DIAGNOSIS OF BONE LOSS AT THE DISTAL FEMUR AND PROXIMAL TIBIA IN PERSONS WITH SPINAL CORD INJURY: DEVELOPMENT OF A YOUNG-HEALTHY BONE MINERAL DENSITY REFERENCE DATASET

By

Christopher M. Cimigliaro, MS, CEP, CBDT

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Department of Rehabilitation and Movement Sciences
Rutgers, the State University of New Jersey
School of Health Professions

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ABSTRACT:

Objectives: Persons with traumatic spinal cord injury (SCI) have severe bone loss below the level of lesion with the distal femur (DF) and proximal tibia (PT) being the skeletal regions appreciated to have the highest risk of fracture. While a reference areal bone mineral density (aBMD) database is available at the total hip (TH) using the combined National Health and Nutrition Examination Survey (NHANES) III study and General Electric (GE) combined (GE/NHANES) to calculate T-score (T-score GE/NHANES), no such reference database exists for aBMD of the DF and PT. The primary objectives of this study were (1) to create a reference dataset of young-healthy able-bodied (YHAB) persons to calculate T-score (T-score YHAB) values at the DF and PT, (2) to explore the impact of time since injury (TSI) on relative bone loss in the DF and PT regions using the two computation models to determine T-score values, and (3) to determine agreement between T-score values for a cohort of persons with SCI using the T-score YHAB and T-score GE/NHANES reference datasets. Participants: A normative reference aBMD database at the DF and PT was collected in 32 male and 32 female Caucasian YHAB participants (n=64) and then applied to
calculate T-score values at the DF and PT in 105 SCI participants from a historical cohort. The SCI participants were then grouped based on TSI epochs (E-I: TSI < 1y, E-II: TSI 1-5y, E-III: TSI 6-10y, E-IV: TSI 11-20y, E-V: TSI > 20y).

**Main Outcome Measures:** The knee and hip aBMD values were obtained by dual energy X-ray absorptiometry (GE Lunar iDXA). **Results:** There were no significant differences in mean aBMD values across the four YHAB age subgroups at the TH, DF, and PT, and mean aBMD values were higher in men compared to the women at all skeletal regions of interest. Using the mean YHAB aBMD values to calculate T-score values at each TSI epoch for persons with SCI, T-score values decreased as a function of TSI and continued to decline for 11-20 years (E-IV epoch). Moderate kappa agreement was noted between the YHAB and the World Health Organization (WHO) reference datasets for the T-score cutoff criteria accepted to diagnose osteoporosis (e.g., SD < -2.5). **Conclusions:** A homogeneous reference dataset of YHAB aBMD values at the DF and PT was applied to calculate T-score values in persons with chronic SCI. There was a moderate level of agreement at the TH between the GE/NHANES and YHAB reference datasets when applying the conventional T-score cutoff value for the diagnosis of osteoporosis.
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CHAPTER I:
INTRODUCTION AND BACKGROUND

1.1: Overview of Bone Densitometry and Bone Loss in Persons with Spinal Cord Injury:

Histomorphometry of bone biopsies immediately after spinal cord injury (SCI) reveal an increase in osteoblast and osteoclast activity that shifts fairly quickly to an increase in osteoclastic activity and a suppression of osteoblastic activity (Chantraine, Nusgens, & Lapiere, 1986; Minaire et al., 1974). The uncoupling of the osteoblast/osteoclast relationship is supported by the clinical findings of hypercalciuria and dramatically elevated markers of bone resorption (Roberts et al., 1998). The depressed bone turnover leads to a rapid loss in bone mineral density (BMD) and deterioration of the trabecular lattice that is ultimately replaced with fatty marrow (Shields et al., 2006). Thus, contrary to that of normal skeletal remodeling in which the quantity of bone resorption is replaced by new bone formation, after SCI the immediate skeletal unloading result in a pathophysiological scenario of the uncoupling of bone formation and resorption that rapidly results in severe bone loss of the sublesional skeleton. This cascade of events results in the most precipitous bone loss
occurring during the first 12 to 24 months after acute SCI (Biering-Sorensen, Bohr, & Schaadt, 1990; Dauty, Perrouin Verbe, Maugars, Dubois, & Mathe, 2000; Wilmet, Ismail, Heilporn, Welraeds, & Bergmann, 1995). Compared to other well recognized conditions of rapid bone loss, this rate of bone loss after acute SCI is considerably higher than that observed in postmenopausal osteoporosis (3-5% annually) (Recker, Lappe, Davies, & Heaney, 2000), long term bed rest (0.1% per week) (Leblanc, Schneider, Evans, Engelbreton, & Krebs, 1990), and space flight (0.25% per week) (Vico et al., 2000). Several small cross-sectional and prospective reports have quantified the loss of bone in cohorts of persons with acute (<3-months from date of injury) (Frey-Rindova, de Bruin, Stussi, Dambacher, & Dietz, 2000) and chronic SCI (>1-year from date of injury) (de Bruin, Vanwanseele, Dambacher, Dietz, & Stussi, 2005). After acute SCI, a loss of BMD at the distal tibia has been reported to occur at a rate of approximately 4% per month in areas which are richly endowed in trabecular bone and as great as 2% loss per month in areas which are predominantly cortical bone. The reduction in BMD has been reported to approach a steady state for bone turnover approximately 3-8 years post injury, with the bone loss at the cortical diaphyseal sites due to endocortical wall thinning (Eser et al., 2004; Frotzler, Berger, Knecht, & Eser, 2008). Other investigations have observed a slower rate of bone loss that appears to continue into the chronic phase of injury (Bauman, Spungen,
Wang, Pierson, & Schwartz, 1999; Modlesky, Majumdar, Narasimhan, & Dudley, 2004).

The loss of structure and strength in the lower extremity places a person with SCI at an increased risk of fracture. Contrary to the occurrence of fractures in postmenopausal osteoporosis, a condition in which fractures predominantly occur at the femoral neck (FN), total hip (TH), lumbar spine, and distal ulnar/radius, in persons with chronic SCI, the epiphyses of the distal femur (DF) and proximal tibia (PT) remain the regions that are most vulnerable to fracture (Morse, Battaglino, et al., 2009). Even though extreme demineralization occurs throughout the entire lower extremity, the DF and PT epiphysis are the regions most vulnerable to fracture in persons with SCI; because a large fraction of accidents occur while sitting in a wheelchair, this makes the DF and PT region the first point of contact to an externally applied force. These fractures typically occur as a result of torsional stress on the bones of the lower extremity when transferring or when compressive forces occur from a low velocity fall from a wheelchair (Akhigbe et al., 2015; Carbone et al., 2013). There have been a paucity of studies looking at lower extremity fracture risk and the relationship to dual-energy X-ray absorptiometry (DXA) derived areal BMD (aBMD) criteria for osteoporosis and clinical risk factors in persons with SCI. The majority of these cross-sectional studies reported individuals with SCI and a diagnosis of prevalent fragility fractures (fracture discovered from
radiographic diagnosis that occurred anytime throughout the life span) and had significantly lower aBMD at the DF and PT compared to those with SCI who do not have a history of prevalent fracture (D. E. Garland, Adkins, Kushwaha, & Stewart, 2004; Lala et al., 2014; Lazo et al., 2001; Tan et al., 2014; Zehnder et al., 2004). There has been one retrospective study that investigated incident fracture (fracture discovered from radiographic diagnosis that occurred in a specific time interval) in veterans with SCI and found those with a history of incident fracture had significantly lower TH T-score values compared to those individuals with no fracture but the DF and PT regions were not evaluated (Abderhalden et al., 2017). In approximately 50% of all cases, fractures result in contractures at the hip and knee, osteomyelitis, and concurrent pressure ulcers that may further serve to diminish mobility (Carbone et al., 2013; Ingram, Suman, & Freeman, 1989; Ragnarsson & Sell, 1981). These outcomes adversely affect activities of daily living, interfere with the ability to maintain employment, and add significant medical costs to rehabilitative care.

There are currently several different types of densitometers available to quantify BMD in persons with SCI. DXA, quantitative computed tomography (QCT), and peripheral quantitative computed tomography (pQCT) are the more commonly used imaging technologies to quantify BMD of the DF and PT epiphysis and metaphysis, with the latter being used more frequently in the research setting (Khoo et al., 2009; N. Li et al., 2013).
The rate of absolute bone loss has been reported to be highly variable in persons with SCI when applying DXA to capture aBMD as well as QCT and pQCT to capture vBMD (Dauty et al., 2000; del Puente et al., 1996). This observed variability is partly the result of acquiring BMD with distinct technologies employed by densitometer manufacturers, unique software applications, and analysis techniques that acquire similar, but not identical, regions of interest (ROI) of the lower extremity, with the latter resulting in the acquisition of trabecular to cortical skeletal compartments of different proportions. To diagnose osteoporosis in post-menopausal able-bodied (AB) women and men over 50 years of age, fracture risk is expressed in terms of a standard deviation score using a young-healthy AB individuals aBMD as a reference, otherwise known as T-score (Schousboe, Shepherd, Bilezikian, & Baim, 2013). While reference aBMD databases and calculation of T-score is available to understand risk of fracture at the FN and TH regions, no such reference database exists for the DF and proximal tibia PT.

1.2: Significance of the Study:

DXA derived absolute aBMD values cannot be directly utilized to diagnose osteoporosis because these values vary in normal individuals by anatomical region and because different company-specific calibration standards are used by DXA manufacturers to measure aBMD. Thus, to
standardize results across multiple skeletal regions and between DXA manufacturers, a computed statistical value, T-score, was introduced that permitted comparison between the aBMD of a given individual against a reference aBMD in young-healthy adults. The International Society for Clinical Densitometry (ISCD) Task Force on Normative Databases and the WHO diagnosis of osteoporosis established a T-score \( \leq -2.5 \) standard deviations from the mean as the cutoff value at the femoral neck and total hip regions in post-menopausal women and in men \( \geq 50 \) years old (Schousboe et al., 2013; Watts, Leslie, Foldes, & Miller, 2013). The National Health and Nutrition Examination Survey (NHANES) III collected BMD data in young, healthy adults 20-30 years old between 1988 and 1994 that is used as the reference standard to calculate T-scores (Looker et al., 1998). To account for machine calibration differences for imaging centers that utilize GE Lunar densitometers, the manufacturer applied validated conversion equations to the NHANES III data (obtained by Hologic densitometers) and created a GE Lunar equivalent aBMD mean and SD for the TH for men, yielding the combined GE/NHANES reference aBMD dataset at the TH (Binkley et al., 2005; Watts et al., 2013).

In the diagnosis of primary osteoporosis in AB individuals, the T-score has historically been used as a surrogate for fracture risk from epidemiologic studies established by the World Health Organization (WHO) and further supported with data from the NHANES prospective cohort study, which
demonstrated that approximately 1/3rd of postmenopausal women and men over 50 with T-scores below -2.5 SD will experience an incident fracture. The current state of knowledge in predicting fracture risk in primary osteoporosis has extended beyond the use of T-score with the incorporation of clinical risk factors and QCT bone densitometry variables. Contrary to the advances in diagnosing fracture risk in the AB population, bone densitometry in persons with SCI still lacks normative data at the DF and PT regions around the knee that are most prone to fracture. To accurately define the fracture threshold in persons with SCI, prospective cohort studies are needed to validate the T-score value at the DF and PT as soon as possible after the occurrence of incident fracture. Until this work can be completed, an adequately powered reference dataset of mean aBMD values at the DF and PT is necessary to determine T-scores. This contribution to the bone densitometry literature is necessary for future research and would allow clinicians to improve their knowledge of relative fracture risk in persons with SCI.

The introduction of advanced rehabilitation methodologies (i.e. powered exoskeleton-assisted walking (EAW), locomotor training, and standing neuromuscular electrical stimulation) for upright posture and ambulation for use in persons with SCI has made it essential to identify candidates who may be at increased risk for fracture if placed in these activities. Recommendations are needed to identify appropriate
candidates for these interventions because bone deteriorates dramatically below the level of the lesion and fragility fractures of the long bones of the lower extremities occur with increased frequency with longer duration of SCI. Several clinical investigations have been applying fracture threshold values from cross-sectional studies that identified the BMD in small samples of persons with chronic SCI who sustained fragility fractures. These cutoff values to identify the fracture threshold vary considerably, with fractures occurring, not infrequently, above the reported mean aBMD (DF and PT) and T-scores (TH and FN) cutoff values (D. E. Garland, Adkins, Kushwaha, et al., 2004; D. E. A. Garland, H; Stewart, C.H., 2005; Lala et al., 2014). The Department of Veterans Affairs Cooperative Studies Program (NCT02658656) has an ongoing nationwide clinical trial that has the following skeletal exclusion criteria: (1) lower extremity fracture within the past 2 years, (2) DF or PT aBMD <0.6 gm/cm², and (3) TH and FN T-score < 3.5 SD. The exclusion criterion for aBMD at the knee in this multicenter study was based largely on work by Garland et al. in which fractures of the lower extremities were observed to occur more frequently when the aBMD at the DF and PT region was below a value of 0.6 gm/cm² (D. E. Garland, Adkins, Kushwaha, et al., 2004; D. E. A. Garland, H; Stewart, C.H., 2005). The exclusion criterion for the cutoff value for T-score at the TH was determined by the nominal group method from a consensus of experts as to what the predicted hip T-score value would be when the cutoff value at the knee
was $<0.6 \text{ gm/cm}^2$. In the trials in persons with SCI completed to date, the absolute cutoff value for aBMD has utility in preventing potential subjects at a high risk for fracture from participation in upright rehabilitation activities, however, a conservative cutoff value for aBMD may also deny entry to advanced rehabilitation trials that could be of benefit. As with all diagnostic tests, the application of bone densitometry to acquire for the DF and PT values will permit one the ability to discern improvements in aBMD of clinical relevance due to an applied rehabilitation modality. Establishing T-score values at the DF and PT would provide clinicians and researchers the ability to quantitate the optimum therapeutic effect of different interventions, a consideration that is especially relevant in the current healthcare environment in which third-party reimbursement is increasingly difficult to justify prescription of various costly therapeutic interventions for upright activities.

The results of the retrospective pilot data analysis reveals that despite the ability to moderately predict T-scores at the TH and FN from aBMD values at the DF and PT using statistical constructs, there still exists a degree of uncertainty for a given individual, especially if the hip T-scores are $<-2.0$ (retrospective pilot data analysis- specific aim I). These findings highlight the importance of direct measurement of the DF and PT by DXA and the need for reference data, as discussed previously. Furthermore, while the cutoff values at the knee of $<0.6 \text{ gm/cm}^2$ are the best absolute
cutoff values for fracture available at the present time, normative reference data at the DF and PT to calculate T-score would be ideal to control for differences in aBMD calculations between densitometer manufacturers and would allow expression of fracture risk to that of young, healthy AB individuals. Standardized scores would allow clinicians to add greater relevance to aBMD values of the DF and PT and, using generally accepted threshold values, to diagnose osteoporosis in persons with SCI. The relationship between bone loss and time since injury (TSI) confirms prior work examining this association, with additional novel and clinically relevant observations noted from this exploratory analysis. Despite a similar mean participant age, a significant decline in DF and PT mean aBMD and was observed after the first decade of injury, with a continued demineralization 1-20 years after SCI at all hip and knee regions, and a steady state aBMD (aBMDss) that was reached into the second decade after SCI (retrospective pilot data analysis- specific aim II). Furthermore, compared to the AB reference group, the aBMDss in the SCI group was 42% lower at the DF, 49.6% lower at the PT, 40% lower at the FN, and 30% lower at the TH (Cimigliaro et al., 2019). These findings of continued demineralization into the second decade after SCI were somewhat novel findings since several studies have observed a steady state bone loss 2-5 years after SCI (Edwards, Simonian, Troy, & Schnitzer, 2015; Eser et al., 2004; Szollar, Martin, Sartoris, Parthemore, & Deftos, 1998).
The aBMD and epochs of TSI analysis provides the theoretical framework for the proposed dissertation work to determine the relationship between T-score at the DF and PT and TSI. To accomplish this task, prospective data collection will provide normative reference data at the DF and PT in a young-healthy AB cohort to permit the calculation of T-score values at the DF and PT in the retrospective SCI cohort. This will allow the investigators to determine how T-score values at the DF and PT decrease as a function of TSI compared to the T-score values at the FN and TH regions (prospective data collection- specific aim III).

1.3: Research Questions, Specific Aims, and Hypotheses:

Research Questions:

Retrospective Pilot Data Analysis: In a retrospective analysis of aBMD data in a cohort of SCI participants, the following questions have been addressed:

I. What is the relationship between T-score at the FN and TH compared to aBMD values at the DF and PT regions?

II. How do aBMD values decrease as a function of TSI at the FN and TH compared to the DF and PT regions?

III. Prospective Data Collection: The generation of a prospective young-healthy able-bodied (YHAB) normative aBMD dataset at the TH, DF, and PT addressed the following questions:
a) Are the YHAB reference dataset mean aBMD values significantly different at the TH, DF, and PT between the age subgroups for both men and women?

b) Do the YHAB T-score ($T_{scoreYHAB}$) values at the TH, DF, and PT decrease as a function of TSI in the SCI historical dataset, and do these values differ when compared to the TH GE/NHANES T-score ($T_{scoreGE/NHANES}$) values for every TSI epoch?

c) What is the agreement between 2 T-score cutoff values derived from the T-score$_{GE/NHANES}$ reference dataset at the TH (one T-score cutoff value accepted for the diagnosis of osteoporosis and the other one applied as an exclusion criterion for EAW training) and the $T_{scoreYHAB}$ values derived from the reference dataset at the DF, PT, and TH?

**Primary Specific Aims:**

**Retrospective Pilot Data Analysis:**

**Specific Aim I:** To determine if GE/NHANES T-score values at the FN and TH can be used to estimate aBMD values at the DF and PT.

**Hypothesis I:** Established T-score cutoffs for bone demineralization at the FN and TH region are not precise enough to estimate demineralization at the DF and PT in the retrospective cohort of SCI participants.
Specific Aim II: To determine if aBMD values decrease as a function of TSI at the DF and PT compared to the demineralization at the FN and TH regions.

Hypothesis II: Measures of bone demineralization at the DF and PT differ from measures of bone demineralization at the FN and TH, and these differences increase the further out from TSI.

Prospective Data Collection:

Specific Aim III:

a. To determine if the YHAB reference dataset mean aBMD values at the TH, DF, and PT are significantly different between the age subgroups for both men and women;
b. To calculate T-scoreYHAB values at the TH, DF, and PT as a function of TSI in the SCI historical dataset, and to determine if these values differ when compared to the TH T-scoreGE/NHANES values for every TSI epoch; and
c. To determine the diagnostic agreement between two T-score cutoff values derived from the T-scoreGE/NHANES reference dataset at the TH (one T-score cutoff value accepted for the diagnosis of osteoporosis and the other one applied as an exclusion criterion for EAW training) and the T-scoreYHAB values derived from the reference dataset at the TH, DF, and PT.

Hypothesis III:
a. The YHAB aBMD values at the TH, DF, and PT will be similar between the men’s and women’s age subgroups in the YHAB reference dataset;

b. Using the YHAB reference dataset to calculate T-score at the TH, DF, and PT in the historical SCI cohort, T-score values will decrease as a function of TSI. Furthermore, the T-scoreYHAB values at the TH, DF, and PT will be similar to the manufacturer derived GE/NHANES T-scoreGE/NHANES values at the TH for every TSI epoch; and

c. There will be a similar diagnostic agreement for two T-score cutoff values derived from the T-scoreGE/NHANES reference dataset at the TH and the T-scoreYHAB values derived from the reference dataset at the TH, DF, and PT.

1.4: Proposed Manuscript Titles:

**Study Title (retrospective database review- published):** Relationships between T-scores at the Hip and Bone Mineral Density at the Distal Femur and Proximal Tibia in Persons with Spinal Cord Injury

**Overview:** The primary purpose of this retrospective database review was to identify optimum T-score values at the TH and FN that correspond to the aBMD cutoff value (0.60 g/cm²) commonly used to evaluate individuals at heightened risk of fracture at the DF and
The findings from this analysis revealed that despite the ability to predict T-scores at the TH and FN from aBMD values at the DF and PT using statistical constructs, there still exists a degree of uncertainty for a given individual to be able to predict knee aBMD values, especially if the hip T-scores are <-2.0. This work provided empirical data, and thus clarifies what aBMD values at the DF and PT relate to T-score values at the TH and FN. Overall, these findings strongly support the need to diagnose osteoporosis and fracture risk by acquiring aBMD of the DF and PT in persons with SCI. Furthermore, while the cutoff values at the knee of <0.6 gm/cm² are the best cutoff values for fracture available at this time, normative reference data at the DF and PT to calculate T-score is ideal to control for differences in aBMD calculations between densitometer manufacturers and would allow expression of fracture risk in terms of differences to young-healthy AB individuals. These observations have been accepted for publication in the Journal of Spinal Cord Medicine (accepted for publication June 2019).

**Study Title (retrospective database review- published):** Progressive Sublesional Bone Loss Extends into the Second Decade after Spinal Cord Injury

**Overview:** The primary purpose of this retrospective database review was to determine if aBMD loss continues into the second
decade after SCI by comparing subgroups stratified by epochs of time since injury and when fitting the aBMD to an exponential decay curve as a function of TSI. The findings from this analysis revealed that when groups were stratified and compared as epochs of TSI, significantly lower mean aBMD and T-scores were observed for the advancing epochs. Furthermore, the time to reach an aBMD steady state (aBMDss) after SCI for at the DF, PT, FN, and TH was 14.6, 11.3, 14, and 6.2 years, respectively. Furthermore, compared to a small AB reference group (n=17), the aBMDss in the SCI group was 42% lower at the DF, 49.6% lower at the PT, 40% lower at the FN, and 30% lower at the TH. This continued demineralization into the second decade after SCI were somewhat novel findings as many studies have observed a steady state bone loss 2-5 years after SCI. This manuscript was accepted for publication October 2018 in the Journal of Clinical Densitometry (Cimigliaro et al., 2019).

**Study Title (prospective study):** Generation of a Reference Dataset to Permit the Calculation of T-scores at the Distal Femur and Proximal Tibia in Persons with Spinal Cord Injury.

**Overview:**

Using a GE densitometer, a homogeneous YHAB reference database of aBMD values was obtained to calculate T-score values from aBMD values in a historical cohort of SCI individuals to achieve
the following objectives to determine if the YHAB reference dataset mean aBMD values at the TH, DF, and PT are significantly different between the age subgroups for both men and women. In addition, T-scoreYHAB values were calculated at the TH, DF, and PT as a function of TSI in the SCI historical dataset and then compared to the TH T-scoreGE/NHANES values for every TSI epoch. These YHAB T-score (T-scoreYHAB) values at the TH, DF, and PT were then compared to the manufacturer derived GE/NHANES T-score (T-scoreGE/NHANES) values at the TH. Finally, diagnostic agreement was determined for two T-score cutoff values derived from the T-scoreGE/NHANES reference dataset at the TH (one T-score cutoff value accepted for the diagnosis of osteoporosis and the other one applied as an exclusion criterion for EAW training) and the T-scoreYHAB values derived from the reference dataset at the TH, DF, and PT. Several post-hoc analyses will also be presented comparing the mean aBMD values at the DF and PT across the 4 age groups in the AB normative reference dataset (21-25, 26-30, 31-35, and 36-40 years of age.) and between male and female participants. It is anticipated that the observations made from in this manuscript will be submitted for publication in a peer-reviewed journal such as Osteoporosis International, Journal of Clinical Densitometry, or Archives of Physical Medicine and Rehabilitation.
CHAPTER II:

THEORY AND LITERATURE

2.1: Introduction:

To provide a summary of the current literature of the measurement and diagnosis of bone loss in persons with SCI, a literature review was conducted in PubMed (MEDLINE), Cochrane, and CINAHL databases with the following bone loss-related keywords and Boolean operators: “Bone Mineral Density” [MESH] OR “Bone Mineral Content” AND “DXA” OR “Dual Energy X-ray Absorptiometry” AND “Tomography Scanners, X-ray Computed” [MESH] OR “QCT” OR “Quantitative Computed Tomography” OR “Peripheral Quantitative Computed Tomography” OR “pQCT” AND “Spinal Cord Injury” AND “Distal Femur” AND “Proximal Tibia” AND “Femur” and “Tibia”. A secondary search of references from articles found in primary search and an electronic search of the Journal of Clinical Densitometry (all issues since the journal’s inception) on the International Society for Clinical Densitometry website (www.iscd.org). From this search, more than 100 articles were identified for inclusion in this dissertation literature review and published in the journal Osteoporosis International (Cimigliaro et al., 2017). For the purposes of this report, only articles that
assessed the DF and PT as the primary region of bone loss were used in this topical review. Using these criteria, the author incorporated only this information when summarizing the severity of bone loss that occurs at the DF and PT in persons with SCI. To further limit this review, additional criteria used to exclude articles were as follows: DXA studies that measured the knee region using a custom ROI from a total body scan, studies performed in the pediatric SCI population, descriptive case reports and the baseline evaluations from pharmacological and mechanical interventions in persons with SCI. From this compilation of articles describing bone loss at the DF and PT, in cross-sectional and prospective investigations, a synthesis of all topics related to the measurement and diagnosis of bone loss at the DF and PT in acute and chronic SCI is provided.

