	5.6	30733.3	34269.3
14	65533	68499	4.5	39360.9	43486.9	10.5	23685.7	22665.7
AVG	**71714.8**	**74114.5**	**3.6**	**45880.5**	**47824.1**	**4.4**	**23573.4**	**24037.9**
SEM	**4324.5**	**4424.6**	**1.7**	**2402.1**	**2471.2**	**1.2**	**3039.2**	**2935.3**

ID	BMD-TOTAL			BMC-TOTAL		
	PRE	POST	% chg	PRE	POST	% chg
1	1.199	1.135	-5.3	2805.32	2731.4	-2.6
2	1.24	1.183	-4.6	2276.96	2340.25	2.8
3	1.109	1.073	-3.2	1767.75	1780.18	0.7
4	1.207	1.211	0.3	2725.91	2657.97	-2.5
5	1.285	1.294	0.7	2671.94	2668.2	-0.1
6	1.093	1.082	-1.0	2533.02	2506.2	-1.1
7	1.074	1.04	-3.2	2026.06	1946.65	-3.9
8	0.98	0.939	-4.2	1734.61	1755	1.2
9	1.146	1.134	-1.0	2346.24	2372.3	1.1
11	0.991	0.947	-4.4	1957.87	1896.59	-3.1
12	1.008	1.024	1.6	1971.5	1971.6	0.0
13	1.013	1.005	-0.8	2090.48	2042.86	-2.3
14	1.186	1.161	-2.1	2487	2346.8	-5.6
AVG	**1.1**	**1.1**	**-2.1**	**2261.1**	**2232.0**	**-1.2**
SEM	**0.0**	**0.0**	**0.6**	**101.1**	**97.1**	**0.7**

ID	RIGHT PROX. FEMUR BMD			LEFT PROX. FEMUR BMD			BOTH LEGS AVERAGE		
	PRE	POST	% chg	PRE	POST	% chg	PRE	POST	% chg
1	1.004	0.949	-5.5	0.902	0.872	-3.3	0.953	0.911	-4.5
2	0.594	0.627	5.6	0.746	0.78	4.6	0.670	0.704	5.0
3	0.624	0.648	3.8	0.723	0.704	-2.6	0.674	0.676	0.4
4	1.063	1.067	0.4	1.004	1.055	5.1	1.034	1.061	2.7
5	0.783	0.659	-15.8	0.801	0.709	-11.5	0.792	0.684	-13.6
6	0.909	0.912	0.3	0.944	0.971	2.9	0.927	0.942	1.6
7	0.553	0.563	1.8	0.509	0.528	3.7	0.531	0.546	2.7
8	0.611	0.629	2.9	0.556	0.56	0.7	0.584	0.595	1.9
9	0.792	0.78	-1.5	0.844	0.818	-3.1	0.818	0.799	-2.3
11	0.606	0.568	-6.3	0.65	0.631	-2.9	0.628	0.600	-4.5
12	0.468	0.473	1.1	0.434	0.457	5.3	0.451	0.465	3.1
13	0.614	0.631	2.8	0.694	0.676	-2.6	0.654	0.654	-0.1
14	0.617	0.566	-8.3	0.626	0.619	-1.1	0.622	0.593	-4.7
AVG	**0.7**	**0.7**	**-1.4**	**0.7**	**0.7**	**-0.4**	**0.7**	**0.7**	**-0.9**
SEM	**0.1**	**0.0**	**1.7**	**0.0**	**0.0**	**1.3**	**0.0**	**0.0**	**1.4**

ID	PROXIMAL TIBIA BMD			DISTAL FEMUR BMD			LUMBAR SPINE BMD		
	PRE	POST	% chg	PRE	POST	% chg	PRE	POST	% chg
1							0.957	0.986	3.03
2									
3									
4							1.119	1.1	-1.7
5							1.035	1.028	-0.68
6	0.857	0.887	3.5	0.839	0.873	4.1	1.129	1.191	5.492
7	0.488	0.48	-1.6	0.36	0.364	1.1	0.93	0.861	-7.42
8	0.621	0.652	5.0	0.634	0.669	5.5	0.785	0.828	5.478
9	0.641	0.638	-0.5	0.703	0.733	4.3	1.327	1.373	3.466
11	0.631	0.647	2.5	0.539	0.576	6.9	1.092	1.047	-4.12
12	0.735	0.724	-1.5	0.567	0.57	0.5	1.078	1.038	-3.71
13	0.845	0.837	-0.9	0.79	0.798	1.0	0.852	0.856	0.469
14	0.607	0.632	4.1	0.656	0.584	-11.0			
AVG	**0.678**	**0.687**	**1.325**	**0.636**	**0.646**	**1.548**	**1.028**	**1.028**	**-0.049**
SEM	**0.04**	**0.05**	**0.97**	**0.05**	**0.06**	**1.96**	**0.07**	**0.07**	**1.82**

ID	Body Mass			Lean Mass			Fat Mass		
	PRE	POST	% chg	PRE	POST	% chg	PRE	POST	% chg
1CN	84055	91961	9.4	54788	57151	4.3	26512	31989	20.7
2CN	81877	82959	1.3	53991	53915	-0.1	25653	26738	4.2
4CN	79851	73120	-8.4	41798	41230	-1.4	35823	29635	-17.3
AVG	**81928**	**82680**	**0.8**	**50192**	**50765**	**0.9**	**29329**	**29454**	**2.5**
SEM	**1214**	**5441**	**5.2**	**4204**	**4858**	**1.7**	**3256**	**1518**	**11.0**

