Precision analysis of site-specific dual-energy x-ray absorptiometry in persons with spinal cord injury and persons who are able-bodied

by

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Abstract

The purpose of this thesis project was to determine the precision error of dual-energy x-ray absorptiometry (DXA) derived bone mineral density (BMD) at regions of interest (ROI) that are clinically relevant to persons with spinal cord injury (SCI), and secondarily to compare the precision error between a group of persons who are able-bodied and a group of persons with chronic SCI. Over 2 visits, four DXA scans at sites of the distal femur, proximal tibia, and calcaneus were completed in 10 persons who are able-bodied and 10 persons with chronic SCI. Using forearm sub region analysis, we measured the BMD and calculated the precision error for a total of 7 ROI at these sites. Despite a lower BMD at every ROI in the group of persons with chronic SCI compared to the group of persons who are able-bodied (range, 33 – 56%), the relative precision error was similar between groups. However, there was a trend for greater precision error in persons with SCI at a whole bone ROI of the distal femur (RMS-CV of 8.40% vs. 5.63%) and a ROI of the posterior calcaneus body (RMS-CV of 3.52% vs. 1.78%) when compared to persons who are able-bodied. Further, the ROI of the posterior calcaneus body appeared to have a lower precision error in persons who are able-bodied (RMS-CV, 1.78%) than the distal femur and proximal tibia (RMS-CV range 3.26 – 5.63%). The results from this study suggest that the precision error of DXA derived BMD is similar between persons with SCI and persons who are able-bodied, and that the posterior calcaneus body may be a more precise site than the distal femur and proximal tibia.

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Abbreviations

AB – able-bodied

AIS - American Spinal Injury Association impairment scale

BMC – bone mineral content

BMD – bone mineral density

BMI – body mass index

CI – confidence interval

CT – computed tomography

DPA – dual-photon absorptiometry

DXA – dual-energy x-ray absorptiometry

ICC – intraclass correlation coefficient

IOF – International Osteoporosis Foundation

ISCD – International Society for Clinical Densitometry

LSC – least significant change

miRNAs – micro RNAs

mSV – millsieverts

NHANES – National Health and Nutrition Examination Survey

NTSCI – non-traumatic spinal cord injury

pQCT – peripheral quantitative computed tomography

QCT – quantitative computed tomography

RMS-CV – root mean square coefficient of variation

RMS-SD – root mean square standard deviation

ROI – region of interest

s-CTX – serum C-terminal telopeptide of type 1 collagen

s-PINP – serum procollagen type 1 propeptide

SCI – spinal cord injury

SPA – single-photon absorptiometry

TSCI – traumatic spinal cord injury

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Chapter 1: Introduction and Literature Review

Introduction

Osteoporosis is a universal health condition in persons with motor complete spinal cord injury (SCI). Currently, dual-energy x-ray absorptiometry (DXA) measured bone mineral density (BMD) at the lumbar spine, hip, and distal radius is the standard method for osteoporosis assessment. These sites do not account for BMD loss after SCI, which is localized to the lower limbs. Rapid and progressive losses in bone mineral happen at the distal femur, proximal tibia, and calcaneus in persons with SCI. These losses lead to bone mineral values that are up to 70% lower than persons who are able-bodied. This results in an increased risk of low-impact fractures, which most frequently occur at the distal femur and proximal tibia, and lead to both health and economic consequences. Although the calcaneus is not often fractured, it is an area of rapid BMD loss that may provide early clues as to potential treatment effectiveness. To properly design intervention studies to prevent osteoporosis after SCI, protocols need to be developed that can reliably measure BMD at these sites. Therefore, the purpose of this thesis project is to determine the precision error of DXA derived BMD at the distal femur, proximal tibia, and calcaneus in persons with SCI and compare these results to persons who are able-bodied. The results from this project will be used to guide the length of follow-up in future interventions aimed to prevent osteoporosis after SCI.