In clinical investigations, DXA has been used as the primary imaging modality employed to acquire lower extremity BMD in persons with SCI. In one of the early cross-sectional studies of participants with SCI, Biering-Sörensen et al. (Biering-Sörensen, Bohr, & Schaad, 1988) compared bone mineral content (BMC) of the PT in 26 SCI subjects 2 to 25 years after acute injury and observed that the PT was greater than 50% lower than the values obtained in an able-bodied cohort. In agreement with these findings, Garland et al. (D. E. A. Garland, H; Stewart, C.H., 2005) compared the combined aBMD of the DF and PT in 28 chronic SCI participants (T1= 3-43 years) to 10 able-bodied controls; the mean aBMD
of the knee region was 50% lower when compared to an age-matched able-bodied control group, with similar findings documented in a cross-sectional study of women with chronic SCI (D. E. Garland, Adkins, Stewart, Ashford, & Vigil, 2001). In another cross-sectional study of 31 patients with chronic SCI and 31 healthy able-bodied control subjects, Dauty et al. (Dauty et al., 2000) documented demineralization of 52% at the DF and 70% at the PT. These cross-sectional findings support those of other investigators who compared lower extremity aBMD values in those with SCI to healthy able-bodied controls (Shields et al., 2005), and in a subgroup of persons with SCI who had a history of fragility fracture (Lala et al., 2014). The major limitation of these cross-sectional studies is in the inability to determine the appropriateness of comparison to the reference group and the inherent inability to know peak bone mass of the SCI group prior to paralysis.

Longitudinal studies using DXA have documented a loss in aBMD over time in acute and chronic SCI populations. To evaluate loss of aBMD shortly after SCI, Warden et al. (Warden et al., 2002) performed a longitudinal study in 15 SCI patients who had sublesional bone loss observed within 6 months of SCI and observed a rapid decrease in aBMD of 5.3% in the PT six weeks after the baseline measurement. In another study documenting the rapid loss of bone that occurs within the first two years after initial motor-complete SCI, Biering-Sorensen et al. (Biering-
performed follow-up DXA scans on 6 men and 2 women with initial scans performed 9-167 days (median 43) after acute paralysis; two years after the initial DXA evaluation, BMC at the PT was 40-50% lower than it was at the baseline assessment. In a study documenting bone loss within the first 2 years after injury, Garland et al. (D. E. Garland, Adkins, Scott, et al., 2004), determined aBMD of the DF and PT soon after injury (33.5 ± 10.8 days) with follow-up measurements at 523 ± 96.2 days post injury in 5 patients with acute motor-complete SCI; results from the follow-up assessment revealed aBMD at the DF and PT were reduced by 27% and 32%, respectively. In a study of 31 individuals with chronic motor-complete SCI (duration of injury 14.6 ± 8.7 years), Garland and colleagues (D. E. Garland, Adkins, & Stewart, 2008) observed an annual loss in aBMD of 1.1% at the DF and 1.5% at the PT over a 5-year period, demonstrating the continued loss of bone several years after acute immobilization. Because the majority of reports that documented bone loss in persons after SCI have relatively small sample sizes, and participants had varying degree of motor function below the level of lesion, the rate and absolute loss in aBMD has varied considerably among studies to date. Despite the varying degrees of bone loss reported among these reports, it is essential for clinicians to understand the precipitous loss in the sublesional skeleton that results from the abrupt onset of paralysis, as well as the likely accelerated continued loss over a lifetime of immobilization, with the
longevity of those with SCI approaching that of the able-bodied population. Thus, there exists the obvious need for safe and efficacious prescription of rehabilitative or pharmacological interventions once they become available to preserve or reverse osteoporosis in the SCI population.

### 2.2: Overview of Knee Anatomy:

A brief review of knee anatomy is warranted as it pertains to the DXA imaging of the knee and the ROI used to obtain the aBMD at the DF and PT. The DF and PT are the regions constituting approximately 30% of the distal region of the femur bone length and 30% of the proximal region of the tibia bone length, with this 30% area further stratified into the epiphyseal (0-10%), metaphyseal (11-20%), and diaphyseal (>20%) subregions (Edwards, Schnitzer, & Troy, 2014a). These regions were validated by CT scan as primarily trabecular bone [epiphyseal (0-10%)], combination of trabecular and cortical bone [metaphyseal (11-20%)], and primarily cortical bone [diaphyseal (>20%)] (Appendix A, Figure 1).

The most prominent features of the distal femur include the medial and lateral condyles and epicondyles, and the articular capsule (Appendix A, Figure 2A). The lateral and medial condyles articulate with the tibia while the lateral and medial epicondyles are essential for muscle attachment. Below the distal femur is a sesamoid bone known as the patella, that
serves to increase the leverage of the quadriceps femoris and protects the front of the knee joint (Appendix A, Figure 2B). The most prominent features of the tibia include the lateral and medial condyles and the intercondylar eminence (Appendix A, Figure 2C). The most proximal end of the tibia portrays the medial and lateral condyles with the surfaces of their superior aspect articulating with the matching femoral condyles. Positioned between the 2 tibial condyles is a protuberance known as the inter-condylar eminence. The fibula is positioned on the lateral side of the tibia and is the smaller of the 2 bones. At the time of a low-impact fragility fracture of the PT the fibula can also be fractured but the aBMD of this region is not of concern at this time since the tibia is the primary weight bearing (approximately 80% of body weight) long bone during upright and ambulatory activities and this region is difficult to reliably measure by DXA. To prevent the confounding effect of the patella when placing the ROI box to capture the aBMD of the DF measurement, the ROI box is positioned above the patella and epicondyles. To prevent the confounding effect of the fibula when placing the ROI box to capture the aBMD of the PT measurement, the ROI box is positioned below the upper most point of contact of the tibia and fibula. This methodology is preferred by the investigators to capture aBMD of the DF and PT since these ROI boxes are not confounded by extraneous boney regions and
the ROI box is located within the metaphyseal region and consists of a combination of trabecular and cortical bone (Shields et al., 2005).

2.3: General Overview of Bone Turnover and Metabolism:

Prior to comparing normal bone metabolism to the sequelae of events leading to metabolic bone disease in persons with SCI, a basic overview on the biology of bone tissue and remodeling will be provided. Bone tissue is in a state of constant remodeling which is a highly complex process that is necessary for skeletal adaptation from external loading as well as fracture healing. Through the coordination of 4 different types of cells: osteocytes, osteoclasts, osteoblasts, and bone lining cells; a basic multicellular unit (BMU) is an anatomical structure necessary to maintain the mineralized connective tissue within the bone matrix during the bone remodeling cycle. Bone lining cells cover the bone surfaces where bone resorption and formation does not occur with the exact role of these cells not completely elucidated. These cells are believed to have a role in preventing direct contact between the osteoclast and the bone matrix when bone resorption should not take place as well as having a role in osteoclast differentiation. Surrounded by a calcified matrix, osteocytes make up approximately 90% of total bone cell volume and have a life span of up to 25 years (Seeman, 2013 American Society for Bone and Mineral Research). Derived from mesenchymal stem cell through
osteoblast differentiation, osteocytes cell bodies are located within the bone lacunae with cytoplasmic processes that connect to neighboring osteocytes creating a cell-cell interconnected network known as the lacuna-canalicular system. The osteocytes within this system acts as mechanoreceptors and play a major role in detecting mechanical forces on the skeleton thereby regulating the bone remodeling process by orchestrating osteoclast and osteoblast activity. The state of constant remodeling requires resorption of bone by osteoclasts and formation of new bone by osteoblasts. Thus, to initiate this remodeling process requires a maturation and proliferation of osteoclasts (Florence-Silva, Sasso, Sasso-Cerri, Simoes, & Cerri, 2015). Osteoclasts arise from the monocytic cell lineage and are stimulated by release of receptor activator of nuclear factor-κβ-ligand (RANKL), with RANKL produced by osteocytes after they become encased in bone. Cells from the osteoblast lineage also release an inhibitor of RANKL known as osteoprotegerin (OPG), which has been found to increase bone density by decreasing bone resorption. In summary, RANKL drives bone resorption and stimulates osteoclastogenesis after binding RANKL to its receptor RANK, present in the membrane of osteoclast precursors, while OPG, released by osteoblasts, acts as a “decoy” receptor down regulating osteoclastic activity and function. Osteoblasts are derived from mesenchymal stem cells that have morphological characteristics of protein synthesizing cells that are well
recognized for their role in bone formation (Jiang, Jiang, & Dai, 2006). Primarily synthesized by osteocytes and essential for osteoblastogenesis is the Wingless (Wnt)/β-catenin signaling pathway and its antagonists, sclerostin and Dickkopf (Dkk-1). The Wnt signaling pathway when coupled with mechanical loading stimulate the osteoblast to secrete collagenous and noncollagenous proteins such as osteocalcin, osteonectin, and bone morphogenic proteins that are responsible for mineralization of the bone matrix (Florencio-Silva et al., 2015). The culmination of all these factors allow bone remodeling to occur when elastic deformation of the skeleton is provoked by habitual physical activity that creates bending and torsional forces on the bone. The Mechanostat theory, a derivative of Wolff’s law, states if strains within bone that are kept within certain limits by adding and removing bone tissue, resulting in improved bone strength according to the particular forces that are imposed. However, if force is applied below a certain set point, bone tissue will ultimately be lost (Frost, 1987). This law is clearly dominant when studying SCI as a model of immobilization osteoporosis secondary to paralysis.

2.4: Pathophysiology of Bone Loss after SCI:

At the time of SCI, the process of bone loss is initiated primarily from unloading of the skeleton, with the magnitude of bone loss in proportion
to the time and degree to which the forces of ambulation are interrupted. Suppression of osteoblastic activity is present for the first few months after SCI and returning to pre-injury levels with continued osteoclastic activity and bone resorption into the chronic phase of SCI (Szollar et al., 1998; Zehnder et al., 2004). This uncoupling of the osteoblast/osteoclast relationship is supported by the clinical findings of hypercalcemia and hypercalciuria and dramatically elevated markers of bone resorption (Roberts et al., 1998). Within the first 2-3 years after SCI, this depressed bone turnover leads to a rapid loss in BMD within the first 2 years after SCI and deterioration of the trabecular lattice into the chronic phase of injury that is ultimately replaced with fatty marrow (Shields et al., 2006). In addition to demineralization of the bone matrix increasing fracture risk, age related changes in the bone matrix proteins and the matrix structure can ultimately modify bone geometry to compensate for the decrease in bone mineral density (Bouxsein & Seeman, 2009). These changes in bone geometry can lead to an increase in endosteal resorption (decreased cortical thickness) with a corresponding increase in bone diameter to maintain resistance to bending and torsional forces. The thinning of the endocortical envelope with a corresponding increase in the periosteum in an attempt to preserve total bone strength, is a finding from several high resolution pQCT studies in persons with chronic SCI (Eser et al., 2004;
Rittweger, Goosey-Tolfrey, Cointry, & Ferretti, 2010), a bone state that is analogous to that seen in senile osteoporosis.

As a result of the invasiveness and difficulty of performing bone histomorphometry studies to measure bone formation and resorption in humans, more sensitive and specific biomarkers of bone resorption and formation have been developed, with several studies performed in persons with SCI. Osteocalcin is a non-collagenous protein produced by osteoblasts and exists in the extracellular bone matrix and the serum of blood so it can used clinically as a metric of bone formation (Seeman, 2013 American Society for Bone and Mineral Research). In an early cross-sectional report by Pietschmann et al. (Pietschmann et al., 1992), osteocalcin (OC) levels, were in the low normal range in 41 SCI patients one month after injury that increased to a peak value 7 months after SCI. In addition to OC, N-terminal propeptide of type I procollagen (P1NP), currently preferred marker of bone formation as it is specific of type-I collagen deposition, has been found to follow the same trend as osteocalcin where a transient depression is followed by elevation back to the normal range (Uebelhart et al., 1994). Contrary to markers of bone formation that demonstrate a temporary and mild suppression soon after SCI, C-terminal cross-linking telopeptide of type I collagen (CTX-1) and N-terminal cross-linking telopeptide of type I collagen (NTX-1) are biomarkers of bone resorption. The CTX and NTX tests measure for the presence and
concentration of a cross-link peptide sequence of type I collagen and is found in high concentrations in bone tissue. These collagen derivatives are connected to bone remodeling as they are components cleaved off by osteoclasts during bone resorption (Seeman, 2013 American Society for Bone and Mineral Research).

These biomarkers are dramatically elevated outside of the normal range after SCI and remain elevated in a majority of patients into the chronic phase of SCI (Roberts et al., 1998; Zehnder et al., 2004). In a more recent investigation, Kostovski et al. (Kostovski, Hjeltnes, Eriksen, Kolset, & Iversen, 2015), assessed changes in bone metabolism soon after injury in a cohort of motor-incomplete (ambulatory) and -complete (non-ambulatory) SCI. Compared to the motor complete SCI group, the authors found the group with motor-incomplete injuries demonstrated significantly lower serum P1NP and CTX levels 3 and 12 months after the initial assessment. Studies to date have compared biomarker concentrations to the clinical normal reference range with no studies comparing these biomarkers values prospectively to an age-matched AB cohort. Thus, findings that the degree of immobilization influences systemic markers of bone resorption and formation supports SCI as the source of abnormal bone metabolism.

Several studies have also demonstrated the change in the RANKL/OPG system and Wnt signaling pathway after SCI. Work performed in a rodent
SCI model to suggest that there is a several-fold increase in RANKL expression within 56 days of acute SCI, while the expression of OPG is unchanged, making the ratio of RANKL to OPG highly unfavorable acutely after SCI. In this animal model, there is an approximate 2-fold increase in osteoclast differentiation markers; in contrast, osteoblast differentiation markers are markedly depressed (Qin et al., 2013). The few human studies that have studied the RANKL/OPG system have found that after adjusting for age, compared to the ambulatory group OPG concentrations were significantly lower in those with cervical non-ambulatory SCI, compared, supporting this unfavorable relationship in humans with SCI as lower OPG concentrations would promote bone resorption and lower BMD values over time (Morse et al., 2008).

Supporting the findings of an adverse RANKL/OPG environment, in a prospective observational study performed by Gifre et al. (Gifre et al., 2017), the effect of recent SCI (<6 months from SCI) on serum levels of RANKL and OPG and the evolution on BMD on 23 patients with SCI and 27 healthy AB controls was performed. Compared to the AB control group, the authors found that serum levels of RANKL were significantly higher in the SCI group at baseline with additional increases at the 6 month follow up assessment. In addition to evidence of an unfavorable RANKL/OPG ratio causing increased bone resorption in persons with SCI, the effect of mechanical unloading on the Wnt/β-catenin signaling pathway as a
result of SCI has also been a topic of recent reports in human studies. In a study by Morse et al. (Morse et al., 2013), Sclerostin levels were found to be significantly lower in persons with chronic SCI (n=39) when compared to an age adjusted control group (n=10) and was the only circulating biomarker of bone resorption and formation significantly correlated to leg bone mineral content and BMD of the DF. The lower sclerostin levels were representative of maximal unloading and bone loss in the chronic SCI cohort and support the viability of sclerostin as a biomarker to diagnose bone loss in persons with SCI when more advanced densitometric methods are not available. This finding is supported by other studies that show sclerostin levels are greatest in subjects with short-term SCI (≤5 years) and decrease significantly over the first 5 years post-injury as a result of the dramatic effect of mechanical unloading on sclerostin levels, an elevation that is sustained for as long as 5 years after injury (Battaglino et al., 2012). In another study by Gifre and colleagues (Gifre et al., 2015), the authors compared Wnt/β-catenin signaling antagonists, sclerostin and Dkk-1, in a subacute SCI cohort (n=42) to an age matched control group (n=10), with additional follow up 6 and 12 months after the initial assessment in the SCI group. Contrary to these findings of lower sclerostin levels in the chronic SCI cohort reported by Morse et al. (Morse et al., 2013), in this subacute SCI group sclerostin levels were similar to the AB controls but the Dkk-1 levels were significantly higher. Furthermore, while
sclerostin levels were relatively unchanged at the 6 and 12 months after the initial assessment, concentrations of the antagonist Dkk-1 increased significantly a few months after SCI and remained elevated over the course of the study. The reason for the differences between the two antagonists cannot be completely understood at this time. In summary, contrary to that of normal skeletal remodeling in which the quantity of bone resorption is replaced by new bone formation, after SCI the immediate skeletal unloading result in a pathophysiological scenario of the uncoupling of bone formation and resorption that rapidly results in severe bone loss of the sublesional skeleton.

During the acute SCI phase, where skeletal resorption is the greatest (calcium release from bone), serum ionized calcium levels are elevated with an increase in renal clearance of calcium (hypercalciuria and high risk of renal stones). The hypercalciuria observed during the acute phase of injury begins within days of SCI and may stay elevated for several months after injury and returning to baseline levels within a year (Stewart, Adler, Byers, Segre, & Broadus, 1982). This model of hypercalciuria is two to four times greater than that observed in non-SCI subjects during long-term bed rest (Leblanc et al., 1990), and corresponds to SCI having the most rapid rate of BMD loss when compared to post-menopausal osteoporosis (Recker et al., 2000) and space flight (Vico et al., 2000). As a result of elevated ionized and serum calcium levels there is a suppression
of parathyroid hormone (PTH) release and reduced conversion of 25 (OH) vitamin D (25OHD) to the more biologically active 1,25-dihydroxyvitamin D (1,25(OH)₂D), ultimately reducing intestinal calcium absorption (Heaney, 1962). During this acute period calcium intake is often restricted by health care professionals due to the erroneous belief that increased calcium intake will increase the risk of renal stones. This may lead patients to avoid calcium rich dairy products that are also fortified with vitamin D, further exacerbating the inevitable bone loss due to immobilization and establishing vitamin D deficiency soon after SCI. In chronic SCI, as in the general population, secretion of PTH and the increase of circulating 1,25(OH)₂D are subject to control by negative feedback mechanisms related to the level of serum calcium, which is, in tum, influenced by 25(OH)D levels (Florencio-Silva et al., 2015). The disordered calcium metabolism in persons with SCI has been well documented. In a study by Bauman et al. (Bauman, Zhong, & Schwartz, 1995), 32 out of 100 chronically injured SCI participants compared with eight of 50 control participants had 25(OH)D levels below the lower limit reference range. Furthermore, forty percent of those with SCI compared with 20 percent of controls had elevated values for serum 1,25(OH)₂D, a significant difference between the groups. The SCI participants with absolute elevations in serum PTH levels had significantly lower serum 25(OH)D levels and significantly higher 1,25(OH)₂D and alkaline phosphatase levels.
Furthermore, the elevated PTH level in the SCI group was positively correlated with 1,25(OH)2D and negatively correlated with 25(OH)D. This relationship reflects an augmented PTH effect upon the 1 alpha-hydroxylase activity (responsible for conversion of 25(OH)D to biologically active 1,25(OH)2D) in the renal tubular cell. These higher levels of PTH would be expected to accelerate bone resorption of a skeleton already regionally osteoporotic as a consequence of the bone mineral loss due to acute immobilization.

There are several local and systemic factors that influence the complex actions of bone cells within the BMU to continuously remodel bone. The local factors consist of paracrine and autocrine molecules such as cytokines, prostaglandins and growth factors. The role of OPG, a cytokine receptor of the tumor necrosis factor (TNF) receptor superfamily, and RANKL, a TNF-related cytokine necessary for osteoclastogenesis and osteoclastic function, have been studied in persons with SCI and were reviewed in earlier sections of this report. The role of prostaglandins such as bone morphogenic proteins and bone growth factors have not been thoroughly studied in humans with SCI and are beyond the scope of this discussion. Additional systemic factors that are important to normal bone metabolism include androgens, estrogens, and glucocorticoids are of particular significance in persons with SCI. It is appreciated that androgens have a pivotal role in regulating bone
homeostasis by the inhibition of osteoblastic release of local stimulating factors for osteoclastogenesis. There have been several reports of relative or absolute androgen deficiency in small cohorts of men with SCI (Tsitouras, Zhong, Spungen, & Bauman, 1995; Wang, Huang, & Lien, 1992), with more recent retrospective analysis by Bauman et al. (Bauman, La Fountaine, & Spungen, 2014) demonstrating the prevalence of low total testosterone levels in 243 healthy men with chronic SCI. The authors demonstrated a 0.6%/year decline in serum total testosterone compared to a 0.4% decline in men in the Massachusetts Male Aging Study. In addition, low serum testosterone levels were observed at earlier decades of life and a higher prevalence in men with SCI than in nondisabled control subjects. The authors performed additional investigations to determine the mechanism of low serum testosterone levels by provocative testing of the hypothalamic-pituitary-gonadal axis (Bauman, La Fountaine, Cimigliaro, Kirshblum, & Spungen, 2017, 2018). The results from provocative testing revealed an absence of a primary testicular dysfunction but suggested a central dysfunction in a majority of the participants with SCI. Therefore, if androgen replacement is given in combination with mechanical loading additional bone loss may be prevented.

The role of estrogens in bone homeostasis is well-recognized as the decrease in estrogen at menopause in women is the primary cause of
osteoporosis. Several studies have shown that estrogen maintains bone homeostasis by inhibiting osteoblast and osteocyte apoptosis as well as suppressing osteoclast formation by RANKL inhibition and preventing excessive bone resorption (Emerton et al., 2010; Tomkinson, Reeve, Shaw, & Noble, 1997). The ability of estrogens to prevent bone cell apoptosis may be just as significant in the model of immobilization osteoporosis as animal models have shown that hind limb unloading results in immediate osteocyte and osteoblast apoptosis (Balemans et al., 2001) and increased osteoclastic bone resorption (X. Li et al., 2008), pre-clinical observations that may prove important if confirmed in humans with SCI. In SCI women, serum estrogen levels have been shown to be significantly lower than AB controls and may be contributory to bone loss years after injury (Rosenquist, 1950). Furthermore, in a cross-sectional comparative study of 10 male pairs of monozygotic twins discordant for SCI, Bauman et al. (Bauman, Spungen, Wang, Pierson, & Schwartz, 2006) investigated the relationship between bone below the level of lesion, adiposity, and circulating estrogenic compounds. The investigators found a strong correlation between total body fat and leg fat and leg BMC and BMD in the twin with SCI, although this relationship was not observed in the AB twin. This same relationship was found between total serum estradiol and leg BMC and BMD in the twin with SCI but again not in the AB twin. Furthermore, a trend toward higher serum total estradiol was observed in
SCI twin. These findings are evidence of a hormonal mediated effect on bone in persons with SCI and is in agreement with the regulatory effect that adipose tissue has on BMD in the general population.

2.5: Imaging to Acquire BMD at the DF and PT: DXA, QCT, and pQCT

The most commonly used technology to quantify aBMD is DXA (Alhava, 1991). To quantify aBMD of the central axial skeletal sites using DXA, an X-ray tube emits photon energy at two distinct photoelectric peaks, one energy peak preferentially attenuates bone mineral, while another energy peak attenuates soft tissue mass (fat and lean mass). The attenuation of soft tissue is then subtracted from the total X-ray attenuation and the difference compared to the bone mineral attenuation standard obtained from ashed bone, providing a 2-dimensional quantitative assessment of aBMD (Bonnick, 2006). The aBMD by DXA is not a “true BMD” value but rather the bone mineral content (per image pixel) divided by the bone area in square centimeters. Currently, the majority of empirical evidence from epidemiological studies for the diagnosis of osteoporosis and fracture risk was obtained using DXA technology. The 1994 WHO guidelines have been held as the gold standard in diagnosing osteoporosis, with recent guidelines indicating a history of fragility fracture as a diagnosis of osteoporosis regardless of bone density testing results (Baim S, 2008). In addition, DXA is a widely available and practical method that has
excellent precision with very low doses of ionizing radiation, giving this measurement excellent external validity from a research perspective.

Since the early 1980s, DXA has been the primary imaging modality employed to acquire BMD of the DF and PT in persons with SCI. In a cross-sectional cohort of participants with SCI, Biering-Sorensen et al. (Biering-Sorensen et al., 1988) compared BMC of the PT in 26 SCI subjects 2 to 25 years after acute injury and observed that PT was more than 50% lower than the values obtained in an AB cohort. Garland et al. (D. E. A. Garland, H; Stewart, C.H., 2005) compared the combined BMD of the DF and PT in 28 chronic SCI participants (DOI 3-43 years) to 10 AB controls and found that the mean BMD of the combined area was 50% lower when compared to an age-matched AB control group; similar findings were reported by Garland et al. in a cross-sectional study of women with chronic SCI (D. E. Garland et al., 2001). The major limitation of these cross-sectional studies is the appropriateness of comparison to the reference group and the inability to know peak bone mass of the SCI group prior to paralysis. Longitudinal studies using DXA have documented a loss in BMD over time in the acute and chronic SCI populations. Utilizing first generation DXA technology, Garland et al. (D. E. Garland, Adkins, Scott, et al., 2004) assessed BMD of the DF and PT soon after injury (33.5 ±10.8 days) with follow-up measures at 523 ±96.2 days post injury in 5 patients with acute motor-complete SCI; BMD was reduced by 27% at the DF and
by 32% at the PT at the follow-up assessment. In another study of 31 individuals with chronic motor-complete SCI (duration of injury 14.6 ± 8.7 years), Garland and colleagues (D. E. Garland et al., 2008) performed a prospective cohort study and observed an annual loss of BMD of 1.1% at the DF and 1.5% at the PT over a 5-year period. Similar to the small precision error DXA has demonstrated in the AB population, several studies have demonstrated excellent reliability in persons with SCI at the hip and knee regions (Bakkum et al., 2014; Morse, Lazzari, et al., 2009; Peppler, Kim, Ethans, & Cowley, 2016), and one study has validated aBMD at the DF and PT against CT technology. In a cross-sectional validation study by McPherson et al. (McPherson et al., 2014), based on the ROIs defined by Edwards et al. (Edwards et al., 2014a) aBMD was obtained by DXA and vBMD by pQCT in 3 custom ROIs in 12 persons with acute and 34 persons with chronic SCI and found that the RMS-CV% aBMD was more precise in the cohort with acute than with the chronic SCI at the DF epiphysis [(DFE) 1.7 vs. 3.12%], DF metaphysis [(DFM) 1.39 vs. 4.7%], and PT epiphysis [(PTE) 1.66 vs. 3.4%], with all custom ROIs by DXA being highly correlated to the same regions measured by QCT (r ≥ 0.93). A comprehensive summary of descriptive studies that obtained DXA derived aBMD values has been provided (Appendix B, Table 1).