ID	RIGHT PROX. FEMUR BMD			LEFT PROX. FEMUR BMD			BOTH LEGS AVERAGE		
	PRE	POST	% chg	PRE	POST	% chg	PRE	POST	% chg
1CN	0.84	0.844	0.5	0.837	0.858	2.5	0.8385	0.851	1.4908
2CN	0.551	0.584	6.0	0.581	0.59	1.5	0.566	0.587	3.7102
4CN	0.584	0.568	-2.7	0.59	0.531	-10.0	0.587	0.55	-6.388
AVG	**0.658**	**0.665**	**1.2**	**0.669**	**0.660**	**-2.0**	**0.664**	**0.663**	**-0.4**
SEM	**0.091**	**0.089**	**2.5**	**0.084**	**0.101**	**4.0**	**0.088**	**0.095**	**3.1**

ID	PROXIMAL TIBIA BMD			DISTAL FEMUR BMD			LUMBAR SPINE BMD		
	PRE	POST	% chg	PRE	POST	% chg	PRE	POST	% chg
1CN	0.904	0.942	4.2	0.937	0.929	-0.9			
2CN	0.434	0.414	-4.6	0.669	0.629	-6.0	1.069	1.019	-4.7
4CN	0.594	0.549	-7.6	0.699	0.639	-8.6	1.393	1.403	0.7
AVG	0.644	0.635	-2.7	0.768	0.732	-5.1	1.231	1.211	-2.0
SEM	0.138	0.158	3.5	0.085	0.098	2.3	0.132	0.157	2.2

ID	BMD-TOTAL			BMC-TOTAL		
	PRE	POST	% chg	PRE	POST	% chg
1CN	1.232	1.239	0.6	2756	2822	2.4
2CN	1.083	1.096	1.2	2234	2307	3.3
4CN	1.028	1.057	2.8	2231	2255	1.1
AVG	**1.114**	**1.131**	**1.5**	**2407**	**2461**	**2.3**
SEM	**0.061**	**0.055**	**0.7**	**174**	**181**	**0.6**

							CROSS-SECTIONAL GEOMETRY, AREA-WEIGHTED						CORTICAL VARIABLES			
ID	CSA PRE	CSA POST	BMD PRE	BMD POST	BMC PRE	BMC POST	IMAX PRE	IMAX POST	IMIN PRE	IMIN POST	IPOL PRE	IPOL POST	CSA PRE	CSA POST	BMD PRE	BMD POST
1	604	607	819	800	2472.9	2426.1	51542	51108	44799	44595	96341	95703	545.8	540	858	847
2	510	449	755	717	1922.8	1608.4	41558	40064	24305	23298	65862	63362	449.4	374	799	782
3	310	315	863	815	1337.0	1283.9	16600	16948	12178	12633	28778	29581	261.5	261	936.4	892
4	546	549	769	759	2100.0	2081.8	42876	43886	29462	29281	72338	73167	474.9	470	824.6	818
5	471	469	849	853	1999.1	2001.1	36757	37377	29560	29612	66316	66989	410.8	412	910.2	914
6	324	322	834	860	1349.3	1385.1	12648	13171	8247	8274	20895	21446	292	290	872.3	897
7	430	420	794	791	1706.1	1660.3	30292	30061	21626	20810	51918	50871	370.2	359	852.9	856
8	324	323	729	739	1181.1	1194.2	25680	22795	18254	16870	43934	39665	247.9	251	822.4	834
9	459	463	758	773	1739.8	1787.2	29838	30458	27986	28238	57823	58696	375.2	379	852.5	868
11	417	420	696	680	1450.3	1426.6	30759	31166	17523	18096	48282	49263	325	325	790.7	779
12	404	386	498	499	1007.3	961.5	35248	33385	26163	26083	61411	59468	191.1	181	764.7	761
13	388	387	813	796	1577.6	1541.2	24518	24624	18087	17922	42605	42546	328.3	327	882.7	863
14	477	458	761	749	1812.8	1715.9	35427	35073	24717	24707	60145	59780	413.7	387	815	818
AVG	**435.6**	**428.3**	**764.4**	**756.0**	**1665.9**	**1621.0**	**31826.4**	**31547.4**	**23300.5**	**23109.2**	**55126.8**	**54656.7**	**360.4**	**350.5**	**844.7**	**840.8**
SEM	**24.5**	**23.9**	**25.9**	**25.6**	**112.6**	**109.6**	**2944.8**	**2926.6**	**2546.5**	**2539.2**	**5378.3**	**5351.5**	**27.6**	**26.3**	**13.5**	**13.3**

MID-FEMUR - RIGHT SIDE

							CROSS-SECTIONAL GEOMETRY, AREA-WEIGHTED						CORTICAL VARIABLES			
ID	CSA PRE	CSA POST	BMD PRE	BMD POST	BMC PRE	BMC POST	IMAX PRE	IMAX POST	IMIN PRE	IMIN POST	IPOL PRE	IPOL POST	CSA PRE	CSA POST	BMD PRE	BMD POST
1	565	563	800	739	2259.2	2082.6	46644	47440	38809	39741	85454	87181	510.7	489	837	791
2	533	494	797	767	2124.4	1894.7	40621	40444	23975	23981	64596	61238	483	435	838	820
3	308	323	901	848	1386.9	1368.0	15364	16153	11644	12422	27008	28575	266	274	966.3	922
4	522	525	782	766	2041.2	2010.3	39203	39204	27525	28450	66728	67654	454.1	453	837.6	823
5	479	474	848	848	2028.3	2011.5	35923	36152	31288	31372	67211	67524	421.2	415	904	908
6	289	301	819	788	1185.4	1184.9	10691	10772	6701	7010	17392	17782	258.4	270	858.5	823
7	433	436	823	805	1782.4	1753.9	29860	30616	23750	24100	53610	54716	374.6	376	882.5	863
8	317	319	737	745	1166.4	1189.2	19641	20050	16093	16075	35734	36125	241.8	249	839	847
9	476	474	774	757	1842.0	1793.4	31681	31669	29255	29060	60937	60729	396.5	389	859.3	846
11	416	420	716	716	1488.4	1503.3	33169	33198	16792	17021	49961	50219	333.1	339	797	807
12	398	393	542	528	1076.8	1036.0	33590	33530	25951	25833	59541	59363	203.8	201	776	770
13	410	404	811	829	1663.4	1675.4	25212	24845	20255	19658	45467	44503	352	347	874.5	893
14	489	459	744	748	1819.7	1717.3	36902	35852	25664	24646	62566	60498	424.7	386	795.1	819
AVG	**433.4**	**429.7**	**776.4**	**760.2**	**1681.9**	**1632.3**	**30653.9**	**30763.5**	**22900.2**	**23028.4**	**53554.2**	**53546.7**	**363.1**	**355.5**	**851.1**	**841.0**
SEM	**24.6**	**22.4**	**23.8**	**22.6**	**108.5**	**96.3**	**2880.9**	**2859.2**	**2380.2**	**2391.4**	**5113.7**	**5075.7**	**27.1**	**23.9**	**13.9**	**12.6**