Advanced imaging methodologies such as QCT and pQCT are photon absorptiometric technique like DXA with the unique ability to provide
vBMD, also known as the “true” BMD, of a bone region (Engelke et al., 2008). While DXA utilizes the attenuation of x-rays as they pass through bone and soft tissue mass to estimate the depth of a bone region being measured, QCT and pQCT can utilize 3-D imaging technologies and measure the depth of bone directly to calculate an Archimedean vBMD in cubic centimeters (cm³) (Lee et al., 2015). Although DXA has been the most widely studied method to measure BMD at the knee, QCT and pQCT is regarded as the scientific gold standard method to quantify bone density at the DF and PT. Only QCT and pQCT can capture trabecular and cortical vBMD separately, which may have greater clinical utility in diagnosing osteoporosis (Engelke et al., 2008). As stated earlier, only QCT and pQCT can provide vBMD of the trabecular and cortical bone compartments of multiple, which has direct relevance to the SCI population because loss of trabecular and cortical bone occurs at different rates over the duration of injury (J. D. Dolbow, Dolbow, Gorgey, Adler, & Gater, 2013). Using pQCT, initiating cuts for vBMD imaging from the most distal end of the femur and tibia, the following slices are standard for capturing trabecular and cortical vBMD along the femur and tibia: distal tibia metaphysis (4% region, trabecular vBMD), distal tibia diaphysis (38% slice, cortical vBMD), PT epiphysis (96% slice, trabecular vBMD), DF epiphysis (4% slice, trabecular vBMD), and distal femoral
diaphysis (25% slice, cortical vBMD) (Cervinka, Giangregorio, Sievanen, Cheung, & Craven, 2018).

In recent years, the use of QCT and pQCT bone densitometers has increased in clinical investigations, which has permitted investigators to differentiate between trabecular and cortical bone compartment changes and changes in bone microarchitecture in persons with SCI. As a result, there have been several cross-sectional reports comparing vBMD in those with SCI to an age- and gender-matched AB cohort. In a cross-sectional study to assess bone loss in separate compartments of trabecular and cortical bone, Eser et al. (Eser et al., 2004) used pQCT to measure the distal epiphysis and midshafts of the femur and tibia in 89 men with motor complete SCI (24 tetraplegia and 65 paraplegia with a DOI between 2 months and 50 years); the loss of bone mass in the epiphyses was approximately 50% in the DF and 60% in the PT, and vBMD loss in the diaphyseal shafts was approximately 35% in the femur and 25% in the tibia which was due to endosteal resorption. In a study by Giangregorio et al. (L. Giangregorio, Lala, Hummel, Gordon, & Craven, 2013), the precision of pQCT of the DF and PT in 12 chronic SCI and 21 AB participants was determined; compared to AB controls, total vBMD in the DF and PT was, as expected, significantly lower in the SCI group. In addition to cross-sectional reports, a few longitudinal studies have employed pQCT to capture the intra-patient loss of bone after SCI. In a
prospective cohort study that evaluated vBMD along the entire length of the tibia in 10 patients with acute SCI who completed initial scans 5-weeks after acute injury, de Bruin et al. (de Bruin et al., 2005) found that the loss in tibia trabecular vBMD ranged from -7.8% to -83% and cortical vBMD range of 3% to -40%. In a report by Edwards et al. (Edwards et al., 2014a), QCT was used to quantify changes in vBMD and bone geometry and torsional stiffness at the trabecular and cortical region at the epiphyseal and metaphyseal region of the DF and PT in 13 SCI subjects soon after injury (mean measurement, 3.8 months after acute injury). The authors observed dramatic rates of monthly decline for BMC (−3% to −4%) and vBMD (−3% to −5%) at the epiphyseal trabecular region, as well as cortical diaphyseal BMC (−4% to −5%), with significantly smaller reductions in cortical vBMD of (−0.6% to −0.8%). The authors suggested that the loss of cortical bone was due to the thinning of the endocortical envelope. From these measurements, computation of bone strength and stiffness was performed and a two-fold greater reduction in these parameters was found when compared to reductions in vBMD.

The primary limitation of using QCT and pQCT as a measurement modality is the limited availability and high cost associated with acquiring scans. From a research perspective, both methodologies tend to be cost prohibitive if subcontracting to a radiology center or if acquiring the machine to perform the scans within a research laboratory. The latter
being the more common obstacle as the expense of the machine and acquiring the expertise to perform the scans as technical expertise is hard to find as this technology has limited commercial availability to date. A comprehensive summary of descriptive studies that obtained QCT and pQCT derived vBMD values has been provided (Appendix B, Table 2).

2.5: DXA Software and Analysis Methods to Capture Knee aBMD:

Contrary to the occurrence of fractures in postmenopausal osteoporosis, a condition in which fractures predominantly occur at the TH, FN, lumbar spine, and distal ulnar/radius. In persons with chronic SCI the epiphyses of the DF and PT are the regions that are most vulnerable to fracture (Ingram et al., 1989; Martinez, Cuenca, Herrera, & Domingo, 2002; Ragnarsson & Sell, 1981). Previous DXA studies measuring aBMD at the DF and PT have used software applications that were nonspecific for the knee (e.g., forearm and lumbar spine) (Bohr & Schaadt, 1987; M. G. Li, Nilsson, & Nivbrant, 2004; Murphy, Bresnihan, & FitzGerald, 2001). Adaptation and application of non-knee specific software programs to measure BMD of the DF and PT (lumbar spine and forearm) has been a practical reality because validated software programs to measure BMD of the knee region have not been offered by DXA manufacturers until recently (J. D. Dolbow et al., 2013; D. E. Garland et al., 1992). These indirect software programs may yield considerably different aBMD values
as the soft tissue (lean and fat tissue mass) algorithms are specific to the region they are programmed to measure. Recently, an orthopedic knee software program was developed to monitor BMD after knee arthroplasty, which has received approval by the Food and Drug Administration for commercial use and is now available to reliably acquire BMD of the knee; this software application has been used in rehabilitation and pharmacological clinical trials in persons with SCI (Morse, Lazzari, et al., 2009; Tan et al., 2014). Standard ROIs, regrettably, have not been uniformly developed and adopted by clinicians and investigators, which has created a variability and uncertainty in the effort to compare BMD findings at the knee among reports. Using non-knee specific software, Shields et al., (Shields et al., 2005) developed a widely used method to isolate the DF and PT region of interest, an approach which has been demonstrated to be highly reliable between multiple raters with ICC values for the DF of 0.98 and for the PT of 0.89 (Shields et al., 2005); this methodology has been applied in subsequent studies of the knee in persons with SCI (Lauer et al., 2007). Slightly different custom ROIs that capture more of the epiphyseal region have been reported, and they have also proven to be highly reliable (McPherson et al., 2014). More recent studies have investigated the reliability of different custom ROIs along the DF and PT metaphysis and epiphysis (Lobos et al., 2018; Peppler
et al., 2016), with all custom ROIs demonstrating good precision error values.

In a study by Gilchrist et al. (N. Gilchrist et al., 2013), the authors addressed the precision of the orthopedic knee software in 20 AB adult patients who received knee prosthesis; BMD in 7 custom ROIs were obtained around the implant with the CV% ranging from 0.55% to 4.04%, with no significant difference in precision between the implanted and non-implanted knee (N. Gilchrist et al., 2013). In a cohort of subjects with chronic SCI, Morse et al. (Morse, Lazzari, et al., 2009) tested the precision (RMS-CV%) of the orthopedic knee software and found that the precision of the distal femur (3.01%) had better reproducibility than that of the proximal tibia (5.91%). Using non-knee specific software (e.g., forearm software), McPherson et al. reported similar findings in a validation study performed in subjects with acute or chronic SCI (McPherson et al., 2014). The aBMD was obtained by DXA and vBMD by pQCT in 12 persons with acute and 34 persons with chronic SCI and found that the RMS-CV% aBMD was more precise in the cohort with acute than with the chronic SCI at the DF epiphyses (1.7 vs. 3.12%), DF metaphysis (1.39 vs. 4.7%), and PTepiphysis (1.66 vs. 3.4%), with all custom ROIs by DXA being highly correlated to the same regions measured by pQCT (r ≥ 0.93); the intra- and inter-rater reliability estimates (2 raters) were also determined in the groups with acute and chronic SCI with the intra-class correlation
coefficients (ICCs) were found to exceed 0.97. McPherson et al. concluded that the better precision in the acute SCI cohort may be attributed to the lower absolute mean BMD in the chronic cohort rather than a systematic difference in precision between the 2 groups. Another report supporting the fine reproducibility of the knee software, Bakkum et al. demonstrated excellent inter- and intra-rater reliability (ICCs between 0.97-0.98) using this methodology in an AB cohort (Bakkum et al., 2014).

Future studies performing repeat DXA scans of the knee using “on and off the table” and “different test days” methodologies, as well as validation studies comparing the different capturing methodologies to CT and pQCT, are necessary to understand the optimal precision of performing DXA scans of the knee in persons with SCI. The absence of standardized protocols to acquire aBMD at the distal femur and proximal tibia is a major source of the variability between values reported by investigators. In addition to the standardized protocols to capture and analyze aBMD at the DF and PT, programs should be standardized among the various manufacturers to permit the acquisition of these regions of interest to be compared across studies and between clinical imaging facilities.

Because validated software programs have not been commercially offered by DXA manufacturers, the majority of clinical investigations to date have relied on the adaptation of software applications of the forearm and lumbar spine to measure aBMD of the knee region (M. G. Li
et al., 2004; Murphy et al., 2001). However, the orthopedic knee software used in this study, as well as other clinical trials in persons with SCI (Bauman et al., 2015; Oleson, Marino, Formal, Modlesky, & Leiby, 2020) was recently approved by the FDA to monitor BMD in patients after knee arthroplasty. This knee software package is now commercially available and should be used when available at an imaging facility to acquire the knee in persons with SCI. In addition, acquiring an adequately powered reference dataset of aBMD values at the DF and PT in young-healthy AB persons is essential to diagnose osteoporosis at the knee in persons with chronic SCI or in any other population sample. Only after this work has been completed can prospective cohort studies be conducted to validate T-scores at the DF and PT by obtaining DXA measurements as soon as possible after a fracture in persons with SCI.

2.6: Fragility Fracture and Diagnosis of Osteoporosis in SCI:

Contrary to the occurrence of fractures in postmenopausal osteoporosis, a condition in which fractures predominantly occur at the femoral neck, hip, lumbar spine, and distal ulnar/radius, in persons with chronic SCI the epiphyses of the DF and PT are the regions that are most vulnerable to fracture (Ingram et al., 1989; Martinez et al., 2002; Ragnarsson & Sell, 1981). These “fragility fractures” tend to occur after insignificant trauma, such as bending, transfers, and physical therapy.
exercises. As first described by Comarr et al. (Comarr, Hutchinson, & Bors, 1962) a cohort of 1,363 individuals with SCI were studied with 119 pathological fractures documented. From this subgroup, a total of 97 (82%) fractures occurred at the DF or PT. A high prevalence of fractures at the DF and PT were reported subsequently by other investigators (Freehafer & Mast, 1965). The incidence of fracture increases with time after SCI, with a mean yearly fracture incidence of 1% per year during the first year after SCI and with the incidence increasing to 4.6% per year at 20-29 years after acute SCI (Zehnder et al., 2004). In a retrospective cohort study, Morse et al. (Morse, Battaglino, et al., 2009) addressed if sociodemographic and health-related factors predicted hospitalization due to low impact fracture; in 328 veterans, the most common fracture requiring hospitalization was a PT or fibula fracture (47.5%), followed by the DF metaphysis (20%), and then the proximal femur (15%). A fall from a wheelchair was identified as the most common cause of fracture, followed by transfers, and striking the lower extremity during wheelchair propulsion (Zehnder et al., 2004).

There is agreement in the literature that lower extremity bone density is lower in individuals with SCI that have a prevalent fragility fracture. Fragility fractures are most common at the DF and PT after SCI (Ingram et al., 1989; Ragnarsson & Sell, 1981). Initial reports focused on bone density at the hip; a skeletal site easily obtained by standard clinical DXA scanning protocols. In
a cross-sectional analysis of 41 men with SCI, Lazo et al. (Lazo et al., 2001) reported that those with a prevalent fragility fracture had 37% lower bone density at the FN than those with no fracture (mean BMD= 0.504 g/cm² vs. 0.786 g/cm², p < 0.001). The risk of having had a fracture increased 2.2 times for each 0.1 g/cm² of bone density at the FN. Garland et al. (D. E. Garland, Adkins, Kushwaha, et al., 2004) were among the first to report changes in bone density at the knee after SCI. Using a technique that measured regional knee bone density and included both DF and PT combined, the authors reported a 16% reduction in knee bone density in individuals with a fracture history compared to those with no fracture history (mean = 0.6287 g/cm² vs mean= 0.5279 g/cm²). In agreement with these findings, a more recent study by Tan and colleagues found that non-ambulatory men with SCI with prevalent fractures had significantly lower bone density at both traditional and SCI-specific skeletal sites (Tan et al., 2014). The authors found that bone density was 24-25% lower at the TH and FN and 43-44% lower at the DF and PT in the fracture group compared to the no fracture group. The larger differences in DF/PT bone densities in the Tan paper are unclear but may be attributed to differences in participant demographics or scanning methodology. Consistent with these findings, additional studies have found that Z- and T-scores are significantly lower at the FN, DF, and PT in individuals with prevalent fractures (Zehnder et al., 2004). Furthermore, in one of the first reports of incident fracture in persons
with SCI, Abdelhadi et al. (Abderhalden et al., 2017), performed a retrospective study of veterans with SCI stratifying risk for fracture by T-score into normal, osteopenia, and osteoporosis groups. The authors reported that hip T-score was significantly lower in veterans with incident fracture compared to those without fracture (-2.71 vs -2.24, \( p=0.05 \)). In another study by Lala (Lala et al., 2014), significantly lower mean BMD values were observed in those with history of fracture (n=6) compared to those with no fracture (n=21). After adjusting for injury completeness, the authors found the risk of having had a fracture increased 4.9 times for each standard deviation decrease in bone density at the distal femur and 6.1 times for each standard deviation decrease in bone density at the proximal tibia. Because of the exceptionally high loss of bone substance that occurs after acute SCI at the DF and PT, absolute aBMD at these regions is currently used found to serve as the primary risk factor for the prediction of fracture (D. E. Garland, Adkins, Kushwaha, et al., 2004; Vestergaard, Krogh, Rejnmark, & Mosekilde, 1998). That being said, the generalizability of absolute aBMD values are limited since they are dependent on the densitometer manufacturer and cannot be compared across studies and clinical treatment centers, a reality that makes standardized scores at the DF and PT necessary at this time.

The fracture threshold is defined as the BMD below which there is an increased risk of fracture. LaLa et al. (Lala et al., 2014) performed DXA
and pQCT in a cohort of 70 SCI individuals (19 with history of fracture) and found that participants who had a prior history of fragility fracture had significantly lower aBMD compared to individuals without a history of fracture. Furthermore, there was an increasing risk of fracture for every standard deviation unit decrease in aBMD by DXA or by pQCT derived vBMD and geometry parameters. Garland et al. (D. E. Garland, Adkins, Kushwaha, et al., 2004) calculated the fracture threshold by determining the 95% confidence interval in 9 subjects with a history of fracture at the DF and PT and found that those with a combined DF and PT aBMD \( \leq 0.5755 \text{ g/cm}^2 \) were prone to fracture, with the authors concluding the fracture threshold should be considered aBMD value \(< 0.6 \text{ g/cm}^2 \). Utilizing similar methodology, Eser et al. (Eser, Frotzler, Zehnder, & Denoth, 2005) found that determination of trabecular vBMD of the femur and tibia distal epiphyses was effective in identifying subjects with a history of fracture, and that subjects with a history of fracture had trabecular vBMD that was \(<114 \text{ mg/cm}^3 \) and \(<72 \text{ mg/cm}^3 \) for the DF and PT, respectively. In a study by LaLa et al. (Lala et al., 2014) DXA and pQCT scans were performed in a cohort of 70 SCI individuals (19 with history of fracture) and found that participants who had a prior history of fragility fracture had significantly lower aBMD and vBMD compared to individuals without a history of fracture. Furthermore, there was an increasing risk of fracture for every standard deviation unit decrease in aBMD by DXA or by pQCT derived
vBMD and geometry parameters. The aBMD values reported by LaLa et al. were 0.454 g/cm² at the DF and 0.371 g/cm² at the PT, values that are considerably lower than those reported by Garland et al. (D. E. Garland, Adkins, Kushwaha, et al., 2004) but may not be of consequence as the authors did not indicate how soon after fracture the DXA scans were obtained. These studies have provided guidance on fracture threshold by DXA and pQCT in the SCI population but, to date, no longitudinal prospective cohort studies in persons with SCI have been performed that have acquired aBMD and calculated T-scores form a reference dataset for the DF and PT at or near the time that the fracture has occurred.

Absolute aBMD values, though, cannot be directly utilized to diagnose osteoporosis because aBMD values in normal individuals vary by anatomical region and different company-specific calibration standards to measure aBMD are used by DXA manufacturers. Thus, to standardize results across multiple skeletal regions and between manufacturers, a computed statistical value, or score, was developed that permitted comparison between the aBMD of a given individual against a reference aBMD, expressed as standard deviation score. The young adult T-score is the most commonly applied clinical metric used to provide a normative reference value to diagnose osteoporosis. The International Society for Clinical Densitometry (ISCD) Task Force on Normative Databases and the World Health Organization (WHO) diagnosis of osteoporosis established a T-
score \leq -2.5 \) standard deviations from the mean as the cutoff value at the femoral neck, total hip, L1-L-4 lumbar vertebrae, and 33% forearm regions in post-menopausal women and in men \( \geq 50 \) years old. The National Health and Nutrition Examination Survey (NHANES) III BMD data collected between 1988 and 1994 is the reference standard used to calculate the T-score (Watts et al., 2013). This cutoff was established by the WHO and supported with data from the NHANES prospective cohort study that demonstrated approximately 1/3rd of postmenopausal women and men over 50 with T-scores below this threshold will experience an incident fracture. The ISCD also recommends the use of Z-scores (age-matched reference) in men less than 50 years of age, premenopausal women, and special populations such as SCI, with the diagnosis of “low bone mass for a given age” defined as a Z-score \( \leq -2.0 \) SD from the mean. The Z-score compares the BMD value of a skeletal region of interest to that of an age and gender matched individual grouped per decade (20-49 y), and matched by ethnicity when possible (Looker et al., 1998). If local reference data are available, they should be used to calculate Z-scores to better represent the demographic being studied (Watts et al., 2013). The investigators appreciate that premenopausal woman and men under the age of 50 years are recommended to be compared to age-matched cohorts using Z-scores. However, because the investigators are attempting to identify persons with SCI who are at heightened risk of fracture, and persons with SCI represent
a unique population sample, T-scores values which have been defined to identify persons with osteoporosis, will be the primary aim of this analysis, rather than Z-scores. As demonstrated in the retrospective pilot data analysis that helped develop the dissertation project, TSI is the primary driver of bone loss in persons with SCI, a finding that supports the use of a young healthy AB reference mean aBMD, T-score, and not an age-matched reference mean aBMD, Z-score, as previously suggested by the ISCD. Conceptually, one may approach the diagnosis of bone loss after SCI in a manner that is analogous to that performed in postmenopausal women (regardless of age of the woman) by assessing the aBMD at the skeletal regions of interest and comparing this value to that of a young, healthy, ethnically-matched able-bodied dataset—that is, by T-score.

2.7: Rehabilitation and Pharmacological Treatment of Bone Loss at the DF and PT:

The mechanostat theory, a derivative of Wolff’s law, states that there are strains within bone that are kept within certain limits by adding and removing bone tissue, resulting in improved bone strength according to the particular forces that are imposed (Frost, 1987). However, if force is applied below a certain set point (i.e., due to immobilization secondary to paralysis), bone tissue will ultimately be lost (Isaacson & Brotto, 2014). Over the last 2 decades, several rehabilitation initiatives have implemented various types of ambulation and electrical stimulation [ES=
elicitation of muscle contraction using electric impulses] and functional
electrical stimulation (FES= elicitation of muscle contraction
using electric impulses to artificially generate body movements
of paralyzed limbs to produce functions such as grasping, walking,
bladder voiding and standing) programs in an attempt to improve bone
mass and strength in persons with SCI (Biering-Sorensen, Hansen, & Lee,
2009; D. R. Dolbow, Gorgey, Gater, & Moore, 2014; Pacy et al., 1988). In
persons with SCI who have had severe bone loss at the DF and PT, there is
an increased risk of fracture by participation in rehabilitation interventions,
albeit this level of risk has not been well described for specific interventions
in the literature. The clinical implications for educating rehabilitation
professionals about low aBMD at the DF and PT in persons with SCI is an
awareness of the potential risk of fracture from rehabilitation and/or
exercise training modalities, which currently includes robotic approaches
for ambulation.

The clinical application of activity-based therapy programs, have
employed locomotor training using body-weight supported treadmill
training to activate the neuromuscular system below the level of lesion.
The primary purpose of the program is to optimize the recovery of function
in persons with SCI with clinically motor-incomplete neurological injuries
(Guertin, 2009), with the potential endpoint of the preservation of BMD in
the lower extremities. While these programs have proven effective in
strengthening the neural circuitry responsible for locomotion in those with motor-incomplete SCI, there is emerging evidence that this therapy alone is largely ineffective in attenuating the rapid bone loss at the DF and PT (de Bruin et al., 1999; L. M. Giangregorio et al., 2005). There is considerable evidence that the use of cyclical muscle contraction induced by FES is at least partially effective in preserving BMD at the DF and PT soon after SCI, with FES cycle ergometry the primary modality utilized to date. Preservation of site-specific bone tissue from FES cycling has also been documented in cohorts of individuals with chronic SCI. Using DXA as the imaging modality to assess aBMD of the DF and PT, Bloomfield and colleagues (Bloomfield, Mysiw, & Jackson, 1996), conducted a 9-month FES cycling program in 9 individuals with motor-complete SCI. The authors found that in a subset of subjects who achieved high power outputs (≥ 18 watts) after 6-months of FES training, aBMD of the DF and PT was significantly increased (Bloomfield et al., 1996). In a longitudinal intervention using high volume FES cycling, Frotzler et al. (Frotzler, Coupaud, et al., 2008), examined the change in vBMD at the DF and PT in 12 motor-complete individuals with chronic SCI who completed 180 minutes per week of FES cycling for 12-months. The final pQCT measurement revealed a significant increase in cross-sectional area (1.2%), trabecular (14.4%) vBMD, and total vBMD (7.0%) at the DF epiphyses, but no significant change was observed at the proximal tibia.
The evidence previously presented supporting the use of FES in those with SCI should be balanced with a brief review of studies that have found FES exercise to have limited therapeutic value in the preservation of bone at the DF and PT. Of clinical relevance, preservation of bone or any reversal of bone loss is rapidly lost once FES exercise is discontinued in cohorts with acute (Eser et al., 2003; Lai et al., 2010) or chronic (Belanger, Stein, Wheeler, Gordon, & Leduc, 2000; Dudley-Javoroski et al., 2012) SCI. Furthermore, a degree of controversy persists with regard to the ability of FES cycle training (30-minute sessions performed 3 times a week) to attenuate vBMD loss at the tibial diaphysis in persons with SCI after acute traumatic motor-complete SCI (Eser et al., 2003). In a follow-up report by Frotzler et al. (Frotzler, Coupaud, et al., 2008), the authors assessed the effect of detraining on bone and found that 64% of the vBMD gained during training was retained 12 months after discontinuing FES cycling. The authors assessed the effect of detraining on bone and found that 64% of the vBMD gained during training was retained 12-months after discontinuing FES cycling. In a study by Lai et al (Lai et al., 2010), aBMD of the DF was assessed in 24 motor-complete acutely injured (26-52 days post injury) patients with SCI, with 12 participants receiving FES cycling and 12 age- and gender-matched participants in the control group; the rate of bone loss at the DF in the FES cycling group was significantly less than that of the control group, with the bone sparing effect of FES completely
lost once FES cycling was discontinued. These findings were supported in a study by Chen et al. (Chen et al., 2005) in which 15 participants with, 15 chronic SCI performed FES cycling 30 minutes a day, 5 times a week, for 6-months. At baseline, the DF and PT aBMD was 39% and 47% lower compared to 15 age-matched AB control participants. After 6-months of FES cycling, aBMD of the DF and PT increased by 11% and 13% respectively, with the aBMD of both regions decreasing significantly 6-months after FES was discontinued. Furthermore, in a study by Mohr et al. (Mohr et al., 1997), 12 months of FES cycling on average 2.3 times a week increased aBMD of the PT by 10%. However, a reduced training exercise prescription of 0.9 times per week was insufficient to maintain these positive changes in aBMD. In a relatively large cohort of acutely injured patients (4-5 weeks after SCI), 19 patients completed FES cycling 3 times a week for 6-months and 19 patients served as controls. The results of this study revealed a small, nonsignificant increase in tibia shaft vBMD in the FES group compared to controls; the authors concluding that FES cycling immediately after SCI did not attenuate bone loss (Eser et al., 2003). A possible explanation for these contradictory findings from previous studies may be that the mid shaft of the tibia, which was the site imaged for changes in vBMD, is composed primarily of cortical bone, whereas in prior studies that showed FES to be effective, the DF and PT were the sites imaged, which are sites rich in trabecular bone.
In recent years, the effect of ES on bone formation/resorption in persons with SCI has been studied while loading in the standing position. In a pilot study of 4 chronically injured persons with SCI, Shields et al. (Shields & Dudley-Javoroski, 2007) studied the effect of a 6 to 11 months of an FES training intervention on the soleus muscle using plantar flexion ES to deliver a mean estimated load to the tibia of 110% of body weight to one leg, while using the other leg as a control limb. The authors found that aBMD of the PT in 3 of the participants increased by 19% and 31% at months 3 and 4 respectively, with no additional increases noted for 2 participants that continued 11 months into the training program. In addition to this early pilot work, in a study in 28 individuals with motor complete SCI with varying durations of injury (0.2 to 24.3 years since date of injury), Dudley-Javoroski et al. (Dudley-Javoroski et al., 2012) compared the effect of bone compressive loads using 0% body weight (no standing), 40% body weight (passive standing), and 150% body weight (ES of the quadriceps delivered compressive loads) on the change in vBMD at the DF and PT; the slope of BMD loss in the high-dose group was 3-times lower at the DF, and 25.1% lower at the PT than that of the low-dose or control groups. In addition to the DF and PT sites, generating 150% of body weight using standing ES of the quadriceps increased vBMD at the 12% femoral length region (Dudley-Javoroski & Shields, 2013). In persons with chronic SCI (mean duration of injury ~10 years), Belanger et al. found a ~30%
restoration in BMD of the DF and PT with stimulation of the quadriceps against an isokinetic load (1 hour per day, 5 days a week for 24 weeks) compared to stimulation against gravity alone (Belanger et al., 2000). This preliminary evidence supports the hypothesis that a combination of loading and intense cyclic muscle contractions against resistance in the upright position may be sufficient to elicit the minimum essential strain required to stimulate positive bone tissue adaptations at the DF and PT in persons with SCI, suggesting the value of the application of this approach in the clinic to ameliorate bone loss. Additional interventions have examined the effect of regular standing in a supportive frame, pulsed ultrasound at specific bone regions, and low-magnitude whole body vibration therapies on the preservation of BMD in the lower extremities (Ben et al., 2005; Warden et al., 2001; Wuermser, Beck, Lamb, Atkinson, & Amin, 2014), with none of these approaches demonstrating efficacy in the treatment of immobilization osteoporosis.