MID-FEMUR BOTH LEGS AVERAGED

							CROSS-SECTIONAL GEOMETRY, AREA-WEIGHTED						CORTICAL VARIABLES			
ID	CSA PRE	CSA POST	BMD PRE	BMD POST	BMC PRE	BMC POST	IMAX PRE	IMAX POST	IMIN PRE	IMIN POST	IPOL PRE	IPOL POST	CSA PRE	CSA POST	BMD PRE	BMD POST
1	584	585	810	769	2366.1	2254.3	49093	49274	41804	42168	90898	91442	528	515	848	819
2	521	472	776	742	2023.6	1751.5	41090	40254	24140	23640	65229	62300	466	404	819	801
3	309	319	882	831	1362.0	1326.0	15982	16550.5	11911	12528	27893	29078	264	267	951	907
4	534	537	776	762	2070.6	2046.1	41040	41545	28493.5	28866	69533	70411	465	462	831	821
5	475	472	848	850	2013.7	2006.3	36340	36764.5	30424	30492	66764	67257	416	413	907	911
6	307	312	826	824	1267.4	1285.0	11670	11971.5	7474	7642	19144	19614	275	280	865	860
7	431	428	809	798	1744.3	1707.1	30076	30338.5	22688	22455	52764	52794	372	368	868	859
8	320	321	733	742	1173.8	1191.7	22661	21422.5	17173.5	16473	39834	37895	245	250	831	841
9	468	468	766	765	1790.9	1790.3	30760	31063.5	28620.5	28649	59380	59713	386	384	856	857
11	416	420	706	698	1469.3	1464.9	31964	32182	17157.5	17559	49122	49741	329	332	794	793
12	401	389	520	513	1042.0	998.7	34419	33457.5	26057	25958	60476	59416	197	191	770	766
13	399	396	812	812	1620.5	1608.3	24865	24734.5	19171	18790	44036	43525	340	337	879	878
14	483	459	753	748	1816.3	1716.6	36165	35462.5	25190.5	24677	61356	60139	419	387	805	818
AVG	**434.5**	**429.0**	**770.4**	**758.1**	**1673.9**	**1626.7**	**31240.2**	**31155.4**	**23100.3**	**23068.8**	**54340.5**	**54101.7**	**361.8**	**353.0**	**847.9**	**840.9**
SEM	**24.4**	**23.0**	**24.7**	**23.6**	**109.5**	**100.9**	**2891.5**	**2879.3**	**2445.1**	**2451.5**	**5211.6**	**5193.4**	**27.2**	**24.9**	**13.4**	**12.0**

REFERENCE CONTROL GROUP

MID-FEMUR - RIGHT SIDE

							CROSS-SECTIONAL GEOMETRY, AREA-WEIGHTED						CORTICAL VARIABLES			
ID	CSA PRE	CSA POST	BMD PRE	BMD POST	BMC PRE	BMC POST	IMAX PRE	IMAX POST	IMIN PRE	IMIN POST	IPOL PRE	IPOL POST	CSA PRE	CSA POST	BMD PRE	BMD POST
1CN	519	512	826	837	2145.1	2141.9	42645	41889	30247	29366	72891	71264	458	451	879	890
2CN	390	383	724	734	1411.8	1406.0	41115	41021	24877	24901	65992	65923	291	291	842	842
4CN	449	444	652	663	1463.4	1471.9	39585	38090	32689	32416	72274	70506	340	344	747	755
AVG	453	446	734	745	1673.4	1673.2	41115	40333	29271	28894	70386	69231	363	362	823	829
SEM	18.0	17.9	24.2	24.3	113.5	112.9	424.3	552.1	1108.4	1048.3	1058.8	801.5	23.9	22.6	18.9	19.0

REFERENCE CONTROL GROUP

MID-FEMUR - LEFT SIDE

							CROSS-SECTIONAL GEOMETRY, AREA-WEIGHTED						CORTICAL VARIABLES			
	CSA PRE	CSA POST	BMD PRE	BMD POST	BMC PRE	BMC POST	IMAX PRE	IMAX POST	IMIN PRE	IMIN POST	IPOL PRE	IPOL POST	CSA PRE	CSA POST	BMD PRE	BMD POST
1CN	533	533	852	831	2269.3	2215.4	43680	44093	31067	30840	74747	74932	477	474	900	880
2CN	368	367	703	701	1293.5	1287.4	36057	36672	23999	24144	60056	60816	258	261	839	830
4CN	444	420	648	650	1436.9	1364.0	44356	39335	31930	30162	76287	69497	332	314	751	750
AVG	**448.1**	**440.1**	**734.3**	**727.3**	**1666.6**	**1622.3**	**41364.3**	**40033.3**	**28998.7**	**28382.0**	**70363.3**	**68415.0**	**355.7**	**349.3**	**830.0**	**820.0**
SEM	**22.9**	**23.5**	**29.3**	**25.9**	**146.1**	**142.9**	**1278.2**	**1042.7**	**1206.8**	**1022.3**	**2484.9**	**1974.7**	**30.9**	**30.7**	**20.8**	**18.2**