Bisphosphonates belong to a class of anti-resorptive compounds that are widely used in the treatment of post-menopausal, glucocorticoid-induced, and senile osteoporosis (Bultink, Baden, & Lems, 2013). Investigators who have reported the administration of bisphosphonate preparations have had varying degrees of success in preventing sublesional bone loss in the SCI population (Chappard et al., 1995; N. L. Gilchrist et al., 2007; Nance et al., 1999; Pearson, Nance, Leslie, & Ludwig,
1997). The main confounding variable in these studies has been that persons with SCI had varying degrees of completeness of motor lesion and that the differences in weight bearing activities/ambulation were not quantified over the course of the study. In a small randomized study investigating the effect of cyclical etidronate on preservation of bone within 6 weeks of acute SCI, Pearson et al. (Pearson et al., 1997) found that persons with SCI who became ambulatory and received etidronate treatment (n=2) had preservation of bone density compared to ambulatory SCI participants who did not receive drug (n=3) and non-ambulatory SCI participants who received etidronate (n=3), which supports further work to evaluate the efficacy of bisphosphonates to prevent bone loss in a larger sample of individuals who are capable of weight-bearing activities and/or ambulation after acute SCI. In a double-blinded, randomized, placebo-controlled study by Bauman et al., despite repeated administration of pamidronate (60 mg intravenously administered at baseline, 1, 3, 6, 9 and 12 months) after acute SCI, at 1-year aBMD was not significantly different from the placebo group at the hip and knee (Bauman et al., 2005). The administration of zoledronic (ZA) shortly after SCI has been demonstrated to have a bone-sparing effect at the spine and hip regions in reports by Bubbear et. al. and Shapiro et al. (Bubbear et al., 2011; J. Shapiro et al., 2007), but in these reports BMD at the DF and PT was not evaluated. Bubbear and colleagues (Bubbear et
al., 2011) administered ZA in an open label study to 14 patients with acute SCI, 7 treated with ZA and 7 receiving placebo, and noted significant benefit to aBMD after 12 months at the lumbar spine (~3%), total hip (~12%), and greater trochanter (~13%), but no significant benefit was observed at the femoral neck. In 17 persons with acute SCI, Shapiro et al. (J. Shapiro et al., 2007) administered ZA and observed a benefit at 6 months post therapy at the hip, a transient positive result which was almost totally lost by 12 months for aBMD, cross-sectional area, and measures of predicted bone strength (i.e., section modulus and buckling ratio) by DXA. In a more recent report by Bauman and colleagues (Bauman et al., 2015), ZA was administered in an open label study to 13 patients with SCI, 6 treated with ZA and 7 receiving no treatment.

Compared to the treatment group, the control group lost a significantly greater percentage of aBMD at the total hip at month 6 (-3.2% vs. -13.9%) and at month 12 (-7.5% vs. -20.1%). However, contrary to the findings at the hip, the treatment group had a greater loss in aBMD compared to the control group at the DF and PT at month 6 (-7.9% vs. -2.7% and -10.5% vs. -4.8%) and at month 12 (-18.5% vs. -8.4% and -20.4% vs. -7.9%). The authors concluded that it would not be prudent at the present time to recommend the use of ZA in an effort to reduce bone loss after acute SCI due to this lack of efficacy at the knee region and the fact that patients with chronic SCI fracture more at the DF and PT than at the hip. Similar to
these findings, Schnitzer et al. (Schnitzer et al., 2016) administered ZA at baseline in a double-blinded, randomized, placebo-controlled trial to 12 patients with SCI, 6 treated with ZA and 6 receiving placebo, with 6-months being the primary time point for drug efficacy after BL drug administration. Compared to the treatment group, the placebo group lost a significantly greater percentage of aBMD at the total hip (right: -2.2% vs. -8.6% and left: -3.7% vs -12.3%). However, contrary to the report by Bauman et al. (Bauman et al., 2015), the placebo group had a greater percentage of aBMD loss at the DF, but this loss was not significant compared to the treatment group 6-months after BL drug administration. Until adequately powered, well designed, randomized control trials have been completed, the safety and efficacy of prescribing ZA shortly after SCI remains questionable. A recent single arm investigation looking at the effectiveness of denosumab (60 mg every 6-months) to ameliorate bone loss in 14 patients with acute SCI found this treatment was highly effective at preventing aBMD loss at the lumbar spine and total hip, but aBMD of the DF and PT was not measured, so the efficacy of denosumab at these ROIs and comparisons to trials using ZA cannot be made at this time (Gifre et al., 2016). Given the small size of these cohorts and the limitations inherent when performing a bone loss intervention clinical trial shortly after SCI [e.g. corticosteroid administration, individual variation in bone loss after SCI, and limited access to advanced imaging methods (CT and/or
MRI), future randomized control trials are warranted that compare multiple anti-resorptive agents with appropriate imaging modalities to more fully understand the drug with the greatest efficacy to preserve bone mass and architecture at the DF and PT. Finally, identification of T-scores at the DF and PT can provide clinicians and researchers the reference values necessary to understand the optimum therapeutic effect of the different ambulatory and pharmacological interventions described above that hold promise to improve bone health at the DF and PT, the regions most prone to fracture in persons with SCI.

2.8: Significance of Literature Review to Specific Aims:

Previous studies that have acquired aBMD at the DF and PT in a comparative AB cohort enrolled a heterogeneous sample of participants not controlling for age, ethnicity, and gender (see Appendix B, Table 1). Since the average age of persons with SCI is 42 years old, mostly male (~80%), and non-Hispanic white ("Spinal Cord Injury (SCI) 2016 Facts and Figures at a Glance," 2016), an AB young-healthy cohort reference dataset would need to control for age, gender, and ethnicity in order to calculate meaningful T-scores at the DF and PT. While the use of pQCT to obtain vBMD and microarchitecture of the DF and PT is well appreciated by clinicians and researchers, the fact remains that pQCT is not widely available and the possibility of third-party reimbursement in the USA is, to
date, not a tenable option when one considers that even reimbursement for DXA imaging has diminished to a great extent over the past decade (Krueger, 2015; Schousboe, 2014). Over the past three decades, the success of bone densitometry to diagnose primary osteoporosis in the AB population is largely due to the accumulation of normative data at the hip, lumbar spine, and forearm regions. There is ample evidence that activities while seated in a wheelchair place a person with SCI at greatest risk for low impact fragility fracture at the DF and PT. The future of routine densitometry in the SCI population is dependent on the development of normative databases to calculate T-scores and improved cutoff values for fracture at the DF and PT. The successful evaluation of any novel intervention to prevent sublesional osteoporosis is contingent upon obtaining accurate, reproducible, and high-quality imaging of the DF and PT. This imaging technology is currently available to clinical investigators and should provide the ability to properly address questions of the efficacy of newer interventions to prevent bone loss at the knee. Specifically, with the advent of robotically powered exoskeletal-assisted ambulation for persons with SCI, as well as other advanced rehabilitation medicine interventions that position the individual in an upright posture and place increased forces on the lower extremities, the increased relevance of identifying those individuals who are at heightened risk of fracture is becoming ever more apparent. Because there is a paucity of
evidence supporting the aBMD or T-score cut-off to exclude persons with SCI from rehabilitation interventions, several studies have incorporated DXA-derived cut-off criteria using a T-score range between -2.5 to -4.0 SD below the mean at the TH or FN (Gorgey et al., 2017), and when imaging is available at the knee for cut-off criteria, aBMD at the DF or PT in the range of 0.60 to 0.70 g/cm² (Ramanujam et al., 2018), with several studies applying these afore mentioned cutoff criteria at both the hip and knee regions to qualify for study entrance (Asselin et al., 2015; Lester & Gorgey, 2018; Yang, Asselin, Knezevic, Komfeld, & Spungen, 2015). While it has become imperative to appropriately evaluate aBMD of the DF and PT in potential candidates to ensure their safe participation, normative databases to calculate T-scores at the DF and PT are currently unavailable. However, the majority of epidemiological prospective cohort studies have utilized DXA to obtain measures of aBMD at or near the time that fracture occurred in a population-based sample of young healthy individuals, men over 50, and post-menopausal women to validate standardized cutoff values at the relevant fracture sites (Watts et al., 2013). In summary, like all diagnostic tests, the application of bone densitometry to assess the efficacy of rehabilitation modalities or pharmacological interventions would require reference data for the DF and PT regions to determine if improvements in aBMD have clinical relevance. Establishing T-score values at the DF and PT would provide
clinicians and researchers the reference values necessary to understand the optimum therapeutic effect of different ambulatory and pharmacological interventions, a point that is especially relevant in the current healthcare environment where third-party reimbursement is increasingly difficult to justify prescription of these therapeutic interventions.

2.9: Conceptual and Operational Definitions:

- **Spinal Cord Injury**: Damage to the spinal cord that results in a loss of function, such as mobility and/or feeling that is frequently causes by trauma or disease. The population that will be compared to the young healthy able-bodied normative dataset in this study.

- **Acute SCI**: As a result of trauma or disease to the spinal cord, a medical emergency that causes loss or decreased feeling, motor function, and spinal reflexes. The acute SCI time frame is classified as the first 3 months after initial injury.

- **Chronic SCI**: Trauma or disease to the spinal cord that causes loss or decreased feeling, motor function, and spinal reflexes that has been present for at least 1-year after initial injury.

- **Time Since Injury (TSI)**: The amount of time since the date of SCI usually expressed in years. In context of the study, the time from the date of injury to the date the DXA study is completed.
- **Motor Complete SCI** - Absence of motor function below the neurological level of spinal cord injury.

- **Motor Incomplete SCI** - Partial motor function intact below the neurological level of spinal cord injury. Motor incomplete SCI has varying patterns of moving one arm or leg more than the other or one side of the body more than the other.

- **Bone Remodeling** - The process where bone tissue is taken away (bone resorption) and new bone tissue is added (bone formation). During the acute phase of SCI and immobilization, there is an uncoupling of the bone resorption/formation process where increased bone resorption dominates resulting in a precipitous loss of bone over the first 2-3 years after SCI that continues into the chronic phase of SCI.

- **Areal Bone Mineral Density (aBMD)** - The BMC measured by standard (projection) DXA per unit of projected surface. This is not a "volumetric" but a "surface-related" density and is expressed per area unit, in g/cm².

- **Volumetric Bone Mineral Density (vBMD)** - A 3-dimensional measurement known as the “true” BMD that is the BMC of the sum of cortical and trabecular bone in the studied bone site per unit volume of all bone plus marrow space. The greatest value in the evaluation of immobilization osteoporosis in persons with SCI is the
ability to measure the trabecular and cortical compartments separately.

- **Trabecular vBMD**: The vBMD of the trabecular compartment of a bone section that can only be measured by 3-dimensional such as QCT, pQCT, and MRI imaging technologies. The trabecular vBMD is important to assess in persons with SCI as this is the compartment most susceptible to fracture and the compartment where resorption is the greatest during the acute phase of bone loss.

- **Cortical vBMD**: The vBMD of the cortical compartment of a bone section that can only be measured by 3-dimensional QCT, pQCT, and MRI imaging technologies.

- **Distal Femur (DF) and Proximal Tibia (PT)**: A region that comprises approximately 30% of the distal area of the femur and 30% of the proximal area of the tibia. In context of reporting aBMD values of the DF and PT in persons with SCI reported in the literature, these regions of interest are at the epiphysis, metaphysis, or diaphysis section of the DF and PT and contain different ratios of trabecular to cortical bone.

- **Epiphysis**: The rounded end of a long bone that meets at the joint of a long bone. The epiphysis contains primarily trabecular bone, is the compartment with the greatest rate of loss after SCI, and is the area most susceptible to fragility fracture at the DF and PT. As a
result of this being the most susceptible region, the majority of knee analysis protocols that report aBMD values at the DF and PT define the ROI at the epiphysis as a separate value.

- **Metaphysis** - The narrow section of a long bone that lies between the epiphysis and diaphysis and contains both the epiphyseal plate and a bony component. The epiphyseal plate calcifies in adulthood into solid cortical bone and the bony component of the metaphysis functioning to transfer loads from weight-bearing joint surfaces to the diaphysis. Several protocols that capture and report aBMD at the DF and PT in persons with SCI use ROI that overlap between the epiphysis and metaphysis.

- **Diaphysis** - The diaphysis comprises the midsection (shaft) of a long bone and is made up of an endocortical envelope with a bone marrow and adipose tissue tubular center. The diaphysis contains primarily cortical bone and is lost slowly after SCI, with continuing loss into the chronic phase of SCI long after trabecular bone mineral in the epiphyseal region is depleted.

- **Bone Densitometry** - A test to measure BMD performed by DXA, QCT, pQCT, or MRI using specialized computer software.

- **Dual Energy X-ray Absorptiometry (DXA)** - A 2-dimensional method of measuring that uses an X-ray beam to determine the attenuation coefficient of soft tissue (fat and lean tissue mass) and bone mineral
content per imaging pixel and finally subtracting soft tissue mass to calculate aBMD. The use of DXA derived aBMD at the hip and knee region are the areas of greatest importance in persons with spinal cord injury, with the latter being the most important region to assess fracture risk.

- **Quantitative Computed Tomography (QCT)** - is 3-dimensional imaging technique using a standard X-ray CT scanner and a bone density calibration standard that is used to convert Hounsfield Units (HU) of the CT to obtain vBMD expressed as mg/cm³. In the SCI population, total leg QCT can obtain vBMD of the trabecular and cortical compartments and the trabecular microarchitecture. Total leg QCT has a significantly higher effective radiation dose compared to DXA and pQCT and is used less frequently in clinical trials.

- **Peripheral quantitative computerized tomography (pQCT)** - a QCT imaging method that uses a small effective radiation dose that is lower than DXA to obtain measurements trabecular and cortical vBMD and bone geometry of the periphery such as the DF and PT as well as the distal tibia and distal radius/ulna. This technology has become commonly used in clinical trials in SCI to obtain vBMD and bone geometry indices and has the benefit of limiting radiation exposure to the extremities and not vital organs. The currently
available commercial device comes with a sliding gantry that can obtain slices along the femur and tibia metaphysis and epiphysis, as well as at sites along the tibia diaphysis (XCT 2000/3000 scanner; Orthometrix/Stratec, White Plains, NY).

- **Standardized Scores** - A standard score is calculated by taking a raw score and transforming it to a common scale that is based on a normal distribution with a mean and a standard deviation. In bone densitometry this is the calculation of age-matched (Z-score) and young healthy adult between the ages of 20-40 (T-score) mean aBMD values at a site and region of interest.

- **T-score** - A computed statistical value to standardize aBMD results across multiple skeletal regions and among DXA manufacturers that permits comparisons between the aBMD of a given individual against that of a reference aBMD in young-healthy adults.

- **Z-score** - A computed statistical value to standardize aBMD results across multiple skeletal regions and among DXA manufacturers that permits comparisons between the aBMD of a given individual against that of a reference population aBMD that is matched for age.

- **Third National Health and Nutrition Examination Survey (NHANES III)** - total hip aBMD data of US adults based on a nationally representative
sample that was examined from 1988-1984 that has been the universally recognized gold standard reference dataset.

- **GE/NHANES** - General Electric (GE) and NHANES III (GE/NHANES) combined reference dataset utilized by GE densitometers.

- **Young-Healthy Able-Bodied** - a reference population between 20-40 years of age used to represent the normal range of aBMD values.

- **Bone-Specific Physical Activity Questionnaire** - the BPAQ is a validated physical activity questionnaire geared toward understanding the relationship between BMD and the exercise history of an individual throughout the lifetime.
CHAPTER III:

RETROSPECTIVE PILOT DATA ANALYSIS:

The purpose of the retrospective data review was to compile DXA scans from four separate VA IRB-approved clinical protocols that were performed at the JJ PVAMC and KIR. The analysis examined the relationship between hip (TH and FN) and knee (DF and PT) aBMD and reference scores, demographic characteristics such as TSI, between an age-matched SCI and AB group. This project was performed in conjunction with my participation in the doctoral program at the Rutgers School of Health Professions which is under the direction of Drs. Mary Jane Myslinski; J. Scott Parrott; and William A. Bauman. The results of the retrospective data analysis yielded valid hip and knee DXA scans on 105 persons with SCI and 17 AB control participants and yielded 2 specific aims that created the theoretical framework for the prospective primary aim dissertation aim. All aspects related to the study design and methods were reviewed and approved by the institutional review boards at both the JJ PVAMC and KIR. After meeting the inclusion criteria, participants were provided information about the study and informed consent was obtained before participation in these studies. At the time of informed
consent, participants were informed that their DXA data could be used in future cross-sectional exploratory analyses to better understand bone health after SCI. To ensure institutional regulatory compliance, additional institutional review board (IRB) approval from the respective institutions was obtained to extract this information from de-identified and coded databases to perform the retrospective review and to analyze the data. Demographic and DXA data were collected from previous investigations addressing bone health in persons with SCI. Extensive demographic information, such as physical activity, smoking, alcohol consumption, calcium intake, vitamin D levels, presence of spasticity, and fracture history, was not available for analysis. The prior studies, from which data were analyzed for this work, were conducted in accordance with the Declaration of Helsinki. Participants Data on 105 unique individuals with SCI and on 17 able-bodied reference (ABref) controls were obtained from our research center’s database and used for analysis. In the parent studies, the inclusion criteria were male and female participants with traumatic SCI who were non-ambulatory (wheelchair reliant 100% of the time) or healthy AB controls, and all participants were between the ages of 18 and 65 years. In those with SCI, neurological level of injury between cervical level-5 (C5) and lumbar level-2 (L2), and International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) grade A, B, or C (28). Exclusion criteria for participants with SCI were the absence of
severe spasticity and contracture that would interfere with acquiring a DXA scan of the hip and knee regions, pressure ulcer(s), current use of bisphosphonates or other medications known to affect bone metabolism, history of alcohol abuse (>2 drinks a day), chronic glucocorticoid use, hormone replacement therapy, current treatment for a acute medical condition, bilateral knee and/or hip replacement, metal hardware in both lower extremities, history of heterotopic ossification at the hip and knee, history of heart disease, and a history of diseases known to affect bone metabolism (e.g., ankylosing spondylitis, rheumatoid arthritis, Paget’s disease, hyperparathyroidism, diabetes mellitus, and/or cancer).

**SPECIFIC AIM I: To determine if T-score values at the FN and TH can be used to estimate aBMD values at the DF and PT.**

**3.1: Introduction and Background:**

Advanced rehabilitation medicine interventions that position the individual in an upright posture and place increased forces on the lower extremities, the increased relevance of identifying those individuals who are at heightened risk of fracture is becoming ever more apparent. Because there is a paucity of evidence supporting the aBMD or T-score cut-off to exclude persons with SCI from rehabilitation interventions, several studies have incorporated DXA-derived cut-off criteria using a T-score range between -2.5 to -4.0 SD below the mean of a young-healthy
AB reference population at the TH or FN (Gorgey et al., 2017). In the clinical research environment where imaging is available to image the knee region, aBMD at the DF or PT in the range of 0.60 to 0.70 g/cm² has been used to exclude persons with SCI from advanced upright rehabilitation strategies (Ramanujam et al., 2018), with several studies applying these afore mentioned cutoff criteria at both the hip and knee regions to qualify for study entrance (Asselin et al., 2015; Lester & Gorgey, 2018; Yang et al., 2015). The threshold aBMD value of 0.60 g/cm² at the DF and PT was first described by Garland et al., below which aBMD value fractures were observed more likely to occur at these skeletal sites (D. E. Garland, Adkins, Kushwaha, et al., 2004; D. E. Garland, Maric, Adkins, & Stewart, 1993). Appreciating that these cut-off values have not been validated in studies that assessed incident fracture, it is important to note that recent studies of prevalent fracture have supported the cut-off value at the knee for aBMD of < 0.60 g/cm² (range 0.20 to 0.55 g/cm²) (Lala et al., 2014; Tan et al., 2014). Measurement of the TH and FN is routinely obtained, has excellent reliability, and standardized with well-established reference young normal (T-score) datasets to account for calibration differences between DXA manufacturers. As opposed to the use of T-score criteria at the TH and FN that are used to diagnose osteoporosis in other populations, the aBMD values at the DF and PT are the more appropriate regions to use to diagnosis low aBMD and to identify those
with SCI who are at increased risk of fracture (Frotzler, Cheikh-Sarraf, Pourtehrani, Krebs, & Lippuner, 2015; Ingram et al., 1989). However, aBMD measurements at the DF and PT are not routinely available to the clinician and are rarely obtained by clinical DXA technicians. If the aBMD at the DF and PT and T-score at the TH and FN have a proven strong association, then calculation of the optimum T-score cutoff values at the TH and FN that correspond to commonly used aBMD cutoff values at the DF and PT where fracture most commonly occurs could be identified. The purpose of this report was to characterize the T-score values at the TH and FN that best represent the DF or PT aBMD cutoff value (<0.60 g/cm²) commonly used to identify persons with SCI at the greatest risk of fracture.

3.2: Statistical Analysis:

Results are expressed as group mean ± standard deviation (SD). Independent sample t-tests were performed to determine the difference in aBMD and T-score values between the hip and knee regions and the confounding effect of demographic variables on aBMD and T-score variables presented. To determine the relationship between the hip and knee regions, simple linear regression models were used to determine the associations between the DF and PT aBMD and T-scores at the TH and FN. The direction of this relationship was intentional to focus on the clinically relevant aBMD that has been identified at the DF and PT (0.60 g/cm²) and the well appreciated fact that T-score at the TH and FN is the commonly
performed and clinically relevant measurement used by physicians to diagnose osteoporosis. The linear relationship was established by visual inspection of scatterplots with a superimposed regression line and normal distribution of residuals was established by visual inspection of probability plots. Homoscedasticity was evaluated by visual inspection of standardized residuals versus standardized predicted values plot and tested using the Breusch-Pagan test. To determine agreement between the predicted and measured T-score values at the TH and FN, a Bland-Altman analysis was completed with the proportion of the sample likely to be accurately predicted calculated. Multiple regression analysis was also used to determine the effect of other predictors (weight, BMI, age, level of lesion, motor completeness, and Sex) of aBMD at the TH and FN.

Applying the commonly used aBMD cutoff value at the DF or PT (< 0.60 g/cm²) to dichotomize participants with low bone mass at heightened risk of fracture at the knee, a receiver operating characteristic (ROC) curve was used to identify the optimum T-score cutoff at the TH and FN (i.e., maximum sensitivity and specificity) using Youden Index [YI; intersecting point or “turn” along the curve that provides the best balance between sensitivity and specificity]. The AUC, sensitivity, and specificity were interpolated for the TH and FN T-score cutoff value from the coordinates of the curve of the YI and then compared to the sensitivity and specificity of the World Health Organization (WHO) criteria to diagnose osteoporosis
(-2.5 SD from the reference mean). Additional sensitivity and specificity analysis was determined for the TH and FN cutoff values of <-3.5 SD (a value for aBMD below which entry into EAW upright ambulation protocols has been denied), <-2.5 SD (WHO criteria to diagnose osteoporosis), and <-3.1 SD (obtained from ROC curve T-scoreY1 and linear regression predicting T-score at the TH from the DF) using the aBMD cutoff value at the DF or PT(< 0.60 g/cm^2) as the reference standard and the number of false negative and false positive cases presented. Statistical analyses were completed using IBM SPSS Statistics version 22, IBM, Armonk, NY and graphs were generated by Prism GraphPad, version 6.0 for Windows, GraphPad Software, San Diego, CA. An a priori level of significance was set at p≤0.05. Findings from this analysis were published in the Journal of Spinal Cord Medicine (Cimigliaro et al., 2020).

3.3: Results:
To determine the ability of the DF and PT to predict T-score at the TH and FN, linear regression analysis was performed. The aBMD at the DF and PT regions were significant predictors of T-score at the TH (R^2 = 0.63, P < 0.001 and R^2 = 0.65, P < 0.001, respectively) and FN (R^2 = 0.55, P < 0.001 and R^2 = 0.58, P < 0.001, respectively). Additional analysis of the regression coefficients revealed that for a 0.1 g/cm^2 change in aBMD at the DF and PT there was a 0.63 and 0.46 change in T-score at the TH, respectively,
(Figure 3 A and B) and a 0.49 and 0.36 change in T-score at the FN, respectively, (Figure 3 C and D). Furthermore, using the DF and PT aBMD value of 0.60 g/cm² to designate heightened risk of fracture, the predicted T-score was -3.1 and -3.5 at the TH, respectively, (Figure 3 A and B) and -2.6 and -2.9 at the FN, respectively (Figure 3 C and D). An additional multivariate regression model was performed to account for other factors that may contribute to predicting aBMD at the DF and PT, but no significant contributions were found the additional covariates of age, BMI, level of lesion, motor completeness, and gender into the model.

The Bland-Altman analysis was used to examine agreement between predicted and measured values based on the linear regression equations obtained. A small mean difference (bias) between the predicted and measured values was observed for all regions (<0.1 SD). Analysis of the 95% CI limit of agreement (LOA) using the DF and PT to predict T-score ranged from -1.991 to 1.990 and -1.938 to 1.939 at the TH, respectively, (Figure 4 A and B, light dashed lines) and -1.825 to 1.827 and -1.770 to 1.770 at the FN, respectively (Figure 4 C and D, light dashed lines). For TH and FN regions, the regression estimates from the DF and PT predicted between 93 and 94% of the total sample of T-scores, with the greatest error observed when T-score was <-2.0 SD (Figure 4 A-D). A second clinically relevant LOA (LOA_{clin}) was set at the acceptable threshold (±1
SD) with the DF and PT accurately predicting 75% and 71% of TH T-score values, respectively, and 76% of FN T-score values, respectively (Figure 4 A-D). Despite the overall good levels of agreement between the difference and measured values, the greater error was observed when measured T-score values are between -1.0 and -3.0 SD, evidence that measures at the hip and knee region should be obtained separately in populations with a high prevalence of osteoporosis that are at increased risk for fracture.

Using the aBMD cutoff value at the DF or PT (<0.60 g/cm²) to diagnosis participants with high fracture risk (HFR) and lower fracture risk (LFR), a receiver operating characteristic (ROC) curve was used to identify the sensitivity and specificity of the T-score at the point of the YI (T-scoreYI). The sensitivity and specificity along the curve was also identified for the WHO T-score cutoff (T-scoreWHO) of -2.5 used to diagnose osteoporosis at the hip. From the ROC curve a T-score of -3.1 (AUC = 0.95, SE = 0.021, 95% CI = 0.911-0.995, P < 0.001) was identified at the TH and -2.4 (AUC = 0.92, SE = 0.027, 95% CI = 0.868-0.974, P < 0.001) at the FN. Furthermore, interpolation of the TH T-scoreYI and T-scoreWHO along the curve revealed a sensitivity and specificity of 81% and 92% and 94 and 80%, respectively. At the FN, T-scoreYI and T-scoreWHO had a sensitivity and specificity of 69 and 86% and 69 and 90%, respectively. By interpretation of the AUC in the ROC curve, the T-score cutoff value at the TH and FN can be used to
diagnose osteoporosis 95 and 92% of the time, with the best balance between sensitivity and specificity demonstrated at the TH (Table 3).

Using the TH or FN T-score criterion of -3.5 SD [a value for a BMD below which entry into exoskeleton-assisted walking (EAW) upright ambulation protocols has been denied], and using the same cutoff criteria at the DF or PT (<0.60 g/cm2), 89 (85%) participants reached or exceeded eligibility by either the TH/FN or DF/PT cutoff criteria, 5 (6%) did not meet eligibility by both the TH/FN or DF/PT cutoff criteria, 3 (3%) did not meet the eligibility cutoff criteria at the DF or PT but reached or exceeded the cutoff criteria at the TH or FN, and 10 (10%) did not meet the eligibility cutoff criteria at the TH or FN but reached or exceeded the eligibility criteria at the DF or PT; thus using a T-score criterion at the hip of -3.5, the sensitivity and specificity was 33% and 97%, respectively (Table 4). Applying the TH or FN T-score WHO criterion (-2.5 SD) and the cutoff value at the DF or PT to this dataset, 68 (65%) participants reached or exceeded eligibility by either the TH/FN or DF/PT cutoff criteria, 17 (16%) did not meet eligibility by both the TH/FN or DF/PT cutoff criteria, 20 (19%) did not meet the eligibility criteria at the DF or PT but reached or exceeded the cutoff criteria at the TH or FN. There were no participants who reached the eligibility criteria at the DF or PT and did not meet the eligibility cutoff criteria at the TH or FN. Thus, using a T-score criterion at the hip of -2.5, the sensitivity and specificity was 100% and 77%, respectively (Table 4). Finally, applying the
TH or FN T-score criterion of -3.1 SD (obtained from ROC curve T-score \(_{YI}\) and linear regression analysis at the TH from the previous analysis), and using the same cutoff criteria at the DF or PT, 82 (78%) participants reached or exceeded eligibility by either the TH/FN or DF/PT cutoff criteria, 13 (12%) did not meet eligibility by both the TH/FN or DF/PT cutoff criteria, 6 (6%) did not meet the eligibility cutoff criteria at the DF or PT but reached or exceeded the cutoff criteria at the TH or FN, and 4 (4%) did not meet the eligibility cutoff criteria at the TH or FN but reached or exceeded the eligibility criteria at the DF or PT; thus using a T-score criterion at the hip of -3.1, the sensitivity and specificity was 76% and 93%, respectively (Table 4).

**Specific Aim II: To determine if aBMD values decrease as a function of time since injury at the DF and PT compared to the demineralization at the FN and TH regions.**

**3.4: Introduction and Background:**

A few DXA studies have demonstrated that bone loss in the lower extremities is a function of time since injury (TSI), with several studies reporting bone loss reaching a steady state 2-5 years after SCI. However, these studies generally had relatively small sample sizes that lacked a sufficiently broad distribution of TSI \(^{16}\), were not performed at the DF and PT \(^{20}\), acquired BMD using CT imaging \(^{21,22}\), or used the earlier generations
of DXA technology. Furthermore, there appears to be some disagreement as to the time to reach steady state values of aBMD after SCI. This lack of agreement as to the length of time to reach a plateau with regard to bone deterioration in skeletal regions of interest (ROI) after SCI requires further investigation with the application of the latest generation DXA technology with aBMD regions captured at the hip [femoral neck (FN) and total hip (TH)] and knee (DF and PT) that use software applications that are specially designed to account for soft tissue distribution around the knee. In persons with SCI who were stratified into subgroups based on TSI epochs and in an able-bodied reference (ABref) group, aBMD at the DF, PT, FN, and TH was measured by DXA and displayed as a function of duration of injury.

3.5: Statistical Analysis:

Results are expressed as group mean ± standard deviation (SD). To determine the effects of increasing TSI on changes in aBMD after SCI, participants were stratified into 5 subgroups by TSI epoch (E) (Szollar et al., 1998; Zehnder et al., 2004): E-I: TSI <1 yr, E-II: TSI 1-5 yrs., E-III: TSI 6-10 yrs., E-IV: TSI 11-20 yrs., E-V: TSI >20 yrs. Separate factorial ANOVAs were used to determine the differences between the ABref and 5 SCI groups for each of the continuous variables [age, height, weight, BMI, TSI, aBMD for the DF, PT, FN, TH, and Z- and T-scores for the hip ROI]. Furthermore, a chi-squared
analysis was performed to determine the confounding effect of completeness of motor lesion, level of lesion, and gender on the degree of bone loss after SCI. The subgroups were compared using a one-factor (6 levels) multivariate analysis of covariance (ANCOVA), with BMI used as a covariate. To further identify specific subgroup differences, a Bonferroni post-hoc analysis was used to control for multiple comparisons. A chi squared analysis was performed to determine if significant differences existed between groups for the proportion of participants with FN and TH reference scores below the ISCD thresholds to identify low aBMD for a given age and to diagnose osteoporosis. To determine the first order exponential decay (Edwards, Schnitzer, & Troy, 2014b; Eser et al., 2004), curves were fit to the aBMD of the DF, PT, FN, and TH as a function of TSI using the following equation (Coupaud, McLean, & Allan, 2009; Edwards et al., 2015):

\[ Y = A \exp(-bt) + C \]

Defined where \( Y \), is the aBMD ROI; \( A \), is the loss amplitude; \( b \), the loss rate; \( C \), aBMD steady state (BMDSS); and \( t \), time in years. For each aBMD region, the mean time period where 95% of the decrement (\( t_{95} \)) occurred was then calculated:

\[ t_{95} = -\ln(0.05)/b \]

For participants with TSI ≥ \( t_{95} \) and aBMD value that has reached the new steady state, the mean and standard deviation was calculated for each
Taking into account participant variance for the time to reach steady state ($t_{ss}$), the actual $t_{ss}$ was defined as the mean ± 0.55 SD for those participants with TSI $\geq t_{95}$ (Edwards et al., 2015; Eser et al., 2004).

$$t_{ss} = -\ln(0.5 \times SD/A)/b$$

The calculated steady state values for $aBMD$ ($aBMD_{ss}$) for regions in the $SC_{total}$ groups were compared to the $AB_{ref}$ group using independent sample t-tests and percent difference calculated. Statistical analyses were completed using IBM SPSS Statistics version 22, IBM, Armonk, NY and graphs were generated by Prism GraphPad, version 6.0 for Windows, GraphPad Software, San Diego, CA. An a priori level of significance was set at $p \leq 0.05$.

**3.6: Results:**

A significant omnibus group main effect was found for all continuous $aBMD$ and reference scores when the SCI group was stratified by TSI epochs ($P < 0.001$). With exceptions of the comparisons between E-III vs. E-II, E-V vs. E-IV, and $AB_{ref}$ vs. E-I, post-hoc analysis revealed significantly lower mean $aBMD$ and reference scores as the SCI TSI epochs increased (Appendix B, Table 5). Compared with the $AB_{ref}$ group, significantly lower $aBMD$ and reference scores were noted at all comparisons except for the E-1 subgroup, as previously noted. The same paired subgroup comparisons were performed to identify the percent of participants in
each TSI with ISCD cutoff values for identifying bone loss for a given age (Z-scores ≤ -2.0 SD) and for diagnosing osteoporosis (T-scores ≤ -2.5 SD); a significantly greater proportion of participants fell below these threshold values for Z- and T-scores as TSI epochs increased (P < 0.001). Post-hoc comparisons revealed a greater number of group comparisons falling below the Z-score threshold compared to the T-score threshold. No participants in the ABref and E-I subgroup had reference scores below the thresholds of Z- and T-scores for low bone mass or osteoporosis. In the other SCI groups, chi squared comparisons between the Z- and T-scores demonstrated a significantly greater percent of participants with Z-scores ≤ -2.0 SD in the E-II epoch but there was a similar number of participants below the thresholds for Z- and T-scores to diagnose low bone mass or osteoporosis in all the other SCI subgroups (Appendix B, Table 5).

There was a precipitous decrement followed by an eventual aBMDss as demonstrated by the exponential decay curves as a function of TSI. TSI was a significant factor affecting bone loss at the DF, PT, FN, and TH with the curves explaining 46%, 49%, 32%, and 43% of the variance in aBMD loss with a tss observed for each region at 14.6, 11.3, 14, and 6.2 years after SCI, respectively (P < 0.001, Appendix A, Figure 5 A-D). Furthermore, compared to the ABref group, the aBMDss was 42% lower at the DF (ABref aBMD = 1.037 g/cm² vs. SCI aBMDss = 0.677 g/cm², P < 0.001), 49.6% lower at the PT (ABref aBMD = 1.320 g/cm² vs. SCI aBMDss = 0.795 g/cm², P <
0.0001), 40% lower at the FN (AB_{ref} aBMD=1.075 g/cm^2 vs. SCI aBMD_{ss}=0.718 g/cm^2, P < 0.001), and 30% lower at the TH (AB_{ref} aBMD=1.023 g/cm^2 vs. SCI aBMD_{ss}=0.714 g/cm^2, P < 0.0001, Appendix A, Figure 5 A-D).

Findings from this analysis were published in the *Journal of Clinical Densitometry* (Cimigliaro et al., 2019).
CHAPTER IV:

PROSPECTIVE STUDY:

4.1: Introduction

Using quantitative analysis of tissue from bone biopsies immediately after spinal cord injury (SCI), an uncoupling of the osteoblast/osteoclast relationship that is supported by the clinical findings of hypercalciuria and dramatically elevated markers of bone resorption (Chantraine et al., 1986; Minaire et al., 1974; Roberts et al., 1998). The dissociation of bone resorption from bone formation results in the most precipitous bone loss occurring during the first 12 to 24 months after acute SCI at a rate that may be as great as 4% per month in areas rich in trabecular bone and 2% per month in areas that are predominantly cortical bone (Biering-Sorensen et al., 1990; Dauty et al., 2000; Wilmet et al., 1995). The loss of structure and strength in the lower extremities places a person with SCI at an increased risk of fracture, with the epiphyses of the distal femur (DF) and proximal tibia (PT) being the skeletal regions most vulnerable to fracture with falls from a wheelchair (Morse, Battaglino, et al., 2009). Even though extreme demineralization occurs throughout the entire lower extremity, the knee is most vulnerable to fracture in persons with SCI because a large fraction of
accidents occur from low velocity falls from wheelchairs that makes the DF and PT the first points of contact to absorb the loading forces upon impact with the ground (Akhigbe et al., 2015; Carbone et al., 2013).

DXA-derived aBMD values cannot be directly utilized to diagnose osteoporosis because these values vary in normal individuals by skeletal region assessed (Schousboe, Tanner, & Leslie, 2014) and because different company-specific calibration standards are used by DXA manufacturers [General Electric (GE) Lunar (now GE Healthcare), Hologic Inc., and Norland] (Watts et al., 2013) to measure aBMD. Thus, to standardize results across multiple skeletal regions and among DXA manufacturers, a computed statistical value, the T-score, was introduced and accepted to permit comparisons between the aBMD of a given individual against that of a reference aBMD in young-healthy adults. The National Health and Nutrition Examination Survey (NHANES) III data collected between 1988 and 1994 [Caucasian (non-race adjusted) female (ages 20-30 years old)] aBMD reference dataset in young-healthy adults is the International Society for Clinical Densitometry (ISCD) recommended universal reference database to calculate T-scores (Looker et al., 1998; Watts et al., 2013), and the generation of a new reference dataset should ideally be in agreement with NHANES III. Furthermore, the ISCD Task Force on Normative Databases and the World Health Organization (WHO) diagnosis of osteoporosis established the guideline of a T-score ≤ 2.5 standard deviations (SD) from the mean as
the cutoff value at the femoral neck (FN) and total hip (TH) regions in post-menopausal women and in men ≥50 years old (Schousboe et al., 2013; Watts et al., 2013). While the introduction of T-score cutoff criteria has improved assessment of fracture risk considerably compared to aBMD, several studies have demonstrated that the use of T-score values have not been completely successful in reaching agreement in T-scores among the DXA densitometer manufacturers (Faulkner, Roberts, & McClung, 1996; Kiebzak, Binkley, Lewiecki, & Miller, 2007) and a reference dataset (Binkley et al., 2005). Furthermore, while the use of a female Caucasian reference dataset to calculate T-scores and to diagnose osteoporosis in men is standard practice because men and women fracture at similar aBMD values (Watts et al., 2013), this practice is remains somewhat controversial in the AB population (Faulkner & Orwoll, 2002; Richy, Gourlay, Garrett, Hanson, & Reginster, 2004) with no evidence to support the application of this concept in persons with traumatic SCI.

The advent of powered exoskeletal-assisted walking (EAW) for persons with SCI, as well as other advanced rehabilitation interventions, has made well-defined metrics of fracture risk of clinical relevance to minimize the risk of causing a fragility fracture. The existence of reference aBMD values to calculate T-score values at the DF and PT would allow clinicians and therapists to evaluate aBMD of a given individual against a reference aBMD in young-healthy adults. The first ISCD Task Force guidelines entitled
“Bone Mineral Density Testing in Spinal Cord Injury” recommended a local reference dataset and calculator from a university research center to obtain T-score values at the DF and PT in persons with SCI (Morse et al., 2019); however, this aBMD reference dataset has not been peer reviewed and published. In addition to this local reference dataset which was generated using a Hologic densitometer, it is important for investigators to report manufacturer-specific, homogeneous young-healthy able-bodied (YHAB) reference aBMD datasets at the DF and PT. Using a GE densitometer, a homogeneous YHAB reference database of aBMD values was obtained to calculate T-score values from aBMD values in a historical cohort of SCI individuals to achieve the following objectives: (1) to determine if differences exist for aBMD values at each of the regions of interest (e.g., TH, DF, and PT) among subgroups stratified by age in the YHAB reference dataset; (2) to calculate T-score values at the TH, DF, and PT in the SCI group as a function of TSI epochs using the YHAB reference dataset and to compare the YHAB T-score (T-score_{YHAB}) values at the TH, DF, and PT to the manufacturer derived GE/NHANES T-score (T-score_{GE/NHANES}) values at the TH; and (3) to determine the diagnostic agreement using two T-score cutoff values derived from the GE/NHANES reference dataset at the TH (one T-score cutoff value accepted for the diagnosis of osteoporosis and the other one applied as an exclusion criterion for EAW training) and the YHAB reference dataset at the DF, PT, and TH.
4.2: General Procedures:

A prospective homogeneous dataset of YHAB aBMD values at the hip (TH) and knee (DF and PT) regions were obtained to use as a reference dataset to calculate T-score values from previously published aBMD values at the DF and PT in a cohort of individuals with SCI (Cimigliaro et al., 2019). Calculation of T-score values in persons with SCI at the DF and PT score using the YHAB mean aBMD values were the regions of interest (ROI) for the primary outcomes, with YHAB T-score values of the TH also obtained to permit comparisons to be made to GE/NHANES T-score values at the TH. Premenopausal woman and men under the age of 50 years are recommended to be compared to an age-matched aBMD reference dataset for the calculation of the Z-score. However, because T-score values have been used in the general population to identify persons with osteoporosis at heightened risk to sustain a fracture and the purpose of this study was to more accurately identify persons with SCI who are at heightened risk of fragility fracture, T-score values were applied, rather than Z-score values, in persons with SCI represent who are appreciated to be a unique population with severe sublesional bone loss. All data were collected from the clinical research units at the James J. Peters VA Medical Center (JJ VAMC) and the Kessler Institute for Rehabilitation (KIR), and administrated at Rutgers University (RU). All aspects related to the study design and methods were reviewed and approved by the
institutional review boards at the JJP VAMC, KIR, and RU (administrative approval only). After meeting the inclusion criteria, participants were provided information with respect to the study and informed consent was obtained before participation in these studies. All data analyzed for this work were collected in accordance with the Declaration of Helsinki.

**Participants:** DXA scanning of the total body (TB), DF and PT, and TH was performed in 32 AB male and 32 female Caucasian YHAB participants (n=64 total) between the ages of 20-40 years with the purpose of developing a local reference database of aBMD values at the DF and PT. A TB scan was performed to control for differences in body composition (fat and lean tissue) that may have the potential to influence BMD values. To ensure adequate representation within the YHAB dataset, 8 participants were matched into each of the following 4 age subgroups: 21-25, 26-30, 31-35, and 36-40 years of age. To be included in the study, participants were Caucasian AB male and female persons between the ages 20 and 40. The exclusion criteria included: a history of metabolic bone disease; history of fragility fracture; glucocorticoid therapy for ≥ 3 months; thyroid disease; parathyroid disease; hypercalciuria; renal failure; chronic liver disease; rheumatoid arthritis; gastrointestinal disease; hypogonadism; amenorrhea; cardiovascular and pulmonary disease; cancer; chronic use of heparin, opioids, anticonvulsants, lithium, loop diuretics; alcohol
consumption defined as ≥ 3 alcoholic drinks per day; current cigarette smokers; current use of illegal narcotics; history of anabolic steroid use or clinical testosterone replacement therapy; body mass index (BMI) ≥ 35 (obesity class II) for both men and women ("Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health," 1998); bilateral knee and/or hip replacement; metal hardware in both lower extremities; and joint contractures. The mean aBMD values prospectively collected in the YHAB cohort were then used to calculate T-scores in a historical cohort of traumatic, chronically injured, non-ambulatory (wheelchair reliant 100% of the time), motor complete and motor incomplete [International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) A, B, and C] persons with SCI (n=105) [20], who were stratified into 5 epochs of TSI (E-I: TSI <1 year, E-II: TSI 1-5 years, E-III: TSI 6-10 years, E-IV: TSI 11-20 years, E-V: TSI >20 years).

4.3: Data Collection Methodology:

**Dual Energy X-ray Absorptiometry (iDXA, GE Lunar):** The aBMD values and reference scores were obtained by DXA (GE iDXA, all software versions 16.0; EnCORE, GE Medical Systems, Madison, WI). To minimize inter-rater variability, one International Society for Clinical Densitometry (ISCD) certified technician with more than 15 years of experience performing DXA
imaging in persons with SCI, at both the KIR and JJPVAMC sites, performed the acquisition and analysis of all DXA scans in the historical SCI cohort and current studies. As part of quality assurance procedures in our unit, a spine phantom (i.e., aluminum spine L1-L4 encased in acrylic) was scanned >300 times over a 5-year period and the coefficient of variation was found to be <1% (CV = 0.54 ±0.06). Thus, there was negligible drift in the precision of DXA technology during the period of time during which these scans were performed. Appreciating that there were no significant differences observed between the right and left sides for each ROI, the values for aBMD of the sides were averaged. For acquisition of the DF and PT aBMD, and their use in the subsequent computation of T-scores, DXA scans were acquired with the use of a Food and Drug Administration (FDA)-approved orthopedic knee software program with a custom ROI, as previously used in persons with SCI (Bauman et al., 2015; Cimigliaro et al., 2019; Oleson et al., 2020); to obtain accurate assessment of aBMD at the DF and PT metaphysis, this method was employed to avoid overlap with the patella and fibula and has been shown to have a high reliability among multiple raters with intra-class correlation coefficient values of 0.98 for the DF and 0.89 for the PT (Shields et al., 2005); with this custom ROI was applied to exclude SCI participants from participating in EAW training in a recent large nationwide clinical trial (Spungen et al., 2020). The TH aBMD values were obtained using the standard proximal femur software provided by the
manufacturer. To account for machine calibration differences for imaging centers that utilize GE Lunar densitometers, the manufacturer applied validated conversion equations to the NHANES III data (obtained by Hologic densitometers) and created a GE Lunar equivalent aBMD mean and SD for the TH for men (mean = 1.101 and SD = 0.144) and women (mean=1.008 and SD=0.126) (Lu, Fuerst, Hui, & Genant, 2001), yielding the combined GE/NHANES reference aBMD dataset at the TH (Binkley et al., 2005; Watts et al., 2013). This combined GE/NHANES reference dataset has been shown to have strong diagnostic agreement when compared to using the NHANES III reference dataset alone at the TH region (Binkley et al., 2005).

**Questionnaires:** In the YHAB reference group, data on general exercise habits were collected utilizing the bone-specific physical activity questionnaire (BPAQ) index. The BPAQ is a validated physical activity questionnaire geared toward understanding the relationship between BMD and the exercise history of an individual throughout the lifetime from that of sports to general exercise, and the BPAQ has been shown to be significantly correlated with aBMD at the TH as well as volumetric BMD and metrics of bone strength at the distal tibia in men and women (Bolam et al., 2014; Kim, Baker, Sharma-Ghimire, Bemben, & Bemben, 2018). The BPAQ was created by considering bone-relevant mechanical loading by incorporating load intensity and the number of years and frequency of a
given activity (Weeks & Beck, 2008). Once the survey was completed by each participant that was part of the YHAB reference dataset, a single investigator was used to enter the BPAQ values into an online calculator (www.fithdysign.com/BPAQ/) and a single total BPAQ (tBPAQ) score was generated. In addition to the tBPAQ, calcium and vitamin D intakes were obtained in the YHAB reference group using the Block Calcium and Vitamin D screening questionnaire (Nutrition Quest, Berkeley, CA) to assess if our YHAB cohort had adequate nutritional intake over the previous 12 months of these two essential items to maintain bone health. This truncated questionnaire and has been highly correlated to validated more comprehensive assessments of calcium and vitamin D intakes (Cummings, Block, McHenry, & Baron, 1987).

**Pregnancy Testing:** All female participants were asked to complete a pregnancy test to ensure they were not pregnant. If the test shows that a female participant is pregnant then she will be withdrawn from the study.

**Risks and Discomforts:** The study may involve the following risks and/or discomforts:

**DXA:** There is minimal discomfort associated with the bone density studies. Bone density studies are associated with a small risk from radiation; the total amount of radiation exposure from the DXA will be no greater than 31 µSv, which is approximately half as much radiation exposure than a routine chest x-ray (PA film, ~ 60 µSv). This quantity of
radiation exposure is approximately four times the amount of radiation received daily from normal background radiation (~ eight µSv). In addition, it has been estimated that the average person in New York City receives approximately 300mR/year (e.g., conversion to 3000 µSv/year); thus, the subjects would have to have DXA imaging performed 97 times to receive an equivalent dose of radiation exposure (see Table 4 for a breakdown of effective radiation exposure).

**Limits for radiation exposure:** Occupational effective dose (20mSv/annum); Public (1mSv/annum) [ICRP Publication 60. 1991. 1990 Recommendations of the International Commission on Radiographic Protection. P1-201]

**4.4: Statistical Analysis:**

All data in the YHAB population was used to calculate the T-score values (T-score$_{YHAB}$) in each TSI epoch and in the total SCI cohort. Demographic and clinical characteristics (e.g., age, gender, ethnicity, height, weight, BMI, duration of injury, tBPAQ scores, calcium and vitamin D intake, risk factors for fracture risk (smoking and alcohol consumption), and T-scores at the DF, PT, and TH and were obtained and expressed as frequency and percent or mean ±SD as appropriate.