Osteocalcin

Exercise Group

ID	Baseline	3 Months	6 Months	12 Months
1	4.310		4.061	9.316
2	14.018		19.093	15.251
3	8.495		9.733	7.748
4	5.505	8.879	8.511	7.886
5	19.635	8.723	15.448	11.798
6	14.921	13.690	8.522	15.330
7	6.202	7.281	7.979	8.950
8	10.919	9.695	10.453	12.208
9	7.082	6.000	6.817	7.119
11				
12	6.817	9.196	8.361	12.715
13	7.395	7.606	8.475	6.839
14	13.465	12.547	14.905	14.268
AVG	9.897	9.291	10.196	10.785
SEM	**1.3502**	**0.81925**	**1.2159**	**0.91978**

Reference Group

ID	Baseline	3 Months	6 Months	12 Months
1CN	9.193		7.391	5.208
2CN	9.844	7.868	7.027	8.582
4CN	8.987		9.356	9.552
AVG	9.341		7.924	7.781
SEM	0.2585		0.7235	1.31668

Deoxypyridinoline

Exercise Group

ID	Baseline	3 Months	6 Months	12 Months
1	6.9993		5.13123	4.14346
2	15.34		6.98763	12.4548
3	8.9874		8.22002	20.1132
4	3.6974	17.635	88.1144	8.51476
5	7.8986	7.5915	5.10468	4.79522
6	6.3499	7.7611	7.40958	7.6277
7	6.7849	6.4069	6.09118	4.72351
8	8.3818	8.9874		8.92768
9	5.4817	2.4281	4.39221	2.82402
11	16.957	10.021	10.2447	13.6015
12	72.409	12.532	16.4859	60.793
13	5.5107	8.518	5.47541	4.83794
14	14.651	15.384	14.1341	9.45772
AVG	13.804	9.727	14.816	12.524
SEM	**5.0169**	**1.4792**	**6.7513**	**4.23283**

Reference Group

ID	Baseline	3 Months	6 Months	12 Months
1CN	6.3736		6.98343	5.89664
2CN	4.745	5.0669	4.58715	3.34916
4CN	15.046		17.9059	21.3018
AVG	8.722		9.825	10.183
SEM	3.197		4.099	5.60807

EXERCISE GROUP

	RIGHT LEG MUSCLE				LEFT LEG MUSCLE				BOTH LEGS AVERAGED			BOTH LEGS AVERAGED		
	MID-THIGH CSA		LOWER LEG CSA		MID-THIGH CSA		LOWER LEG CSA		MID-THIGH CSA			LOWER LEG CSA		
ID	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	%CHG	PRE	POST	%CHG
1	15433.7	16868.9	6800.3	7627.8	11879.1	13342.1	6224.6	7412.0	13656.4	15105.5	10.6	6512.4	7519.9	15.5
2	3859.2	4529.5	3128.5	3479.1	6343.7	8176.9	3455.0	4267.6	5101.5	6353.2	24.5	3291.8	3873.3	17.7
3	9436.9	9325.8	4807.4	4783.3	9900.8	9548.9	4806.4	4782.7	9668.8	9437.4	-2.39	4806.9	4783.0	-0.5
4	17619.9	18371.6	7982.8	7718.7	16733.4	18636.7	7385.9	7877.9	17176.7	18504.1	7.73	7684.3	7798.3	1.48
5	10275.3	10331.9	5422.9	5744.6	10198.9	9997.9	5444.7	5776.2	10237.1	10164.9	-0.71	5433.8	5760.4	6.01
6	9830.9	9984.0	4708.4	4835.2	8798.3	8624.4	4568.1	4387.4	9314.6	9304.2	-0.11	4638.2	4611.3	-0.58
7	10928.2	11603.0	5684.7	5832.6	11153.8	11969.8	6344.1	6359.8	11041.0	11786.4	6.75	6014.4	6096.2	1.36
8	12245.2	11748.2	7619.3	7394.6	12177.5	11851.9	7455.7	7203.2	12211.4	11800.0	-3.37	7537.5	7298.9	-3.17
9	10189.5	10839.7	5106.5	5968.4	10032.6	10219.1	5507.7	6129.7	10111.0	10529.4	4.14	5307.1	6049.0	14
10	4959.2	5605.4	3391.3	3591.2	5519.8	5683.5	3635.8	4065.1	5239.5	5644.4	7.73	3513.6	3828.1	8.95
12	2222.4	2331.8	414.0	445.3	2698.9	2703.1	368.3	451.2	2460.6	2517.4	2.31	391.1	448.2	14.6
13	9230.2	10120.9	5266.2	5323.4	10164.3	11037.3	5397.5	5814.7	9697.3	10579.1	9.09	5331.8	5569.0	4.45
14	3472.6	3481.2	2130.1	3081.5	3552.4	3402.4	3009.8	3461.7	3512.5	3441.8	-2.01	2570.0	3271.6	27.3
AVG	**9207.9**	**9626.3**	**4804.8**	**5063.5**	**9165.6**	**9630.3**	**4892.6**	**5229.9**	**9186.8**	**9628.3**	**4.9**	**4848.7**	**5146.7**	**8.2**
SEM	**1272.5**	**1322.6**	**592.2**	**572.1**	**1061.4**	**1169.2**	**539.3**	**552.1**	**1156.3**	**1229.3**	**2.1**	**563.3**	**560.1**	**2.5**

REFERENCE CONTROL GROUP

	RIGHT LEG MUSCLE				LEFT LEG MUSCLE				BOTH LEGS AVERAGED			BOTH LEGS AVERAGED		
ID	MID-THIGH CSA		LOWER LEG CSA		MID-THIGH CSA		LOWER LEG CSA		MID-THIGH CSA			LOWER LEG CSA		
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	%CHG	PRE	POST	%CHG
1CN	10111	9380.4	6775	6680	9302.7	8799.85	6857	6640.7	9706.8	9090.1	-6.35	6816	6661	-2.28
2CN	9212	9064.8	4592	4507	7404.7	7171.3	2636	2468.7	8308.4	8118.1	-2.29	3614	3488	-3.5
4CN	6426.8	5719.3	4298	3790	5634.3	4315.25	3644	3334.9	6030.5	5017.3	-16.8	3971	3562	-10.3
AVG	**8583.3**	**8054.8**	**5222.0**	**4992.4**	**7447.2**	**6762.1**	**4378.9**	**4148.1**	**8015.2**	**7408.5**	**-8.5**	**4800.4**	**4570.2**	**-5.4**
SEM	**532.8**	**562.7**	**375.3**	**417.5**	**508.8**	**629.6**	**611.3**	**610.6**	**514.7**	**590.0**	**2.1**	**486.6**	**502.2**	**1.2**

Appendix I: Statistical Analyses for Chapter 5

Bone Density and Geometry of the Lower Leg, Measured Using CT

Multivariate

Within Subjects Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	Pillai's Trace	.37208	.5	7.000	6.000	.802	.372	3.554	.114

a Computed using alpha = .05

Bone Density and Geometry of the Mid-Femur, Measured Using CT
Multivariate

Within Subjects Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	Pillai's Trace	.632	1.076	8.000	5.000	.491	.632	8.605	.182

a Computed using alpha = .05

Muscle Cross-sectional Areas at Mid-Femur and 65% Lower Leg, Measured Using CT – Multivariate

Within Subjects Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	Pillai's Trace	.459	4.675	2.000	11.000	.034	.459	9.351	.659

a Computed using alpha = .05

Tests of Within-Subjects Contrasts - Univariate

Source	Measure	FACTOR1	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	FEM	Linear	1267038.775	1	1267038.775	6.331	.027	.345	6.331	.638
	TIB	Linear	577330.305	1	577330.305	8.745	.012	.422	8.745	.775
Error(FACTOR1)	FEM	Linear	2401715.336	12	200142.945					
	TIB	Linear	792175.797	12	66014.650					

a Computed using alpha = .05

Body Composition, Measured Using DXA – Statistical Analysis
Multivariate

Within Subjects Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	Pillai's Trace	.696	3.666	5.000	8.000	.051	.696	18.329	.662

a Computed using alpha = .05

Tests of Within-Subjects Contrasts - Univariate

Source	Measure	FACTOR1	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	WEIGHT	Linear	37428720.850	1	37428720.850	4.537	.055	.274	4.537	.500
	LEAN	Linear	24553110.094	1	24553110.094	13.379	.003	.527	13.379	.918
	FAT	Linear	1402162.939	1	1402162.939	.410	.534	.033	.410	.091
	BMD	Linear	3.531E-03	1	3.531E-03	11.065	.006	.480	11.065	.862
	BMC	Linear	5514.746	1	5514.746	3.575	.083	.230	3.575	.413
Error(FACTOR1)	WEIGHT	Linear	98985624.125	12	8248802.042					
	LEAN	Linear	22021680.646	12	1835140.054					
	FAT	Linear	40996517.726	12	3416376.477					
	BMD	Linear	3.829E-03	12	3.191E-04					
	BMC	Linear	18511.862	12	1542.655					

a Computed using alpha = .05

Lumbar Spine Bone Mineral Density – Tests of Within-Subjects Contrasts

Source	FACTOR1	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	Linear	2.450E-06	1	2.450E-06	.002	.961	.000	.002	.050
Error(FACTOR1)	Linear	8.862E-03	9	9.847E-04					

a Computed using alpha = .05

Upper and Lower Body Composition, Measured Using DXA – Statistical Analysis

Multivariate

Within Subjects Effect		Value	F	Hypothesis df	Error df	Sig.
FACTOR1	Pillai's Trace	.565	7.135	2.000	11.000	.010

Tests of Within-Subjects Contrasts

Source	Measure	FACTOR1	Type III Sum of Squares	df	Mean Square	F	Sig.
FACTOR1	LLLEAN	Linear	9.408	1	9.408	11.836	.005
	UBLEAN	Linear	3.877	1	3.877	4.286	.061
Error(FACTOR1)	LLLEAN	Linear	9.538	12	.795		
	UBLEAN	Linear	10.854	12	.905		

Total Proximal Femur Bone Mineral Density – Statistical Analyses

Multivariate

Within Subjects Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	Pillai's Trace	.102	.623	2.000	11.000	.554	.102	1.245	.129

a Computed using alpha = .05

Distal Femur and Tibia Bone Mineral Density – Statistical Analyses

Multivariate

Within Subjects Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	Pillai's Trace	.266	1.089	2.000	6.000	.395	.266	2.178	.164

a Computed using alpha = .05

Radius and Tibia Speed of Sound – Multivariate

Within Subjects Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	Pillai's Trace	.068	.385	8.000	88.000	.926	.034	3.082	.173

a Computed using alpha = .05

Osteocalcin – Statistical Analyses

Tests of Within-Subjects Contrasts

Source	FACTOR1	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	Linear	7.654	1	7.654	1.911	.194	.148	1.911	.244
	Quadratic	4.287	1	4.287	1.319	.275	.107	1.319	.183
	Cubic	2.006	1	2.006	.187	.674	.017	.187	.068
Error(FACTOR1)	Linear	44.062	11	4.006					
	Quadratic	35.748	11	3.250					
	Cubic	118.172	11	10.743					

a Computed using alpha = .05

Deoxypyridinoline – Statistical Analyses

Tests of Within-Subjects Contrasts

Source	FACTOR1	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
FACTOR1	Linear	.322	1	.322	.007	.935	.001	.007	.051
	Quadratic	18.384	1	18.384	.041	.844	.004	.041	.054
	Cubic	163.649	1	163.649	.888	.366	.075	.888	.139
Error(FACTOR1)	Linear	505.782	11	45.980					
	Quadratic	4986.037	11	453.276					
	Cubic	2027.605	11	184.328					

a Computed using alpha = .05

Appendix J: Attendance data for Chapter 5 (chronic SCI)