**Primary Aim III- A:** To achieve our primary objective to determine the mean aBMD for the DF and PT in a sufficiently powered young-healthy
able-bodied (YHAB) cohort, the mean aBMD difference in a convenience sample of chronic SCI (n=27) and AB (n=12) participants between the age of 20-40 was used to estimate the sample size needed. Using this comparison and a power for the primary outcome set at 80%, with a significance level set at α=0.05, eight subjects for each of the four age subgroups (21-25, 26-30, 31-35, and 36-40 years of age) provided ample power within the groups of men and women (n=32 each group) for a total of 64 participants required for the study (Effect Size $d=1.35$, critical $t=1.76$). All regional YHAB aBMD values that were used to calculate the T-score values were tested for a normal distribution using the Shapiro-Wilk test (S. S. Shapiro & Wilk, 1965) with kurtosis and skewness calculated to determine if they are within the $\pm 1.96 SD$ threshold. Additional visual inspection of histograms, boxplots, normal Q-Q plots were also completed to identify potential outliers. To control for the type-1 error rate across analyses of variance, a multi-variate analysis of covariance (MANCOVA), using fat and lean tissue mass percent as covariates in the analysis, was used and then decomposed into constituent ANOVAs to compare aBMD across the 4 age subgroups (21-25, 26-30, 31-35, and 36-40 years of age) at the DF, PT, and TH.

**Primary Aim III- B:** From this reference YHAB dataset, T-score values at the DF, PT, and TH regions were calculated in the 5 TSI epochs and total
SCI group using aBMD values previously published in this SCI cohort (Cimigliaro et al., 2019) using the following formula:

\[
T\text{-score} = \frac{\text{aBMD (SCI individual)} - \text{mean aBMD [ABYH subgroup (20-40 years)]}}{\text{SD (from the mean aBMD ABYH)}}
\]

The calculated T-score values were compared among these 5 SCI subgroups using a one level (5-factor) MANOVA. Independent sample t-tests were also used to compare the SCI calculated T-score at the DF, PT, and TH using the YHAB reference dataset (T-score_{YHAB}) to the T-score values at the TH that utilized the GE/NHANES combined young-adult aBMD reference dataset (T-score_{GE/NHANES}).

**Primary Aim III- C**: To determine agreement between the reference datasets (T-score_{YHAB} versus T-score_{GE/NHANES}) in the ability to diagnosis osteoporosis, the number of participants classified with osteoporosis was expressed as a percent and then tested using Cohen’s kappa agreement statistics (Landis & Koch, 1977). The kappa statistic estimates the agreement level beyond chance by calculating the expected versus observed cases that agree compared to the null hypothesis. Classification was performed for the total SCI group (1) using the TH GE/NHANES reference dataset to diagnose osteoporosis at the TH by the accepted T-score cutoff value (TH_{GE/NHANES}) and (2) using the YHAB reference dataset the TH T-score (TH_{YHAB}) and the DF and PTT-score
values were determined (DF/PTYHAB) at two T-score cutoff values: <-2.5 SD (WHO criteria to diagnose osteoporosis) and <-3.5 SD (TH T-score cutoff value commonly used to exclude SCI participants from powered EAW training).

Statistical analyses were completed using IBM SPSS Statistics version 22, IBM, Armonk, NY, graphs were generated by Prism GraphPad, version 6.0 for Windows, GraphPad Software, San Diego, CA and calculations to estimate sample size were completed using G*Power [G*Power 3.0.1.0, Franz Faul, Universität, Kiel, Germany (Faul, Erdfelder, Lang, & Buchner, 2007)]. An a priori level of significance was set at p≤0.05 for all analyses completed.

4.5: Results:

Descriptive statistics for the YHAB sample are presented in Tables 7 and 8. The male and female subgroups were not significantly different for smoking, alcohol use, calcium and vitamin D intake, and tBPAQ index scores (Table 7). The total BPAQ index scores reported are similar to those previously reported in physically active young adult men and women (Kim et al., 2018; Rantalainen, Weeks, Nogueira, & Beck, 2015).

**YHAB Reference aBMD Values at the Distal Femur and Proximal Tibia (Primary Aim III- A):** Normality testing using the Shapiro-Wilk test and skewness and kurtosis standards (±2 SD) revealed a normal distribution in
the YHAB study dataset of aBMD values at the DF, PT, and TH within each of the four age subgroups for each of the sexes (Table 1). There was a significant omnibus main effect ($P < 0.001$) among the men’s age subgroups for all body habitus variables [height, weight, BMI, total body fat percent (TBF%), and total body lean percent (TBL%)], but no significant difference observed between the women’s age subgroups (Table 8). In the men’s age subgroups, post-hoc comparisons revealed a significant difference between the 21-25 vs. 31-35 ($p \leq 0.01$), 31-35 vs. 36-40 ($P < 0.05$), and 26-30 vs. 31-35 ($P < 0.05$) age subgroups for all the body habitus variables presented. As a result of these differences, TBF% and TBL% (body habitus variables that have been demonstrated to have the most impact on bone density) were used as covariates when comparing aBMD values at the DF, PT, and TH across the age subgroups in men (Table 7). The results of the MANCOVA revealed that there were no significant differences in mean aBMD across the four age subgroups in either sex; as such, the mean aBMD of the subgroups was calculated and presented as total men and total women at the DF, PT, and TH. The aBMD was, as expected, higher in the total men compared to the total women group for all of the hip and knee regions presented ($P < 0.001$, Table 8), a difference that should be considered when deciding whether to use a sex-specific reference database to calculate T-score in men and women with SCI.
Distal Femur and Proximal Tibia T-score Stratified by Epochs of Time Since Injury (Primary Aim III- B): In the SCI cohort, a significant omnibus group main effect was found across all epochs of TSI for TH T-score\textsubscript{GE/NHANES} values and for the DF, PT, and TH T-score\textsubscript{YHAB} values (P < 0.001, Table 9). Post-hoc analysis of the T-score\textsubscript{GE/NHANES} value for each TSI epoch revealed significantly different values between all comparisons with the exception of the E-IV vs. E-III epochs for the TH (Table 9). Additional post-hoc analysis of the T-score\textsubscript{YHAB} values for each TSI epoch revealed significantly different values between all epoch comparisons for the TH, the E-IV vs. E-III epoch comparison for the DF, and the E-IV vs. E-III and the E-V vs. E-III epoch comparisons for the PT (Table 9). At each TSI epoch and for the total SCI group, the TH paired comparisons revealed T-score\textsubscript{YHAB} values that were significantly lower than the T-score\textsubscript{GE/NHANES} values. Contrary to this finding, DF and PT T-score\textsubscript{YHAB} values were similar to TH T-score\textsubscript{GE/NHANES} values for most of the epochs of TSI, with the exception of the E-V epoch at the DF and the E-III epoch at the PT (Table 9). These findings reveal that T-score values, regardless of the reference dataset applied, decrease as a function of TSI and the TH T-score\textsubscript{GE/NHANES} values are similar to the DF and PTT T-score\textsubscript{YHAB}, but not the TH T-score\textsubscript{YHAB} values.

Diagnostic Agreement: GE/NHANES III vs. YHAB Reference Datasets (Primary Aim III- C): For the total SCI group, percent and Cohen's Kappa agreement for the classification of osteoporosis between the GE/NHANES
reference dataset at the TH and the YHAB reference datasets at the DF, PT, and TH are presented (Table 10). The agreement is expressed as the percent of cases that diagnose osteoporosis using the T-score cutoff of <-2.5 SD WHO criteria. To control for the amount of agreement that could occur by chance, the Kappa agreement statistic was also calculated from the observed and expected count between the two reference datasets at the two T-score cutoff values. The Kappa agreement was considerably lower than the percent agreement when comparing T-score from the THGE/NHANES vs. THYHAB and THGE/NHANES vs. DF/PTYHAB reference datasets, with the <-2.5 SD cutoff value demonstrating only moderate kappa agreement (>0.41) and the <-3.5 SD SD cutoff value demonstrating only fair kappa agreement (0.21-0.40) (McHugh, 2012; Viera & Garrett, 2005) (Table 10). In other words, when controlling the level of agreement that occurs by chance, the -2.5 SD T-score cutoff demonstrated better agreement than the -3.5 SD T-score for diagnosing osteoporosis when comparing the THGE/NHANES vs. DF/PTYHAB and the THGE/NHANES vs. DF/PTYHAB reference datasets. This moderate of agreement supports the use of the YHAB dataset to calculate the T-score at the WHO value to diagnose osteoporosis but only displays a fair level of agreement for a markedly lower T-score cutoff value.
CHAPTER V:

SUMMARY OF RESEARCH FINDINGS:

5.1: Specific Aim- I: Summary of Findings: To determine if T-score values at the FN and TH can be used to estimate aBMD values at the DF and PT.

This is the first report to develop regression equations to predict T-scores at the TH and FN regions from aBMD values at the DF and PT and provide statistics with diagnostic accuracy from this relationship. When using the cutoff value at the knee of <0.6 gm/cm², as described by Garland et al., (D. E. Garland, Adkins, Kushwaha, et al., 2004; D. E. A. Garland, H; Stewart, C.H., 2005) as the independent predictor of risk of fracture, the question arises as to whether the T-score values at the hip are different than the WHO criteria to diagnose osteoporosis, defined as a T-score of < -2.5 SD below the mean. Because fractures of the leg have been reported to occur more frequently below a BMD value at the knee of 0.6 gm/cm², this value for BMD at the DF and PT was found to be equivalent to T-scores at the TH of -3.1 and -3.5 and T-scores at the FN of -2.6 and -2.9, respectively. Despite the moderate strength of our prediction equations, which have been demonstrated to account for between 50% and 60% of the variance in TH and FN T-score values, our model still reveals considerable inaccuracies when comparing the proportion of predicted values outside
the clinically acceptable limits of agreement (±1 SD), with the highest
degree of predicted errors occurring when T-scores are < -2.0 SD from the
mean. This is of concern because of the high proportion of TH and FN T-
scores < -2.0 in persons with chronic SCI (Fattal et al., 2011). In addition to
the linear modeling, utilizing the same aBMD cutoff value of < 0.60 g/cm²
at the DF or PT to differentiate participants with increased risk of fracture,
receiver operating characteristic (ROC) regression was also performed.
Extrapolating the intersecting point along the curve that provides the best
balance between sensitivity and specificity, a T-score cutoff of -3.1 SD at
the TH and -2.4 at the FN could diagnosis osteoporosis at the knee region
95% and 92% of the time. Our work provides empirical data, and thus
clarifies what BMD values at the DF and PT relate to T-score values at the
TH and FN. Overall, these findings strongly support the need to diagnose
osteoporosis and fracture risk by acquiring aBMD of the DF and PT in
persons with SCI. Furthermore, while the cutoff values at the knee of < 0.6
gm/cm² are the best cutoff values for fracture available at this time,
normative reference data at the DF and PT (T-scores) that control for
differences in aBMD calculations between densitometer manufacturers
would allow clinicians to more accurately diagnosis osteoporosis in
persons with SCI.

Having predicted T-score values at the hip from aBMD at the knee, the
reverse may then be performed: the ability to predict aBMD values at the
knee from T-scores at the hip can also be performed. This approach has clinical relevance because the ability to acquire aBMD at the knee is not currently available in most clinical settings. If healthcare professionals had the ability to directly measure aBMD at the knee and exclude patients with SCI who have a heightened risk of fracture from participating in upright ambulatory activities, then this exercise would be unnecessary. However, for clinicians who do not have the resources to capture aBMD of the DF and PT because of the unavailability of knee software, adequate technician knowledge and experience, and/or the absence of third-party reimbursement, identification of a TH or FN T-score value that can be used as a surrogate marker to identify individuals with SCI at the greatest risk of fracture at the DF or PT has clinical utility. A lower sensitivity was observed at the FN, compared to the TH for both the T-scoreWHO and T-scoreYI criteria (See Table 2). Comparing the sensitivity and specificity between the T-scoreWHO (-2.5 SD), the T-score commonly used to exclude participants from EAW upright ambulation (-3.5 SD), and the T-score value identified from the regression and diagnostic accuracy techniques (-3.1 SD), the best balance between sensitivity and specificity was demonstrated at a T-score cutoff of -3.1 SD (See Table 3). Using a T-score cutoff of -2.5 SD at the TH/FN, the number of SCI individuals diagnosed as having a high fracture risk at the DF/PT (true positive cases, n=17), is at the expense of a considerable number of SCI individuals misdiagnosed as
having a high fracture risk [HFR, FP cases (n=20)], a cutoff value that could result in a considerable number of SCI participants denied entry into upright ambulatory EAW. Conversely, a diagnostic scenario of greater clinical impact can be observed using the T-score cutoff -3.5 SD at the TH/FN. Using this cutoff value, a considerable number of SCI participants with HFR at the TH/FN, would be misdiagnosed as having a lower fracture risk [LFR, FN cases (n=10)] at the DF/PT, and would be allowed entry into upright ambulatory EAW. This evidence presented suggests that the use of a T-score of -3.1 SD for TH, a cutoff value predicted at the TH from regression and diagnostic accuracy analysis in the study cohort presented, would yield the greatest balance in maximizing entry into advanced upright rehabilitation therapy who have a LFR, while restricting the majority of participants at the HFR, when direct measurement of aBMD at the DF and PT is not possible. However, each physician must weigh the potential risks and benefits of a more conservative or more liberal cutoff value for the TH T-score for the particular patient being offered the upright rehabilitation therapy. These findings were recently accepted for publication in the Journal of Spinal Cord Medicine (Cimigliaro et al., 2020).
5.2: Specific Aim II: To determine if aBMD values decrease as a function of time since injury at the DF and PT compared to the demineralization at the FN and TH regions.

While the general relationship between bone loss and TSI confirms prior work, there are several novel and clinically relevant observations from the findings in this report. Despite a similar mean age of participants among the first 4 groups, our observations demonstrated that compared with the mean aBMD values in the ABref and E-I group, a significant decline in mean aBMD and reference scores was observed after the first decade of injury (E-II and E-III), demineralization continued for 11-20 years after SCI (E-IV), with no further decrease noted in aBMD and reference scores in the epoch >20 years (E-V) after SCI. As anticipated, a significant number of SCI participants fell below the thresholds for Z- and T-scores to diagnose low aBMD or osteoporosis as TSI epochs increased. Our findings confirm previous reports of severe bone loss after SCI and the effect of duration of injury on bone deterioration, which continues to approach or exceed the fracture threshold as TSI advances. However, and in contrast to earlier reports in the literature that have performed similar cross-sectional studies with DXA or peripheral quantitative computed tomography (pQCT) methodologies, our observations lend strong support to the progressive loss of aBMD into the second decade after the acute immobilizing event.
even in regions where the predominant component of bone is trabecular (e.g., DF and PT).

Several smaller cross-sectional and prospective reports have quantified the degree of bone loss after SCI using earlier generation DXA technology and indirect software applications (forearm and anterior-posterior spine) to measure aBMD at the DF and PT regions (Biering-Sorensen et al., 1990; D. E. Garland, Adkins, Scott, et al., 2004; D. E. A. Garland, H; Stewart, C.H., 2005; Shields et al., 2005). These reports found that DF and PT aBMD values were 50% lower than that of an age-matched AB reference cohort (Biering-Sorensen et al., 1988; D. E. A. Garland, H; Stewart, C.H., 2005). In these prior studies, earlier DXA technology and nonspecific software applications (anterior-posterior spine) were used to measure aBMD at the DF and PT regions. Of note, a DXA machine by a different manufacturer (GE Lunar iDXA) that utilized a proprietary specialized software package (Orthopedic Knee software application) was used in the current study to measure aBMD of the DF and PT; the average aBMD loss in the SCI group was still marked but less severe than that previously reported. These differing reports are most likely attributable to the smaller sample sizes and the large variability of aBMD loss among individuals, but may have also been confounded by differences in DXA technology in acquiring and analyzing the images. One of the initial reports to stratify aBMD by TSI in chronic SCI (>1-year post SCI) revealed a decrease in mean aBMD at the
FN 1-9 years after SCI, with no additional decrease 10-19 and 20-29 years after SCI (Szollar et al., 1998). In a later report that confirmed these earlier findings using a similar study design, Zehnder et al. (Zehnder et al., 2004) studied aBMD of the FN and PT in 100 participants with acute and chronic paraplegia categorized by TSI (<1 year: stratum 1; 1-9 years: stratum II; 10-19 years: stratum III; 20-29 years: stratum IV). Comparison between these strata revealed that bone loss did not significantly decrease past the 1-9 years after SCI in almost all regions, including the femoral neck and distal tibial epiphysis. The curvilinear relationship between FN Z-score and TSI revealed an aBMDss that was reached 1 to 3 years after SCI; however, Z-scores of the tibia diaphysis did indeed continue to decrease >10 years after SCI. By pQCT methodology, Eser et al. reported losses in bone mass at the distal epiphyses of the femur and tibia of 50% and 60%, respectively, with steady state values being achieved after 2.9 years at the femur and 4.8 years at the tibia; loss of bone at the diaphyses of the femur and tibia occurred by endosteal resorption at a rate of approximately 0.25 mm per year and resulted in a total loss of BMD of 35% in the femur and 25% in the tibia at 6.9 years after SCI (Eser et al., 2004).

Our observation of continued demineralization into the second decade after SCI is a somewhat novel finding, because such progressive, insidious loss of BMD has been observed using other study designs that demonstrated bone loss past the first decade after sustaining a SCI
(Bauman et al., 1999; Modlesky et al., 2004). In a cross-sectional study in monozygotic twins, discordant for SCI, Bauman and colleagues (Bauman et al., 1999) regressed DXA intra-pair difference scores for total leg BMD and bone mineral content (BMC) against TSI and found that bone loss continued over at least three decades after SCI; a strength of these findings was the ability to control for differences in bone loss due to genetic variability among individuals. The results of our curve fitting analysis revealed a loss of bone into the chronic phase of SCI that reached values close to the fracture threshold (D. E. A. Garland, H; Stewart, C.H., 2005). The \( t_{ss} \) at the DF, PT, and FN was 14.6, 11.3, and 14 years after SCI, respectively. The finding of a \( \Delta BMD_{ss} \) for most sublesional ROI extends into the second decade after SCI supports the hypothesis that bone deterioration continues for longer durations of time than previously reported in studies that used similar experimental designs and statistical analyses (Edwards et al., 2015; Eser et al., 2004). At the TH, \( t_{ss} \) was achieved much earlier (6.2 years after SCI) and is consistent with findings from earlier studies. While DXA-derived measures of aBMD loss are a combination of trabecular bone loss and thinning of the endocortical envelope, CT- and peripheral quantitative CT- (pQCT-) derived measures of volumetric BMD (vBMD) can differentiate loss at the trabecular and cortical bone compartments. Recent longitudinal studies in persons with SCI documented the accelerated loss in cortical vBMD at the TH. In a
study by Edwards and colleagues (Edwards, Schnitzer, & Troy, 2013), investigators performed repeated CT measurements at the hip and knee early after SCI and reported a cortical vBMD loss of 0.8-1.0 %/month at the TH and a slower rate of loss of 0.5-0.8 %/month at the DF and 0.3-0.6 %/month at the FN. Earlier DXA reports that investigated bone loss as a function of TSI have accounted for greater variance than the model presented herein; in one report, the tss that did not extend beyond the first decade after SCI using slightly different curve fitting methods, but the finding in this model were likely the result of an extremely small sample size (n=9) (Lobos et al., 2018). In another study that found tss was achieved within the first decade after SCI, Edwards et al. (Edwards et al., 2015), completed a cross-sectional study to determine if the reductions in vBMD at the proximal tibia were a function of TSI. Sixty adults with SCI (TSI 0-50 years) and 10 AB controls completed a CT scan of the PT. Using exponential decay curves to fit the data, TSI explained between 52 and 70% of the variance in the prediction of vBMD loss, with new steady state values reached 2.1 to 2.7 years after SCI, and were 52% to 56% lower than the AB control group. These apparently differing findings in the Edwards et al. study to that of ours may be partially explained by the differences in the distribution of participants along the continuum of TSI, methods employed (CT versus DXA), and the variability of loss of BMD in persons with SCI.
These findings have clinical relevance in the diagnosis and potential treatment of bone loss in persons with SCI. If steady state bone loss is not reached until many years later than expected, clinicians and therapists may have a larger window to prescribe pharmacological and mechanical interventions to prevent the insidious progression of sublesional skeletal deterioration than was previously assumed to be the case. The incidence of fracture increases with TSI, with a mean annual fracture incidence of 1% per year during the first year after SCI, with the incidence increasing to 4.6% per year at 20-29 years after acute SCI [26]. Thus, our findings add credence to reports documenting that the majority of fractures occur during the second decade after SCI, corresponding to the epoch with the maximal loss of bone mass (Akhigbe et al., 2015; Eser et al., 2005; Zehnder et al., 2004). In a retrospective historical analysis by Abderhalden et al. (Abderhalden et al., 2017), the ability of DXA to predict fracture in 22,186 persons with SCI was determined. In the 552 patients who received DXA scans, 47 (8.5%) incurred an incident fracture with a mean (SD) TSI of 18.2 (13.2) years. The authors found that SCI participants who had DXA total hip world health organization reference scores in the osteopenia and osteoporosis range were more than 4 times more likely to fracture when compared to participants with reference scores in the normal range. In this non-ambulatory motor-incomplete
cohort, residual neural activation and motor function below the level of injury was not sufficient to attenuate bone loss over the lifetime.

5.3: Specific Aim III:

A) To determine if the YHAB reference dataset mean aBMD values at the TH, DF, and PT are significantly different between the age subgroups for both men and women;

B) To calculate T-scoreYHAB values at the TH, DF, and PT as a function of TSI in the SCI historical dataset, and to determine if these values differ when compared to the TH T-scoreGE/NHANES values for every TSI epoch; and

C) To determine the diagnostic agreement between two T-score cutoff values derived from the T-scoreGE/NHANES reference dataset at the TH (one T-score cutoff value accepted for the diagnosis of osteoporosis and the other one applied as an exclusion criterion for EAW training) and the T-scoreYHAB values derived from the reference dataset at the TH, DF, and PT.

Similar to previous reports in the AB population, the YHAB aBMD values at the DF, PT, and TH regions were significantly higher in men than women (Schousboe et al., 2014). Despite evidence that aBMD values are higher in men, a young-healthy female reference dataset has been used as the reference database to compute T-score values in men because men and women have been reported to fracture at similar aBMD values when all other risk factors are the same (Kanis, Johnell, Oden, De Laet, &
Mellstrom, 2001; Langsetmo et al., 2010). This decision makes sense since the majority of evidence describing the relationship between aBMD and fracture risk is in able-bodied (AB) older women, with limited evidence describing this relationship in AB men (Melton, Atkinson, O’Connor, O’Fallon, & Riggs, 1998; Van Der Klift et al., 2002), making the need for separate aBMD reference datasets for men unclear at this time. At the current time, the ISCD and the International Osteoporosis Foundation recommend the use of a female reference database to calculate T-score values in men (Kanis et al., 2011; Watts et al., 2013). Contrary to this recommendation, the United States Endocrine Society prefers the use of a male reference database to calculate T-score in men because of the concern that the number of men diagnosed with osteoporosis and possibly treated would be reduced if a female reference dataset is used (Watts et al., 2012). As our understanding of fracture risk in persons with SCI improves, it is essential to establish reference datasets of aBMD values separately in men and women at the DF and PT to ensure that the fracture risk is not underestimated in men with SCI by employing female bone values; any given aBMD in men with SCI may represent more severe bone disease than a comparable aBMD value in women with SCI because of the greater loss of bone integrity and strength. The use of separate aBMD reference datasets is an important consideration in persons with SCI since approximately 80% of new SCI cases are men
("Spinal Cord Injury (SCI) 2016 Facts and Figures at a Glance," 2016). In addition to controlling for sex in this preliminary YHAB reference dataset, only Caucasian (non-hispanic) men and women were enrolled in this study to adhere to the ISCD guidelines as a starting point (Watts et al., 2013). However, understanding that race and ethnicity can have a profound effect on peak bone density (Henry & Eastell, 2000), stratifying by race and ethnicity will influence the direction of the YHAB reference aBMD dataset in the future and will necessitate the collection of data in diverse demographic populations.

Similar to the decline in aBMD values previously published in this cohort (Cimiglio et al., 2019), T-score values decreased as a function of TSI. However, novel to this report is the finding of significantly different T-score values at every TSI epoch when comparing the TH region between the two reference datasets (T-scoreYHAB vs. T-scoreGE/NHANES). Surprisingly, because of the greater variability in T-score when comparing values obtained at a different skeletal region, the T-score values were similar at most TSI epochs when comparing T-scoreGE/NHANES values at the TH to the T-scoreYHAB values at the DF and PT. The discordance in T-score values when using different reference datasets has been well documented in postmenopausal women (Binkley et al., 2005; Faulkner, von Stetten, & Miller, 1999). In a study of 115 postmenopausal women, Binkley et al. (Binkley et al., 2005) compared the original NHANES III to the more recent
GE/NHANES combined reference dataset on T-score values at the femoral neck (FN) and trochanter (TR) regions. The authors found that mean T-score values were lower at the FN by 0.48 SD and at the TR by 0.68 SD than when T-score values were calculated with the GE/NHANES combined dataset. After the application of the validated conversion equations to the NHANES III aBMD values to the GE Lunar aBMD values, these differences were reduced to 0.03 SD at the FN and 0.32 SD at the TR.

The YHAB reference dataset can be used to calculate T-score values from the DF and PT aBMD cutoff values commonly used to exclude SCI participants from powered EAW clinical trials. The aBMD cutoff threshold value of 0.60 g/cm² at the DF and PT was first described by Garland et al., and was defined as the aBMD value below which fractures were observed to more likely to occur (D. E. Garland, Adkins, Kushwaha, et al., 2004; D. E. A. Garland, H; Stewart, C.H., 2005; Lala et al., 2014). Similar to the GE/NHANES TH T-score cutoff value of <-3.5 SD used in the kappa agreement analysis, this criterion has been implemented to exclude SCI participants from EAW in a recent large nationwide clinical trial (Spungen et al., 2020), as well as other advanced rehabilitation programs. Using this YHAB reference aBMD and the T-score equation, the aBMD value of 0.60 g/cm² would have a T-score of -4.1 SD at the DF and -4.3 SD at the PT in men and -2.9 SD at the DF and -3.5 SD at the PT in women. The separate T-score values for both men and women, at both the DF and PT, are
coherent with different aBMD values presented from the YHAB reference dataset. Considering that the WHO criteria—further supported with data from the NHANES III prospective cohort study—demonstrated that approximately 1/3rd of postmenopausal women and men over 50 with T-scores <-2.5 SD will experience an incident fracture, the application of these lower T-score cutoff values at the DF and PT using this reference aBMD dataset (i.e. <-3.5 SD at the TH that has been used to exclude participants from ambulatory interventions such as powered EAW), may be appropriate considering the aBMD values between 0.58 and 0.60 g/cm² at the DF and PT have been the standard to exclude participants from powered EAW in these same studies (Asselin et al., 2015; Knezevic et al., 2021; Ramanujam et al., 2018; Spungen et al., 2020).