ID	Possible BWSTT Days	Actual BWSTT Days	% days BWSTT of possible	Average # Sessions per week
1	216	144	66.7	2.0
2	168	144	85.7	2.6
3	152	140	92.1	2.8
4	180	144	80.0	2.4
5	207	144	69.6	2.1
6	177	144	81.4	2.4
7	174	144	82.8	2.5
8	195	144	73.8	2.2
9	210	144	68.6	2.1
11	192	144	75.0	2.3
12	183	144	78.7	2.4
13	171	144	84.2	2.5
14	171	144	84.2	2.5
AVG	184.3	143.7	78.7	2.4
SEM	18.7	1.1	7.5	0.2

Appendix K: BWSTT Intensity Over 144 sessions of Training in Individuals with Chronic SCI

	BWS					Speed					Duration				
ID	0mo	3mo	6mo	9mo	12mo	0mo	3mo	6mo	9mo	12mo	0mo	3mo	6mo	9mo	12mo
1	42	32	0	0	0	0.5	1	1.1	1.3	1.4	13	28	30	30	43
2	93	38	13	13	13	1	1.1	1.7	2.5	3	30	16	30	35	30
3	49	33	0	0	0	0.8	1.2	1.5	1.8	2	45	60	60	60	60
4	83	46	38	30	30	0.1	0.5	0.7	0.9	1	15	20	26	30	40
5	65	51	40	26	16	0.1	0.7	0.7	0.7	0.8	10	26	26	30	40
6	63	38	31	22	22	1	1.8	1.5	1.8	1.8	30	30	35	40	40
7	62	44	30	24	17	0.4	0.7	0.8	0.9	0.9	30	30	30	30	30
8	60	51	33	33	0	0.3	0.7	1.1	1	1.1	17	30	36	40	46
9	75	36	24	0	0	0.5	0.8	1.1	1.2	1.1	15	24	32	45	45
11	75	65	51	46	36	0.9	1	1.4	1.4	1.3	18	32	34	35	36
12	70	70	47	41	24	0.5	0.9	1.1	1.4	1.3	27	30	30	30	30
13	76	62	41	24	13	0.3	0.8	1.2	1.3	1.1	15	30	39	40	30
14	72	58	48	35	23	0.5	1	1.6	1.5	1.5	15	35	60	60	60
AVG	**68.1**	**48.0**	**30.5**	**22.6**	**14.9**	**0.5**	**0.9**	**1.2**	**1.4**	**1.4**	**21.5**	**30.1**	**36.0**	**38.8**	**40.8**
SEM	**3.76**	**3.52**	**4.72**	**4.27**	**3.37**	**0.09**	**0.09**	**0.09**	**0.13**	**0.16**	**2.78**	**2.87**	**3.13**	**2.95**	**2.86**

Appendix L: Attendance data for Chapter 6

ID	Possible BWSTT Days	Actual BWSTT Days	% days BWSTT of possible	Average # Sessions per week
1	50	48	96	1.9
2	58	48	83	1.7
3	65	48	74	1.5
4	64	31	48	1.0
5	54	48	89	1.8
Mean	58.2	44.6	78.0	1.6
SD	6.4	7.6	18.4	0.4
Mean no #4	56.8	48.0	85.4	1.7
SD no # 4	6.4	0.0	9.4	0.2

Appendix M: Raw data for Chapter 6

ID	DXA MASS		LBM-TOTAL			FM-TOTAL			BMD-TOTAL		
	PRE	POST	PRE	POST	% chg	PRE	POST	%chg	PRE	POST	% chg
1	82031	85501	52723	55850	5.9	26601	27114	1.9	1.22	1.19	-2.0
2	61437	67814	46798	47872	2.3	11721	17257	47.2	1.2	1.14	-5.5
3	51961	58228	29429	n/a		20642	n/a		1.14	n/a	
4	71246	86213	34903	36763	5.3	33763	47172	39.7	1.22	1.14	-6.8
5	75053	85050	46932	43785	-6.7	25189	38298	52.0	1.19	1.2	0.8

ID	LUMBAR SPINE			TIBIA: TOTAL BMD			FEM: TOTAL BMD		
	BMD-PRE	BMD-POST	% chg	PRE	POST	% chg	PRE	POST	% chg
1	1.02	1.022	0.2	0.829	0.95	14.6	1.209	1.09	-9.6
2	1.063	1.023	-3.8	0.933	0.88	-5.8	1.032	0.94	-8.5
3	0.972	0.936	-3.7	0.952	0.86	-10.2	0.901	0.84	-6.3
4	1.225	1.134	-7.4	1.105	0.89	-19.1	1.141	0.84	-26.7
5	1.186	1.174	-1.0	0.985	0.97	-1.2	1.211	1.06	-12.8

ID	RIGHT PROXIMAL FEMUR			LEFT PROX. FEMUR		%chg	Average Proximal Femur			
	BMD-PRE	BMD-POST	% chg	BMD-PRE	BMD-POST		BMD Pre	BMD Post	% chg	
1	1.194	1.081	-9.5	1.295	1.23	-5.3	1.245	1.15	-7.3	
2	0.922	0.714	-22.6	0.948	0.78	-17.7	0.935	0.75	-20.1	
3	0.952	0.84	-11.8	0.905	0.83	-8.3	0.929	0.84	-10.1	
4	0.998	0.769	-22.9	0.961	0.75	-22.3	0.98	0.76	-22.6	
5	1.062	1.026	-3.4	1.01	0.96	-5.3	1.036	0.99	-4.3	

ID	Osteocalcin (ng/mL)			DPD (nmol DPD/mmol Cr)		
	Baseline	24 sessions	48 sessions	Baseline	24 Sessions	48 Sessions
1	8.64	13.50	9.68	24.85	14.52	9.35
2	7.54	7.69	9.95	36.91	19.44	21.89
3	6.80	9.40	8.02	59.91	29.31	13.75
4	5.65	7.84	6.73	108.97	34.39	53.39
5	8.71	9.08	7.28	60.68	38.14	31.37

	MUSCLE											
ID	THIGH - RIGHT SIDE			THIGH - LEFT SIDE			CALF - RIGHT SIDE			CALF - LEFT SIDE		
	PRE	POST	CONTROL	PRE	POST	CONTROL	PRE	POST	CONTROL	PRE	POST	CONTROL
1	13934	14794	20388	14023	14312	19694	6957	8970	7946	7044	9184	8142.3
2	9490	9884	18769	9467	9898	19341.2	3467	3506	6853	3325	3543	6894.7
3	5557	9085	10097	5320	7977	9419.8	3783	5304	4711	4060	3285	4241.1
4	5388	7063	9990	5417	6878	10318.4	2361	4030	6511	2813	3917	6984.2
5	9812	10488	13153	9322	10168	12733.7	3818	4609	6389	3950	4935	5768

	THIGH - LEFT AND RIGHT AVERAGE					PRE	POST	CALF - LEFT AND RIGHT AVERAGE					PRE	POST
	PRE	POST	Chg	% INC.	CONTROL	% CON	% CON	PRE	POST	Chg	% INC.	CONTROL	% CON	% CON
1	13979	14553	574	4.1	20040.8	69.8	72.6	7001	9077	2076	29.7	7945.8	88.1	114.2
2	9478	9891	413	4.4	18769.2	50.5	52.7	3396	3524	128	3.8	6853.0	49.6	51.4
3	5438	8531	3093	56.9	10096.6	53.9	84.5	3922	4295	373	9.5	4710.8	83.2	91.2
4	5402	6970	1568	29.0	9989.7	54.1	69.8	2587	3974	1387	53.6	6510.8	39.7	61.0
5	9567	10328	760	7.9	13153.1	72.7	78.5	3884	4772	888	22.9	6078.5	63.9	78.5

	FAT											
	THIGH - RIGHT SIDE			THIGH - LEFT SIDE			CALF - RIGHT SIDE			CALF - LEFT SIDE		
	PRE	POST	CONTROL	PRE	POST	CONTROL	PRE	POST	CONTROL	PRE	POST	CONTROL
1	14258	15691	15558	13331	16727	16426	2983	3739	2993	2906	3648	2490
2	6829	10963	5525	7238	10755	5041	1464	1953	2005	1439	2025	1967
3	13719	14876	8263	13696	15373	8014	3435	4186	1957	3409	5064	2094
4	19030	21221	20377	18116	21742	19318	5652	7724	5958	4886	7114	6187
5	14164	17986	17180	14032	18720	16339	2826	3628	4541	3033	3608	4325

	THIGH - LEFT AND RIGHT AVERAGE					PRE	POST	CALF - LEFT AND RIGHT AVERAGE					PRE	POST
	PRE	POST	Chg	% INC.	CONTROL	% CON	% CON	PRE	POST	Chg	% INC.	CONTROL	% CON	% CON
1	13794	16209	2415	17.5	15992	86.3	101.4	2944	3693	749	25.4	2741	107.4	134.7
2	7033	10859	3826	54.4	5283	133.1	205.5	1452	1989	538	37.0	1986	73.1	100.2
3	13707	15124	1417	10.3	8139	168.4	185.8	3422	4625	1203	35.1	2026	169.0	228.3
4	18573	21482	2909	15.7	19848	93.6	108.2	5269	7419	2150	40.8	6073	86.8	122.2
5	14098	18353	4255	30.2	16760	84.1	109.5	2930	3618	688	23.5	4433	66.1	81.6

***note that shaded cells represent a change that is greater than three times the measured error (SD) for that variable**