5.4: Research Limitations:

In the SCI cohort, information describing the level of physical activity, smoking history, alcohol consumption, fracture history, spasticity, vitamin D concentrations, calcium intake, and medication history was not available to determine the potential effect of these previously reported determinants of skeletal integrity. Despite its obvious clinical feasibility, the use of DXA to evaluate BMD has appreciated drawbacks when applied to identify risk of fracture in both the AB and SCI populations due to the confounding effects of bone size on aBMD (Prentice, Parsons, & Cole,
1994), differences between manufacturer-derived algorithms to calculate aBMD (Tothill, Avenell, & Reid, 1994), and DXA intra/inter-unit problems with accuracy and linearity (Pors Nielsen, Barenholdt, Diessel, Ambrust, & Felsenberg, 1998). It is well known that DXA is a 2D projection scan that does not capture a “true” measure of BMD. Instead, DXA directly measures BMC per image pixel and the area of a specified region to calculate an aBMD in g/cm². Furthermore, DXA does not capture BMD of the cortical and trabecular compartments separately (SL, 2010), a feature that is useful in evaluating bone loss in persons with SCI, because CT studies have demonstrated trabecular and cortical bone is lost at different rates after SCI (Rittweger et al., 2010). The different ratio of cortical and trabecular bone at the different regions will yield significantly different vBMD and T-scores of the cortical and trabecular compartments at the DF and PT. This concept may have a profound effect on bone strength and fracture risk at any given value of total aBMD or vBMD at a specific region (Bouxsein & Seeman, 2009). Obstacles exist in acquiring DXA images of sufficiently high quality in persons with SCI due to heterotopic ossification (extraneous calcification around the hip and knee region), contracture, excessive spasticity, and abnormal soft tissue distribution are obstacles that can also limit the use of DXA in persons with SCI (Troy & Morse, 2015).
There are several limitations associated with the prospective data collection component of this study. Regardless of the power analysis that determined the number of participants 20-40 years of age required for a preliminary homogeneous YHAB reference dataset, the generalizability of these findings is limited by the small sample size. While the young-healthy aBMD values at the FN, TH, and TR from NHANES III (N=409 women and N=382 men) serves as the national reference dataset, several studies have reported normative aBMD data in women and men with smaller sample sizes similar to those reported here, and demonstrated good agreement at the FN and TH (Callreus, McGuigan, & Akesson, 2014; Kroger, Heikkinen, Laitinen, & Kotaniemi, 1992; Paggiosi et al., 2011). This preliminary report should serve as the framework to establish an aBMD reference dataset large enough to achieve substantial kappa agreement scores with the GE/NHANES reference dataset. Furthermore, in the current study, a commonly used custom region of interest (ROI) protocol was used to capture aBMD at the DF and PT in both cross-sectional and longitudinal clinical trials (Bauman et al., 2015; Shields et al., 2005; Spungen et al., 2020). The use of additional DF and PT custom ROI may have different mean and SD aBMD values at the DF and PT and yield T-scores with better agreement than the values presented in this study (McPherson et al., 2014; Shields et al., 2005). To obtain a real-world indication of the relationships of T-scores of the ROI to the risk of fracture, it would be extremely valuable
to obtain T-scores at the DF and PT in prospective cohort study at the time of fracture to the DF or PT. These longitudinal studies are necessary to validate the use of T-score values and the optimum T-score cutoff values where the risk of fragility fracture is has a high probability to occur in the majority of persons with SCI. Despite these limitations, identification of T-scores at the DF and PT by DXA is possible to acquire in an effect to better define the risk of fracture at the knee. This information can guide clinical treatment options and/or research interventions associated with the application of substantial mechanical forces during supervised therapeutic sessions or exercise training routines.

**5.5: Future Directions:**

Future prospective research is needed to expand the sample size of this YHAB homogeneous reference dataset of aBMD values at the DF and PT in both men and women. A YHAB reference dataset of aBMD values at the DF and PT that is similar in size to that used in a national reference dataset, such as NHANES III, would give clinicians and researchers the confidence needed to better evaluate fracture risk in persons with SCI. There is considerable evidence that aBMD values can be confounded by race, ethnicity, and body mass. As a result, it is important to obtain YHAB aBMD values at the DF and PT in different racial and ethnic groups as well as obese individuals [BMI ≥ 35 (obesity class II)], to determine how aBMD
values in these cohorts compare to those presented herein. Furthermore, our investigative group at the JJPVAMC have been developing collaborations with SCI researchers around the world (informal communications), to expanded this YHAB reference dataset by combining local reference datasets of aBMD values at the DF and PT. These initiatives taken as a whole will likely ensure this work continues so a national reference dataset of aBMD values at the DF and PT is ultimately established.

Considerable evidence over the past decade has demonstrated that powered EAW holds promise to improve mobility and medical health and fitness in persons with SCI. Since the inception of these devices into the research environment, the Center for the Medical Consequences for Spinal Cord Injury at the JJPVAMC has been the national coordinating site in the largest nationwide clinical trial sponsored by the Department of Veterans Affairs Cooperative Studies Program investigating the effect of powered EAW on quality of life in persons with chronic SCI. To minimize the risk of fracture to research participants, DXA imaging has been essential to exclude individuals with aBMD values at the DF or PT <0.6 gm/cm² and T-score values <-3.5 SD at the TH. As the senior research coordinator at the JJPVAMC, it has been an honor for me to coordinate all of the DXA imaging in this trial working with more than 10 different VA Medical Centers that utilize GE densitometers to obtain aBMD values at
the DF and PT using the same methodology described in this study. Over the course of this study, aBMD has been obtained on more than 300 individuals with SCI, with the calculation of T-score now possible with the use of this reference dataset. This baseline assessment and collaboration with more than 10 VA Medical Centers, has provided our group the opportunity to establish follow-up assessments by developing a prospective cohort study so aBMD and T-score values can be calculated at the DF and PT at the occurrence of subsequent fractures at these regions, or, as a less acceptable alternative, DXA measurements should be obtained at the knee as soon as possible after a fracture of the leg occurs in persons with SCI. Finally, using this reference dataset of YHAB aBMD values at the DF and PT, a T-score calculator on our research center’s website that is available to the public can hopefully be developed so T-score can be easily calculated by clinicians when aBMD at the DF and PT is available in persons with SCI. This application of the YHAB reference dataset can allow clinicians to better assess fracture risk and more appropriately prescribe ambulatory interventions such as powered EAW.
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Appendix A:

Compendium of Figures:
Figure 1: Illustration of the diaphysis (highlighted green), metaphysis (highlighted yellow), and epiphysis (highlighted blue) that comprises approximately 30% of the distal area of the femur and proximal area of the tibia [adapted from previous work by Edwards et al. (Edwards, Schnitzer, & Troy, 2014)]
Figure 2: Illustration of the anatomy at the (A) distal region of the femur and (B) proximal region of the tibia. Modified from Gray, Henry. Anatomy of the Human Body. Philadelphia: Lea & Febiger, www.bartleyby.com/107/. Figures 245 (A) and 258 (B)
Figure 3. Scatter plots of total hip (TH) T-score regressed against (A) distal femur (DF) \[\text{TH T-score (SD)} = 6.257 \times \text{DF aBMD (g/cm}^2\text{)} + -6.878, \text{slope 95\% CI: 5.320-7.194, } R^2 = 0.63, \text{ SE = 0.472, } P < 0.0001\)] and (B) proximal tibia (PT) \[\text{TH T-score (SD)} = 4.596 \times \text{PT aBMD (g/cm}^2\text{)} + -6.234, \text{slope 95\% CI: 3.936-5.256, } R^2 = 0.65, \text{ SE = 0.333, } P < 0.0001\]. A similar relationship was observed when regressing the femoral neck (FN) T-score against the (C) DF \[\text{FN T-score (SD)} = 4.902 \times \text{DF aBMD (g/cm}^2\text{)} + -5.542, \text{slope 95\% CI: 4.042-5.762, } R^2 = 0.55, \text{ SE = 0.434, } P < 0.0001\)] and (D) PT \[\text{FN T-score (SD)} = 3.635 \times \text{PT aBMD (g/cm}^2\text{)} + -5.073, \text{slope 95\% CI: 3.032-4.237, } R^2 = 0.58, \text{ SE = 0.304, } P < 0.0001\]. Diamonds indicate DF and circles indicate PT. Solid line: line of identity, Thin dashed line: 95\% CI; Thick dashed line: zero T-score.
Figure 4. Bland-Altman agreement analysis: The differences between measured and predicted T-score values were plotted against the average of the measured and predicted T-score value. Prediction of T-score at the total hip and femoral neck using the DF (A and B) and PT (C and D). Solid line: mean difference; Thin dashed line: 95% limit of agreement (LOA$_{95\%}$ mean difference ±2SD); Thick dashed line: clinical limit of agreement (LOA$_{clin}$).
Figure 5. Exponential decay curves best fit demonstrating change in aBMD loss as a function of TSI at the (A) Distal Femur [DF aBMD (cm²) = 0.411 Exp(-0.189t) + 0.677, R² = 0.46, P < 0.001]; (B) Proximal Tibia [PT BMD (cm²) = 0.591 Exp(-0.239t) + 0.795, R² = 0.49, P < 0.001], (C) Femoral Neck [FN BMD (cm²) = 0.310 Exp(-0.159t) + 0.718, R² = 0.32, P < 0.001], and (D) Total Hip [TH BMD (cm²) = 0.391 Exp(-0.4t) + 0.714, R² = 0.43, P < 0.001] regions in 105 persons with SCI. The thin dashed line represents the 95% confidence interval (CI) and the thick dashed line represents the able-bodied reference (ABref) mean BMD value for the DF (1.037 g/cm²), PT (1.320 g/cm²), FN (1.075 g/cm²), and TH (1.023 g/cm²). Diamonds indicate DF, circles PT, squares FN, and triangles the TH regions.
Figure 6: Power calculator to estimate sample size for the primary aim to develop a normative database of mean aBMD values at the DF and PT (G*Power 3.0.1.0, Franz Faul, Universität, Kiel, Germany).
Appendix B:

Compendium of Tables:
### Table 1. Summary of Descriptive Studies using Dual Energy X-ray Absorptiometry to Assess Areal Bone Mineral Density of the DF and PT in Spinal Cord Injured Cohorts.

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>SCI Population (gender), Age, DOI</th>
<th>Study Design</th>
<th>Comparative Group or Variable</th>
<th>Site, Software Application, ROI Method</th>
<th>Primary Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Battaglino et al. 2012</td>
<td>155 SCI (all M)</td>
<td>CS</td>
<td>18 Short term (≤5 years) vs. 137 long term (&gt;5 years) SCI</td>
<td>DF and PT, GE orthopedic knee software; Morse ROI</td>
<td>Short term SCI significantly greater aBMD at the DF and PT</td>
</tr>
<tr>
<td>Biering-Sorensen et al., 1990</td>
<td>8 SCI (6 M, 2 F)</td>
<td>PS</td>
<td>5-13 scans, 31-53 months post SCI</td>
<td>PT, forearm software, Schaadt and Bohr ROI</td>
<td>SCI&lt;AB: PT aBMD ↓ &gt;50%</td>
</tr>
<tr>
<td>Biering-Sorensen et al., 1988</td>
<td>26 SCI (24 M, 2 F)</td>
<td>CS</td>
<td>98 AB (47 M, 51 F)</td>
<td>PT, forearm software, Schaadt and Bohr ROI</td>
<td>SCI&lt;AB: PT aBMD ↓ &gt;50%</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>CS N</td>
<td>Control</td>
<td>SCI&lt;AB:</td>
<td>Notes</td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>-------</td>
</tr>
<tr>
<td>Dauty et al., 2000</td>
<td>31 SCI (all M)</td>
<td>31 AB (all M)</td>
<td>DF and PT, NR, NR</td>
<td>SCI&lt;AB: BMC ↓ 52 % in DF and 70% in PT</td>
<td></td>
</tr>
<tr>
<td>Age: 18-60 years, DOI: &gt;1 year</td>
<td></td>
<td>31 AB (all M)</td>
<td>Age: 18-60 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doherty et al., 2014</td>
<td>149 SCI (all M)</td>
<td>95 wheelchair mobility vs. 54 ambulatory SCI</td>
<td>DF and PT, GE orthopedic knee software, Morse ROI</td>
<td>aBMD ↓ in the DF and PT in wheelchair dependent SCI</td>
<td></td>
</tr>
<tr>
<td>Age: 27-88 years, DOI: 5-61 years</td>
<td></td>
<td>17 AB (all F)</td>
<td>Age: 26-52 years</td>
<td>SCI&lt;AB: aBMD of the DF and PT ↓ in Y (38%), M (41%), O (47%) SCI groups</td>
<td></td>
</tr>
<tr>
<td>Garland et al., 2001</td>
<td>31 SCI (all F)</td>
<td>10 AB (all M)</td>
<td>DF and PT, forearm software, NR</td>
<td>SCI&lt;AB: aBMD of the knee ↓ 50% in SCI</td>
<td></td>
</tr>
<tr>
<td>Age: (Y ≤30, M 31-50, O &gt;50), DOI: 2-44 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garland et al., 2005</td>
<td>18 SCI (all M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Age</td>
<td>DOI</td>
<td>Methodology</td>
<td>Findings</td>
</tr>
<tr>
<td>---------------------------</td>
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</tr>
<tr>
<td>Garland et al., 2004</td>
<td>6 SCI (5 M, 1 F)</td>
<td>21-28 years</td>
<td>24-53 days</td>
<td>PS</td>
<td>523 days post SCI</td>
</tr>
<tr>
<td>LaLa et al., 2014</td>
<td>70 SCI (50 M, 20 F)</td>
<td>37-60 years</td>
<td>6-26 years</td>
<td>CS</td>
<td>Fx, Hx versus no-Fx Hx group</td>
</tr>
<tr>
<td>Modlesky et al., 2004</td>
<td>10 SCI (all M)</td>
<td>34 years</td>
<td>2-20 years</td>
<td>CS</td>
<td>8 AB (all M) Age: 33±10 years</td>
</tr>
<tr>
<td>Morse et al., 2012</td>
<td>39 SCI (all M)</td>
<td>30-78 years</td>
<td>4-43 years</td>
<td>CS</td>
<td>10 AB (all M) Age: 8-80 years</td>
</tr>
<tr>
<td>Morse et al., 2013</td>
<td>39 SCI (all M)</td>
<td>30-78 years</td>
<td>4-43 years</td>
<td>CS</td>
<td>10 AB (all M) Age: 61.7±8.2 years</td>
</tr>
<tr>
<td>Shields et al., 2005</td>
<td>11 SCI (10 M, 1 F)</td>
<td>28-67 years</td>
<td>2-35 years</td>
<td>CS</td>
<td>11 AB (10 M, 1 F) Age: 22-62 years</td>
</tr>
<tr>
<td>Study</td>
<td>Sample Size</td>
<td>Age Range</td>
<td>Duration of Injury</td>
<td>Methodology</td>
<td>Equipment</td>
</tr>
<tr>
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</tr>
<tr>
<td>Warden et al., 2002</td>
<td>15 SCI (all M)</td>
<td>17-40 years</td>
<td>46-153 days</td>
<td>PS</td>
<td>6-week post BL scan</td>
</tr>
<tr>
<td>Zehnder et al., 2004</td>
<td>100 SCI (all M)</td>
<td>18-60 years</td>
<td>3 months - 30 years</td>
<td>CS</td>
<td>Time since injury</td>
</tr>
</tbody>
</table>

Abbreviations: DXA = Dual Energy X-ray Absorptiometry; GE = General Electric; DOI = duration of injury; aBMD = areal bone mineral density; BMC = bone mineral content; TDIA = tibial diaphysis; DF = distal femur; PT = proximal tibia; SCI = spinal cord injury; AB = able-bodied; M = men; F = women; Fx = fracture; Hx = history; CS = cross sectional; PS = prospective study; NR = not reported; NA = not applicable; Y = youngest; M = middle; O = oldest; <= less than
### Table 2. Summary of Descriptive Studies using Advanced Imaging to Assess Volumetric Bone Mineral Density and Bone Geometry of the DF and PT in Spinal Cord Injured Cohorts.

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>SCI Population (gender), Age, DOI</th>
<th>Study Design</th>
<th>Comparative group</th>
<th>Site/Regions Imaged</th>
<th>Primary Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coupau d et al., 2015</td>
<td>26 SCI (21 M, 5 F) Age: 17-76 years DOI: 8-13 months</td>
<td>PS</td>
<td>4, 8, and 12 months post SCI</td>
<td>DF 4% and PT 96% Tb region</td>
<td>vBMD&lt;sub&gt;Tb&lt;/sub&gt; and vBMD&lt;sub&gt;Ct&lt;/sub&gt; ↓ at the same rate (↓ 20% DF and PT) at follow-up</td>
</tr>
<tr>
<td>Coupau d et al., 2009</td>
<td>47 SCI (38 M, 9 F) Age: 37.3±13 DOI: 6 years</td>
<td>PS</td>
<td>Time since injury</td>
<td>DF 4% Epi</td>
<td>vBMD&lt;sub&gt;Tb&lt;/sub&gt; decreased exponentially with time since injury</td>
</tr>
<tr>
<td>Study</td>
<td>Sample Size</td>
<td>Study Design</td>
<td>Follow-Up</td>
<td>Parameters Reported</td>
<td>Findings</td>
</tr>
<tr>
<td>--------------------------</td>
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</tr>
<tr>
<td>Edwards et al., 2014</td>
<td>13 SCI (9 M, 4 F)</td>
<td>PS</td>
<td>3.5 months post BL scan</td>
<td>DF and PT: 0-10% Epi, 10-20% Met, and 20-30% Dia</td>
<td>vBMD&lt;sub&gt;Tb&lt;/sub&gt; ↓ 2-2.7 %/month vBMD&lt;sub&gt;Ct&lt;/sub&gt; ↓ 0.5-0.8%/month Epi and Met IP ↓ 2-3.8% in DF and 0.7-4.2% in PT at follow-up</td>
</tr>
<tr>
<td>Eser et al., 2005</td>
<td>99 SCI (89 M, 10 F)</td>
<td>CS</td>
<td>Fx, Hx versus no-Fx Hx group</td>
<td>DF Epi</td>
<td>vBMD&lt;sub&gt;Tb&lt;/sub&gt; of the DF ↓ 54%</td>
</tr>
<tr>
<td>Eser et al., 2004</td>
<td>89 SCI (all M)</td>
<td>CS</td>
<td>21 AB (all M)</td>
<td>DF Epi</td>
<td>SCI&lt;AB: vBMD&lt;sub&gt;Tb&lt;/sub&gt; of the DF ↓ 55%</td>
</tr>
<tr>
<td>Frotzler et al., 2008</td>
<td>39 SCI (all M)</td>
<td>PS</td>
<td>15 and 30 month timepoints</td>
<td>DF Epi</td>
<td>Small nonsignificant ↓ vBMD&lt;sub&gt;Tb&lt;/sub&gt; at the DF at follow-up</td>
</tr>
<tr>
<td>Study</td>
<td>SCI Count (Sex)</td>
<td>DOI</td>
<td>Follow-up</td>
<td>CS/PS</td>
<td>Age</td>
</tr>
<tr>
<td>-------------------------------------------</td>
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</tr>
<tr>
<td>McCartney et al., 2012</td>
<td>17 SCI (all M)</td>
<td></td>
<td>1-151</td>
<td>CS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age: 20-52 years</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>DOI: 1-151 months</td>
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</tr>
<tr>
<td>Rittwege et al., 2010</td>
<td>9 SCI (all M)</td>
<td></td>
<td>9-32</td>
<td>CS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age: 18-30 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DOI: 9-32 years</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tan et al., 2014</td>
<td>27 SCI (all M)</td>
<td></td>
<td>0.12-38</td>
<td>CS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age: 21-64 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DOI: 0.12-38 years</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dudley-Javoroski et al., 2012</td>
<td>29 SCI (26 M, 3 F)</td>
<td></td>
<td>&lt;6 months</td>
<td>PS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age: 16-72 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>DOI: &lt;6 months</td>
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</tr>
<tr>
<td>Dudley-Javoroski et al., 2010</td>
<td>15 SCI (14 M, 1 F)</td>
<td></td>
<td>0.19-7</td>
<td>PS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age: 18-49 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DOI: 0.19-7 years</td>
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</tbody>
</table>
Abbreviations: DOI= duration of injury; pQCT= peripheral quantitative computed tomography; MRI= magnetic resonance imaging; vBMD= volumetric bone mineral density; Tb= Trabecular; Ct= Cortical; Epi= epiphysis; Met= Metaphysis; Dia= diaphysis; DF= distal femur; PT= proximal tibia; SCI= spinal cord injury; AB= able-bodied; M= men; F= female; CS= cross sectional; PS= prospective study; RS= retrospective study; LS= longitudinal study; IP= polar moment of inertia; DOI= duration of injury; NR= not reported; <= less than
Table 3. Receiver operating characteristic curve to determine sensitivity and specificity for femoral neck and total hip cut-off values categorized by an aBMD cut-off value of

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>Std. Error</th>
<th>P-value</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH</td>
<td>0.95</td>
<td>0.021</td>
<td>&lt;0.0001</td>
<td>0.911</td>
<td>0.995</td>
<td>94</td>
<td>81</td>
</tr>
<tr>
<td>FN</td>
<td>0.92</td>
<td>0.027</td>
<td>&lt;0.0001</td>
<td>0.868</td>
<td>0.974</td>
<td>69</td>
<td>69</td>
</tr>
</tbody>
</table>

Abbreviations: DF = distal femur; PT = proximal tibia; TH = total hip; FN = femoral neck; AUC = area under the curve; ROC = receiver operating characteristic; CI = confidence interval; T-scoreWHO = World Health Organization T-score criteria of -2.5 SD below the young adult reference mean used to diagnose osteoporosis; T-scoreY = Youden Index T-score cutoff of -3.1 SD at the TH and -2.4 SD at the FN.
<table>
<thead>
<tr>
<th>FN/TH T-score cutoffs</th>
<th>DF/PT aBMD (≤ 0.60g/cm²)</th>
<th>HFR</th>
<th>LFR</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; -3.5 SD</td>
<td>HFR 5</td>
<td>3</td>
<td>33%</td>
<td>97%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LFR 10</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HFR LFR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; -2.5 SD</td>
<td>HFR 17</td>
<td>20</td>
<td>100%</td>
<td>77%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LFR 0</td>
<td>68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HFR LFR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; -3.1 SD</td>
<td>HFR 13</td>
<td>6</td>
<td>76%</td>
<td>93%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LFR 4</td>
<td>82</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

FN and TH cutoff values: < -3.5 SD (a value for aBMD below which entry into EAW upright ambulation protocols has been denied), < -2.5 SD (WHO criteria to diagnose osteoporosis), and < -3.1 SD (obtained from ROC curve T-score) and linear regression T-score predicted at the TH from the DF); DF/PT cutoff value used was < 0.60 g/cm².

Abbreviations: aBMD= areal bone mineral density; HFR= high fracture risk; LFR= lower fracture risk; DF= distal femur; PT= proximal tibia; TH= total hip; FN= femoral neck; SD= standard deviation; ROC= receiver operating characteristic; T-score = Youden Index T-score cutoff of -3.1 SD

Sensitivity= True-Positive/(True-Positive + False-Negative)
Specificity= True-Negative/(False-Positive + True-Negative)
<table>
<thead>
<tr>
<th>abMD and Reference Scores</th>
<th>E-I: TSI &lt;1y</th>
<th>E-II: TSI 1-5y</th>
<th>E-III: TSI 6-10y</th>
<th>E-IV: TSI 11-20y</th>
<th>E-V: TSI &gt;20y</th>
<th>ABref</th>
<th>Post-hoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>abMD (g/cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal Femur</td>
<td>1.69±0.176</td>
<td>0.88±0.169</td>
<td>0.83±0.168</td>
<td>0.71±0.148</td>
<td>0.64±0.136</td>
<td>1.03±0.203</td>
<td>1,2,3,4,6,7,8,9,13</td>
</tr>
<tr>
<td>Proximal Tibia</td>
<td>1.35±0.210</td>
<td>1.08±0.259</td>
<td>0.95±0.205</td>
<td>0.78±0.137</td>
<td>0.78±0.197</td>
<td>1.32±0.249</td>
<td>1,2,3,4,6,7,8,9,10</td>
</tr>
<tr>
<td>Femoral Neck</td>
<td>1.06±0.192</td>
<td>0.87±0.141</td>
<td>0.87±0.179</td>
<td>0.72±0.089</td>
<td>0.71±0.131</td>
<td>1.07±0.120</td>
<td>1,2,3,4,6,7,8,9,10</td>
</tr>
<tr>
<td>Total Hip</td>
<td>1.09±0.181</td>
<td>0.82±0.142</td>
<td>0.82±0.168</td>
<td>0.66±0.119</td>
<td>0.66±0.125</td>
<td>1.02±0.131</td>
<td>1,2,3,4,6,7,8,9,10,11,12</td>
</tr>
<tr>
<td>Z-score (SD)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral Neck</td>
<td>-0.02±1.13</td>
<td>-1.37±1.01</td>
<td>-1.34±1.64</td>
<td>-2.02±1.40</td>
<td>-2.00±0.88</td>
<td>0.17±0.82</td>
<td>1,3,6,7,8,9,10</td>
</tr>
<tr>
<td>Total Hip</td>
<td>-0.02±1.01</td>
<td>-1.76±0.97</td>
<td>-1.78±1.48</td>
<td>-2.55±1.42</td>
<td>-2.67±0.82</td>
<td>-0.02±0.73</td>
<td>1,3,6,7,8,9,10</td>
</tr>
<tr>
<td>T-score (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral Neck</td>
<td>0.21±1.37</td>
<td>-1.30±1.07</td>
<td>-1.27±1.29</td>
<td>-2.44±0.70</td>
<td>-2.39±0.89</td>
<td>0.19±0.87</td>
<td>1,2,3,4,6,7,8,9,10</td>
</tr>
<tr>
<td>Total Hip</td>
<td>0.73±1.43</td>
<td>-1.54±1.18</td>
<td>-1.58±1.29</td>
<td>-2.88±0.82</td>
<td>-2.90±0.90</td>
<td>0.29±0.85</td>
<td>1,2,3,4,6,7,8,9,10,11,12</td>
</tr>
</tbody>
</table>

% ≤ Threshold

| Z-score -2.0 | 0 | 34¹ | 40 | 81 | 87 | 0 | 1,2,3,4,6,7,8,10,12 |
| T-score -2.5 | 0 | 12 | 35 | 69 | 60 | 0 | 1,2,3,4,5,7,8 |

Abbreviations: ABref, able-bodied reference group; SD, standard deviation; Z-score: age matched reference score; T-score: young normal reference score; % ≤ Threshold: percent participants with standardized scores below threshold; TSI: time since injury; g/cm²: grams per centimeter squared; E-I: 1st Epoch = TSI <1 yr; E-II: 2nd Epoch = TSI 1-5 yrs; E-III: 3rd Epoch = TSI 6-10 yrs; E-IV: 4th Epoch = TSI 11-20 yrs; E-V: 5th Epoch = TSI >20 yrs.