	Right Femur								
ID	Area Pre	Area Post	Change	BMD Pre	BMD Post	Change	Imax Pre	Imax Post	Change
1	570.4	567.0	-3.4	814.4	744.7	-69.7	38836.7	38571.0	-265.6
2	526.7	526.7	0.0	782.7	783.0	0.3	46532.1	46532.0	-0.1
3	419.2	415.9	-3.3	816.0	798.0	-18.0	19164.0	19305.0	141.0
4	523.1	500.5	-22.6	786.0	698.0	-88.0	31740.0	30512.1	-1227.9
5	579.8	596.8	17.0	773.0	754.1	-18.9	43151.0	45249.9	2098.9
	Left Femur								
ID	Area Pre	Area Post	Change	BMD Pre	BMD Post	Change	Imax Pre	Imax Post	Change
1	624.3	631.1	6.8	819.1	749.5	-69.6	42772.9	43943.2	1170.3
2	562.1	562.1	0.0	768.7	769.0	0.3	47520.9	47521.0	0.1
3	397.7	401.6	3.9	827.0	788.0	-39.0	18766.0	19435.0	669.0
4	524.9	510.1	-14.8	778.0	636.1	-141.9	31756.0	30972.0	-784.0
5	575.0	577.4	2.4	781.0	786.3	5.3	44886.0	45024.0	138.0
	Right Tibia								
ID	Area Pre	Area Post	Change	BMD Pre	BMD Post	Change	Imax Pre	Imax Post	Change
1	468.8	462.7	-6.1	771.5	667.4	-104.1	50062.4	48688.7	-1373.8
2	491.9	491.9	0.0	793.1	793.0	-0.1	51388.6	51389.0	0.4
3	329.4	322.8	-6.6	870.0	866.0	-4.0	24597.0	24597.0	0.0
4	419.3	409.2	-10.1	785.0	701.6	-83.4	38993.0	37001.2	-1991.8
5	473.0	472.0	-1.0	751.0	748.1	-2.9	44314.0	41896.6	-2417.4
	Left Tibia								
ID	Area Pre	Area Post	Change	BMD Pre	BMD Post	Change	Imax Pre	Imax Post	Change
1	464.2	470.4	6.3	781.2	704.7	-76.4	49482.0	52927.7	3445.7
2	467.5	467.5	0.0	774.8	775.0	0.2	54570.6	54571.0	0.4
3	310.1	316.2	6.1	871.0	793.0	-78.0	23025.0	23207.0	182.0
4	415.6	405.2	-10.4	803.0	681.1	-121.9	39683.0	39271.5	-411.5
5	462.0	471.1	9.1	758.0	730.7	-27.3	50134.0	49348.2	-785.8
	Femur - Averaged								
ID	Area Pre	Area Post	Change	BMD Pre	BMD Post	Change	Imax Pre	Imax Post	Change
1	597.3	599.1	1.7	816.8	747.1	-69.7	40804.8	41257.1	452.3
2	544.4	544.4	0.0	775.7	776.0	0.3	47026.5	47026.5	0.0
3	408.5	408.8	0.3	821.5	793.0	-28.5	18965.0	19370.0	405.0
4	524.0	505.3	-18.7	782.0	667.0	-115.0	31748.0	30742.1	-1005.9
5	577.4	587.1	9.7	777.0	770.2	-6.8	44018.5	45137.0	1118.5
	Tibia - Averaged								
ID	Area Pre	Area Post	Change	BMD Pre	BMD Post	Change	Imax Pre	Imax Post	Change
1	466.5	466.6	0.1	776.3	686.1	-90.2	49772.2	50808.2	1036.0
2	479.7	479.7	0.0	783.9	784.0	0.1	52979.6	52980.0	0.4
3	319.8	319.5	-0.3	870.5	829.5	-41.0	23811.0	23902.0	91.0
4	417.5	407.2	-10.2	794.0	691.3	-102.7	39338.0	38136.4	-1201.6
5	467.5	471.5	4.0	754.5	739.4	-15.1	47224.0	45622.4	-1601.6

* shaded areas indicate that the change was greater than 3 times the measured error (SD)

R	femur					
ID	Imin Pre	Imin Post	Change	Ipol Pre	Ipol Post	Change
1	29305.9	28627.4	-678.6	68142.6	67198.4	-944.2
2	31837.7	31838.0	0.3	78369.9	78370.0	0.1
3	16500.0	16558.0	58.0	35664.0	35864.0	200.0
4	24981.0	24401.7	-579.3	56721.0	54913.8	-1807.2
5	30541.0	32045.1	1504.1	73692.0	77295.1	3603.1
L	Femur					
ID	Imin Pre	Imin Post	Change	Ipol Pre	Ipol Post	Change
1	35757.5	35803.4	45.8	78530.5	79746.5	1216.1
2	35378.4	35378.0	-0.4	82899.3	82899.0	-0.3
3	14269.0	14798.0	529.0	33056.0	34233.0	1177.0
4	24909.0	26339.5	1430.5	56665.0	57311.5	646.5
5	30105.0	30772.7	667.7	74991.0	75796.7	805.7
R	Tibia					
ID	Imin Pre	Imin Post	Change	Ipol Pre	Ipol Post	Change
1	20481.1	20026.8	-454.2	70543.5	68715.5	-1828.0
2	27357.4	27357.0	-0.4	78746.0	78746.0	0.0
3	8811.0	8616.0	-195.0	33408.0	33214.0	-194.0
4	12940.0	12728.8	-211.2	51933.0	49730.0	-2203.0
5	23113.0	22705.3	-407.7	67427.0	64601.9	-2825.1
L	Tibia					
ID	Imin Pre	Imin Post	Change	Ipol Pre	Ipol Post	Change
1	20281.0	21322.1	1041.1	69763.0	74249.7	4486.8
2	25007.1	25002.0	-5.1	79577.7	79578.0	0.3
3	7803.0	7525.0	-278.0	30828.0	30732.0	-96.0
4	12922.0	13024.9	102.9	52604.0	52296.4	-307.6
5	24571.0	25200.3	629.3	74205.0	74548.5	343.5
	Femur	Averaged				
ID	Imin Pre	Imin Post	Change	Ipol Pre	Ipol Post	Change
1	32531.7	32215.4	-316.4	73336.5	73472.5	136.0
2	33608.1	33608.0	-0.1	80634.6	80634.5	-0.1
3	15384.5	15678.0	293.5	34360.0	35048.5	688.5
4	24945.0	25370.6	425.6	56693.0	56112.7	-580.3
5	30323.0	31408.9	1085.9	74341.5	76545.9	2204.4
	Tibia	Averaged				
ID	Imin Pre	Imin Post	Change	Ipol Pre	Ipol Post	Change
1	20381.0	20674.4	293.4	70153.2	71482.6	1329.4
2	26182.2	26179.5	-2.7	79161.8	79162.0	0.2
3	8307.0	8070.5	-236.5	32118.0	31973.0	-145.0
4	12931.0	12876.9	-54.1	52268.5	51013.2	-1255.3
5	23842.0	23952.8	110.8	70816.0	69575.2	-1240.8