Values are expressed as group means or marginal estimated means ± SD where applicable. Multivariate ANOVA omnibus results across all groups were significant at a priori level of significance of P = 0.001.

¹E-V vs. E-I = p ≤ 0.01; E-V vs. E-II = p ≤ 0.01; E-II vs. E-I = p ≤ 0.01; E-IV vs. E-I = p ≤ 0.01; E-IV vs. E-II = p ≤ 0.01; E-III vs. E-I = p ≤ 0.01; E-III vs. E-II = p ≤ 0.01; ABref vs. E-I = p ≤ 0.01; ABref vs. E-V = p ≤ 0.01; ABref vs. E-IV = p ≤ 0.01; ABref vs. E-III = p ≤ 0.01; ABref vs. E-II = p ≤ 0.01; E-V vs. E-III = p = 0.05; E-IV vs. E-II = p = 0.05; ABref vs. E-I = p = 0.05; *Z-score ≤ -2.0 vs. T-score ≤ -2.5 = p = 0.01.
<table>
<thead>
<tr>
<th>Site</th>
<th>Irradiation Time (sec)</th>
<th>Estimated skin entrance dose (µSv)</th>
<th>Effective Dose of initial scan (µSv)</th>
<th>Effective dose of initial and repeat scans combined (µSv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual Femur</td>
<td>212</td>
<td>329</td>
<td>12.3</td>
<td>24.6</td>
</tr>
<tr>
<td>*Knee</td>
<td>12</td>
<td>34</td>
<td>No Data</td>
<td>~6.1</td>
</tr>
</tbody>
</table>

*Values of effective dose (absorption) for the forearm and knee scans in µSv do not exist at this time, but considering the intensity and the duration of the scan is approximately half of the dual femur scan, the absorbed amount will also be approximately half (~6.1 µSv; GE Medical systems, Madison WI).*
Table 7. Demographic characteristics for YHAB men and women study participants stratified into age subgroups and for the total group.

<table>
<thead>
<tr>
<th></th>
<th>Men (n=32)</th>
<th>Women (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age*</td>
<td>24.0 ± 1.1</td>
<td>28.8 ± 0.89</td>
</tr>
<tr>
<td>Smoking Hx (Y/N)</td>
<td>1/7</td>
<td>1/7</td>
</tr>
<tr>
<td>Alcohol Wk. (0/1-4/25)</td>
<td>2/4/2</td>
<td>1/4/3</td>
</tr>
<tr>
<td>IBPAQ score (arbitrary units)</td>
<td>39.8 ± 21.1</td>
<td>34.2 ± 19.9</td>
</tr>
<tr>
<td>Calcium Intake (mg/day)</td>
<td>695 ± 380</td>
<td>551 ± 274</td>
</tr>
<tr>
<td>Vitamin D Intake (IU/day)</td>
<td>416 ± 259</td>
<td>406 ± 613</td>
</tr>
</tbody>
</table>

Abbreviations: YHAB= Young Healthy able-bodied; mg= milligrams; IU= international units; Wk= weekly; Hx= history. Values are expressed as group means ± SD. **Multivariate analysis of variance (MANOVA) omnibus results across all subgroups in Men were significant at a priori level of significance of P< 0.05.
Table 8. Body Habitus and Areal Bone Mineral Density Characteristics for YHAB Men and Women Study Participants Stratified Into Age Subgroups and for the Total Group

<table>
<thead>
<tr>
<th></th>
<th>Men (n=32):</th>
<th></th>
<th>Women (n=32):</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YHAB Age Subgroups and Total</td>
<td></td>
<td>YHAB Age Subgroups and Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21-25 (n=8)</td>
<td>26-30 (n=8)</td>
<td>31-35 (n=8)</td>
<td>36-40 (n=8)</td>
</tr>
<tr>
<td>Height (cm)*</td>
<td>175.6±5.5</td>
<td>179.1±4.9</td>
<td>183.2±4.8</td>
<td>177.2±5.7</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>75.1±12.4</td>
<td>84.4±12.1</td>
<td>95.4±12.2</td>
<td>80.3±8.5</td>
</tr>
<tr>
<td>BMI*</td>
<td>23.7±2.7</td>
<td>26.3±3.8</td>
<td>28.8±4.2</td>
<td>25.5±1.8</td>
</tr>
<tr>
<td>TBF (%)*</td>
<td>17.0±6.6</td>
<td>23.3±8.9</td>
<td>30.6±6.7</td>
<td>23.6±5.7</td>
</tr>
<tr>
<td>TBI (%)*</td>
<td>78.7±6.2</td>
<td>73.0±6.5</td>
<td>65.8±6.6</td>
<td>72.6±5.3</td>
</tr>
<tr>
<td>aBMD (g/cm²)</td>
<td>1.16±0.12</td>
<td>1.11±0.115</td>
<td>1.17±0.127</td>
<td>1.00±0.09</td>
</tr>
<tr>
<td>TH*</td>
<td>1.2±0.15</td>
<td>1.225±0.129</td>
<td>1.256±0.352</td>
<td>1.115±0.105</td>
</tr>
<tr>
<td>PT*</td>
<td>1.46±0.202</td>
<td>1.399±0.164</td>
<td>1.693±0.406</td>
<td>1.536±0.190</td>
</tr>
</tbody>
</table>

Abbreviations: aBMD= areal bone mineral density; YHAB= Young healthy able-bodied; SD= standard deviation; g/cm²= grams per centimeter squared; mg/ml= milligrams; *L= international units; **= weekly; TBF/AOQ= total bone-specific physical activity questionnaire; BMI= Body Mass Index; TH= total hip; PT= distal femur; P= p-value from ANOVA. Values are expressed as mean ± SD.

**Multivariate analysis of variance (MANOVA) coefficients resulting across all subgroups in Men were significant at a priori level of significance of P<0.05. MANCOVA Post-hoc comparisons: 21-25 vs. 31-35 = p<0.05; 21-25 vs. 36-40 = p>0.05; 26-30 vs. 31-35 = p<0.05; **TBF/AOQ for Men and Women. *Independent Sample T-test between Men and Women for all aBMD measurements was significant at P< 0.001.
<table>
<thead>
<tr>
<th>SCI T-score</th>
<th>E-I: TSI &lt;1y</th>
<th>E-II: TSI 1-5y</th>
<th>E-III: TSI 6-10y</th>
<th>E-IV: TSI 11-20y</th>
<th>E-V: TSI &gt;20 y</th>
<th>Post-hoc</th>
<th>Total SCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Hip</td>
<td>0.12 ± 1.1</td>
<td>-1.80 ± 1.01</td>
<td>-1.91 ± 1.27</td>
<td>-2.96 ± 0.82</td>
<td>-2.99 ± 0.90</td>
<td>1,2,3,4,6,7</td>
<td>-1.82 ± 1.49</td>
</tr>
<tr>
<td>Distal Femur</td>
<td>-0.36 ± 1.25</td>
<td>-1.72 ± 1.35</td>
<td>-2.30 ± 1.54</td>
<td>-2.92 ± 1.12</td>
<td>-3.54 ± 1.02</td>
<td>1,2,3,4,6,7</td>
<td>-2.02 ± 1.62</td>
</tr>
<tr>
<td>Proximal Tibia</td>
<td>-0.27 ± 1.07</td>
<td>-1.56 ± 1.01</td>
<td>-2.38 ± 1.34</td>
<td>-3.12 ± 0.75</td>
<td>-3.20 ± 1.02</td>
<td>1,2,3,4,6,7</td>
<td>-1.95 ± 1.57</td>
</tr>
</tbody>
</table>

**Table 9.** T-score values stratified by epochs of time since spinal cord injury and total SCI cohort using the YHAB reference mean aBMD values at the hip and knee reported in Table 1.

**SCI T-score**

<table>
<thead>
<tr>
<th>T-scores</th>
<th>E-I: TSI &lt;1y</th>
<th>E-II: TSI 1-5y</th>
<th>E-III: TSI 6-10y</th>
<th>E-IV: TSI 11-20y</th>
<th>E-V: TSI &gt;20 y</th>
<th>Post-hoc</th>
<th>Total SCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Hip</td>
<td>0.12 ± 1.1</td>
<td>-1.80 ± 1.01</td>
<td>-1.91 ± 1.27</td>
<td>-2.96 ± 0.82</td>
<td>-2.99 ± 0.90</td>
<td>1,2,3,4,6,7</td>
<td>-1.82 ± 1.49</td>
</tr>
<tr>
<td>Distal Femur</td>
<td>-0.36 ± 1.25</td>
<td>-1.72 ± 1.35</td>
<td>-2.30 ± 1.54</td>
<td>-2.92 ± 1.12</td>
<td>-3.54 ± 1.02</td>
<td>1,2,3,4,6,7</td>
<td>-2.02 ± 1.62</td>
</tr>
<tr>
<td>Proximal Tibia</td>
<td>-0.27 ± 1.07</td>
<td>-1.56 ± 1.01</td>
<td>-2.38 ± 1.34</td>
<td>-3.12 ± 0.75</td>
<td>-3.20 ± 1.02</td>
<td>1,2,3,4,6,7</td>
<td>-1.95 ± 1.57</td>
</tr>
</tbody>
</table>

**Abbreviations:** aBMD = areal bone mineral density; SCI = spinal cord injury; y = years; SD = standard deviation; T-score = T-score calculated using the study cohort young-healthy able-bodied (YHAB) aBMD values for the DF and FF presented in Table 1; T-score = T-score calculated using the combined General Electric (GE) Lunar and National Health and Nutrition Examination Survey III (NHANES III) combined young-healthy aBMD reference dataset; TSI = time since injury; g/cm²; grams per centimeter squared; E-I: 1st Epoch = TSI <1 y; E-II: 2nd Epoch = TSI 1-5 yrs; E-III: 3rd Epoch = TSI 6-10 yrs; E-IV: 4th Epoch = TSI 11-20 yrs; E-V: 5th Epoch = TSI >20 yrs.

The aBMD values used to calculate T-scores in this SCI cohort were obtained from a previous report using the same study design (Cimigliore et al., 2019). Values are expressed as group means or marginal estimated means ± SD where applicable.

Multivariate ANOVA (MANOVA) omnibus results across all epochs of TSI were significant at a priori level of significance of P<0.001. MANOVA post-hoc comparisons: *E-V vs. E-I = p<0.01; *E-V vs. E-II = p<0.01; *E-V vs. E-I vs. E-II = ps<0.01; *E-V vs. E-I vs. E-II vs. E-III = ps<0.05; *E-V vs. E-I vs. E-II vs. E-III vs. E-IV = ps<0.05; *E-V vs. E-I vs. E-II vs. E-III vs. E-IV vs. E-V = ps<0.05.

Paired comparisons: *T-score vs. T-score total hip T-score = p<0.05.
Table 10. Diagnostic statistics for diagnosing osteoporosis comparing the hip T-score cutoff values using the GE/NHANES III reference dataset to the hip and knee T-score values calculated from the YHAB reference dataset

<table>
<thead>
<tr>
<th>T-score Cutoff Values</th>
<th>THGE/NHANES vs. DF/PTYHAB</th>
<th>THGE/NHANES vs. THYHAB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T-score % Agreement</td>
<td>T-score Kappa Agreement</td>
</tr>
<tr>
<td>&lt; -2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75</td>
<td>0.50</td>
</tr>
<tr>
<td>&lt; -3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Abbreviations: TH = total hip; DF = distal femur; PT = proximal tibia; THGE/NHANES = dichotomous classification of osteoporosis using TH T-score values calculated using the General Electric (GE) National Health and Nutrition Examination Survey III (NHANES III) combined young-healthy aBMD reference dataset; DF/PTYHAB = dichotomous classification of osteoporosis using DF or PT T-score values from the study young-healthy able-bodied reference dataset presented in table 3; THYHAB = dichotomous classification of osteoporosis using TH T-score values from the YHAB reference dataset presented in table 3. T-score osteoporosis cutoff values: < -2.5<sup>a</sup> SD (WHO criteria to diagnose osteoporosis) and < -3.5<sup>b</sup> SD (TH T-score cutoff value commonly used to exclude participants from powered exoskeleton-assisted walking therapy in persons with SCI).
Appendix C:

DXA Acquisition and Analysis Protocols
1. **Scanning the Patient:**

   A. The patient should be given instructions to lie as still as possible and only talk during the scan when necessary.

   B. A pillow must be removed from behind the patient’s head prior to starting the scan and then put back under the head when the scan arm has reached the level of the torso. This may cause slight discomfort for patients that are obese due to the degree of extension that occurs at the cervical vertebrae. If there is too much neck discomfort without a pillow then use one or two towels to keep head elevated (towels do not attenuate the x-ray like the material in a pillow). If necessary, when the scan arm reaches the torso the pillow can be put back under the patient’s head having the patient avoid any other movement.

   C. During the scan attention to detail is needed for any movement that might occur due to spasms or increased tone. If there is movement then the scan should be aborted and redone having the patient repositioned if necessary.

   D. For patients over 6 feet tall, the toes may extend over the terminal scan line due to plantar flexion contracture. If this occurs the DXA will give an error reading under the computer assisted densitometry (CAD) tab of the analysis screen. This is an acceptable error since it will have minimal influence on body composition results.

   E. When the scan is complete it is necessary to check for any movement artifact or other artifact in the scan region. If there is no unexpected artifact due to patient movement or other unexpected material that was scanned then save the image for further analysis.

**Description for Measurements**
The following will describe the procedures used for all the scans.

**Dual Proximal Femur Acquisition and Analysis Protocol**

This scan should only be done once during the screening phase.
Patient preparation is the same as for the total body and therefore easier to be completed on the same day.
Positioning:

- Position the patient as indicated in the GE Lunar iDXA instruction manual paying special attention to the following:
  - Both legs should be internally rotated as much as possible. Assist the patient by not just rotating at the shank but also at the thigh.
  - Feet should be tightly strapped to the triangle foot positioner
  - Place the cross hairs distal from the ASIS. You can estimate the starting point by opening your hand so that it makes an “L”. Then placing your middle finger on the ASIS and your thumb as distal as possible to indicate where the cross hairs should be.
  - When scanning the patient make sure that you have approximately 2 sweeps before the lesser trochanter is visible and 3-4 sweeps before the iliac crest is visible. The scan should continue for another 2-3 sweeps after the top of the trochanter.

- Prior to starting the scan the patient thickness must be assessed by measuring the thickest area anterior to posterior of that patient. Under the “Scan Mode” option the following modes can be used to scan each patient:
  - Thin mode- patient thickness <13 cm
  - Standard mode- patient thickness 13-25 cm
  - Thick mode- patient thickness >25 cm
  - NOTE: if you don’t have enough soft tissue around the bone it will not analyze the scan properly. BEFORE the patient gets off the table to into the analysis mode to make sure that all the bone is visible.
**Scanning:**
- The patient should be given instructions to lie as still as possible.
- They may have a pillow for this test.

**Image should look similar to this:**

Distal Femur and Proximal Tibia BMD Acquisition Protocol
1. Measurement procedures
   a. This scan is obtained using the GE Lunar Orthopedic knee
   b. Patient positioning: Using the stabilizing triangle, secure the foot to it in a similar manner to performing the hip scan. Ensure that internal rotation is obtained to minimize the amount of overlap between the tibia and the fibula.
   c. Ensure that the foam pad provided by GE is used behind the knee.
   d. Ensure that lower leg is by adducting (not sure how to explain that it can’t be abducted) and parallel to midline.
   e. Identification of starting point:
      The starting point of the acquisition is set on the approximately 4 fingers (approximately 10cm) from the bottom edge of patella.

Shields et al. method to capture aBMD at the DF and PT

The protocol to scan the knee was established from the following publication:

a. Measure Femur Length:
   • Measure from Lateral Part of the Greater Trochanter to the lateral epicondyle. Line runs up and down the femur since it is the femur length. (Parallel to Femur). Record this length in centimeters (femur length)

b. Analyzing Knee to capture the DF and PT regions:
   • Open Knee Scan
   • Ensure Bone Is Marked Properly
      i. Select Points
ii. **Determine Whether Each of the Points of Interest (Bone, Tissue, Air, etc) are Marked Properly.**

1. **Image Correction:** Manually correct the image point typing by identifying bone that may have been incorrectly identified. Erase the fibula bone (set to neutral) but leave what overlaps with the tibia. Identify any soft tissue surrounding the bone but user there is a neutral edge around the perimeter of the bone.

- Select ‘Results’ After All Bone is Marked Properly
- Select Analyze Tab
- Select Custom
- Choose ROI

**Line 1:**

i. Select the ‘Create Line’ Option and Create a Line proximally from the Distal Edge of the Lateral Condyle up to 13% of the Femur Length

1. Length of line is calculated by multiplying the Femur Length by 0.13 (13%).

**Box 2:**

i. Using the ‘Rectangle Mode’ Option, Begin Making a Box from the Most Proximal Point of Line 1 up, so that the height of the box measures 7% of the Femoral Length.

1. Height of Box is Calculated by Multiplying the Femur Length by 0.07 (7%).
2. Ensure that the Width of the Box Encompasses all of the Proximal Femur that Begins from the Proximal Point of the Femur up to the 7% Mark.
• Box 3:
  1. Using the 'Rectangle Mode' Option, Begin Making a Box with one Corner Starting at the Uppermost Point of Contact Between the Fibular Head and the Tibia. Similarly to Box 2, Create the Box Distally from the Point of Contact 7% of the Femoral Length.
  2. Height of Box is Calculated by Multiplying the Femur Length by 0.07 (7%).
  3. Ensure that the Width of the Box Encompasses all of the Distal Tibia that Begins from the Tibia/Fibula Point of Contact, Distally to the 7% Mark.
  4. Select “Results’ to Analyze

• Repeat for Analysis of Other Knee if Necessary.
Appendix D:

Demographic and Medical History form:
Demographic and Medical History Intake Form

General Demographic Information:

Date:_______; ID#:___________________;

______________________________ cm  ____________________ lbs / _______ kg
HEIGHT  WEIGHT  AGE

Ethnicity:  Caucasian (not of Hispanic Origin)  African American (not of Hispanic Origin)  Hispanic  American Indian
Alaskan Native  Asian  Pacific Islander  Other__________

Gender: MALE / FEMALE

Emergency Contact:______________________________

Phone Number:______________________________

<table>
<thead>
<tr>
<th></th>
<th>Current</th>
<th>History</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smoking</strong></td>
<td>PPD:____________</td>
<td>PPD:________</td>
<td></td>
</tr>
<tr>
<td></td>
<td>From:__________</td>
<td>From:_______</td>
<td></td>
</tr>
<tr>
<td><strong>Drug Use</strong></td>
<td>Drugs:__________</td>
<td>Drugs________</td>
<td></td>
</tr>
<tr>
<td></td>
<td>__________________</td>
<td>__________________</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>__________________</td>
<td>__________________</td>
<td></td>
</tr>
<tr>
<td>Alcohol Use</td>
<td>Glasses per day / week / month of wine / beer / liquor</td>
<td>Alcoholic from_______ - _______</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comments:________</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### MEDICAL HISTORY (answer yes/no)

<table>
<thead>
<tr>
<th>Condition</th>
<th>YES / NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Disease</td>
<td></td>
</tr>
<tr>
<td>Myocardial Infarction (heart attack) Be</td>
<td></td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>Type I / Type II</td>
</tr>
<tr>
<td>Lipid Abnormality</td>
<td></td>
</tr>
<tr>
<td>Neurological Problems (other than SCI)</td>
<td></td>
</tr>
<tr>
<td>Headaches</td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td></td>
</tr>
<tr>
<td>Bleeding/Coagulation Disorder</td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
</tr>
<tr>
<td>Emphysema</td>
<td></td>
</tr>
<tr>
<td>Chronic Bronchitis</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
</tr>
<tr>
<td>Renal Disease</td>
<td></td>
</tr>
<tr>
<td>Liver Disease</td>
<td></td>
</tr>
<tr>
<td>Hepatitis A / B / C</td>
<td></td>
</tr>
<tr>
<td>Joint / Bone Disease</td>
<td></td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td></td>
</tr>
<tr>
<td>Carpel Tunnel Syndrome</td>
<td></td>
</tr>
<tr>
<td>Tendonitis</td>
<td></td>
</tr>
<tr>
<td>Thyroid Disease</td>
<td></td>
</tr>
<tr>
<td>Family History of Fragility Fracture</td>
<td>YES / NO</td>
</tr>
</tbody>
</table>

| History of High Impact Fractures              | YES / NO |
| Location:                                     |          |
| Location:                                     |          |
| Location:                                     |          |

### E. MEDICATION PROFILE
Veteran of Armed Forces: YES / NO (circle)
_____________________________________________________________________________________________
_____________________________________________________________________________________________

PARTICIPANT IN OTHER STUDIES:

YES / NO (circle):
_____________________________________________________________________________________________
_____________________________________________________________________________________________
Appendix E:
Vitamin D and Calcium Intake Survey
Appendix F:

How often do you usually eat each of the foods listed below? Remember breakfast, lunch, dinner, snacks, and when eating out.

Please use a pencil.

<table>
<thead>
<tr>
<th>ID NUMBER</th>
<th>AGE</th>
<th>SEX</th>
<th>If female, are you pregnant or breastfeeding?</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

**How Often in the Past Year?**

<table>
<thead>
<tr>
<th>Eggs or breakfast sandwiches with eggs</th>
<th>Never</th>
<th>2-3 times per month</th>
<th>1-2 times per week</th>
<th>3-4 times per week</th>
<th>5-6 times per month</th>
<th>Every day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast cereal, like corn flakes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oatmeal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glasses of regular milk, soy milk, chocolate milk or cocoa (Don’t count milk on cereal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinks like Sego, Slimfast, Slender or Ensure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakfast bars, granola bars or energy bars like Power Bar, Slimfast Bar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread, rolls or English muffins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tortillas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamburger or cheeseburger with bun</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pizza</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacos, burritos, enchiladas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaroni &amp; cheese, lasagna or cheese ravioli</td>
<td></td>
<td></td>
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<td>Pinto beans, black beans or chili with beans</td>
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<tr>
<td>Cheese or cheese spread not counted above</td>
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<tr>
<td>Yogurt</td>
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<td>Ice cream or frozen yogurt, regular or low-fat cake or doughnuts</td>
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<tr>
<td>Carbonated soft drinks, like Coke, ginger ale, orange soda or any kind (diet or regular)</td>
<td></td>
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<tr>
<td>Tang or Rolaid</td>
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<td>Multiple-vitamins, like One-a-Day, or prenatal supplements</td>
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<tr>
<td>Calcium supplements</td>
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<tr>
<td>Vitamin D, alone or with calcium</td>
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On the days that you take **calcium supplements** (not including Tang/Rolaids) how many milligrams of calcium do you take in the whole day? 300mg 500mg 600mg 800mg 1000mg 1200mg 1500mg 1600mg
On the days you take **vitamin D supplements** (alone or combined with calcium) how many IU of vitamin D do you take in the whole day? 200 IU 400-500 IU 600-800 IU 1000 IU 2000 IU 5000 IU 10,000+ IU

When you drink **orange juice**, is it usually With added calcium Without added calcium Don’t know

When you eat **burgers** are they usually Hamburger Cheeseburgers Both equally

When you eat **cold cereal** is it usually “Total” brand cereal Some other kind of cereal
Bone-Specific Physical Activity Questionnaire (BPAQ)
Bone-Specific Physical Activity Questionnaire (BPAQ)

1. Please list any sports or other physical activities you have participated in regularly. Please tick the boxes to indicate how old you were for each sport/activity and how many years you participated for.

| Activities | Age: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|------------|--------|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|            |        |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|            |        |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|            |        |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

| Activities | Age: 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 |
|------------|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|            |         |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|            |         |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|            |         |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

| Activities | Age: 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 |
|------------|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|            |         |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|            |         |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|            |         |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

Bone Specific Physical Activity Questionnaire
Developed by B.K. Weeks and B.E. Beck
Griffith University, QLD, Australia
# Bone-Specific Physical Activity Questionnaire (BPAQ)

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<thead>
<tr>
<th>Activity:</th>
<th>Frequency (per week):</th>
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2. Please list the sports or other physical activities (be as specific as possible) you participated in regularly during the last 12 months and indicate the average frequency (sessions per week).