Etiology and epidemiology of spinal cord injury (SCI)

A spinal cord injury (SCI) is a life-altering event that results in neurological damage and subsequent paralysis directly related to the location and severity of the injury. SCI can be categorized as tetraplegia, which are lesions of the cervical segments (C1-C8) that lead to impairment of all four limbs or paraplegia, which are lesions to the thoracic, lumbar, or sacral segments that lead to impairment in the lower body (High: T1-T6; Low: T7 and below). The American Spinal Injury Association impairment scale (AIS) is the current international neurological classification system for SCI. The AIS (Table 1) is divided into five classifications, with each level representing a different form of impairment (Maynard *et al.*, 1997; Marino *et al.*, 2003). In this schematic, AIS A and B refer to motor complete injury, while C and D are refer to motor incomplete.

Table 1: American Spinal Injury Association impairment scale.

	Classification		
AIS	Motor	Sensory	Clinical features
A	Complete	Complete	Neither motor nor sensory function preserved.
B	Complete	Incomplete	Preserved sensory function, but not motor.
C	Incomplete	Incomplete	Preserved motor function. Muscle grade <3 for more than half of key muscles.
D	Incomplete	Incomplete	Preserved motor function. Muscle grade ≥3 for more than half of key muscles.
E	Normal	Normal	Normal motor and sensory function.

Table adapted from (Maynard *et al.*, 1997; Marino *et al.*, 2003).

In Canada, there are over 85,000 people living with SCI. Yearly, the initial incidence rate of SCI is over 4,259 cases, with 1,785 cases due to traumatic SCI (TSCI) (Farry & Baxter, 2010; Noonan *et al.*, 2012). TSCI is a result of an acute event, most commonly motor vehicle accidents (21.2-50%) and falls (16.7%-

30.6%) in the overall population (Sekhon & Fehlings, 2001; McCammon & Ethans, 2011). In older adults, non-traumatic spinal cord injury (NTSCI) is more common, and falls represent the most common cause of TSCI (Selassie *et al.*, 2013). The representative population of TSCI is 4:1 male to female, with the highest incidence rates between 15 and 24 years old, and over 50% of all persons are living with tetraplegia (Farry & Baxter, 2010; Qin *et al.*, 2010; McCammon & Ethans, 2011). In Manitoba, the documented incidence of SCI has increased over time, with an incidence rate of 42.6 persons per million between 2003 and 2007, up from 1981 to 1985 when the rate was just 20.2 persons per million (McCammon & Ethans, 2011).

Secondary health conditions after spinal cord injury (SCI)

Neurological damage and paralysis are the direct consequences of SCI, but in the years after injury, there is an increased risk and prevalence of many secondary health conditions. In the chronic stage of SCI, diabetes or impaired glucose tolerance is common (Duckworth *et al.*, 1980; Duckworth *et al.*, 1983), cardiovascular disease and vascular complications are increased (Bauman *et al.*, 1993), increased intramuscular fat content (Gorgey & Dudley, 2007) and body mass index (BMI) develop (de Groot *et al.*, 2010; Crane *et al.*, 2011), muscle cross-sectional area is reduced (Castro *et al.*, 1999) with muscle type conversion from type I to II also appearing (Burnham *et al.*, 1997; Biering-Sørensen *et al.*, 2009a; Kostovski *et al.*, 2013), bowel irregularities are prevalent (Glickman & Kamm, 1996), autonomic dysreflexia can develop with injuries above T6

(Karlsson, 1999), and urinary tract infections are common (Cardenas & Hooton, 1995).

In persons with motor complete SCI, osteoporosis of the lower limbs is considered to be a universal secondary health condition (Dolbow *et al.*, 2011). This has long been known, and in the last half-century imaging methods such as single photon absorptiometry (SPA), dual photon absorptiometry (DPA), dual-energy x-ray absorptiometry (DXA), and most recently computed tomography (CT) [quantitative CT (QCT) or peripheral QCT (pQCT)], have been able to quantitate this. Quantification is generally via bone mineral density (BMD) or bone mineral content (BMC). It is widely recognized that mechanical unloading and inactivity of the paralyzed limbs may represent a primary factor for osteoporosis after SCI, as BMD losses are localized to the unloaded limbs (Dauty *et al.*, 2000; Qin *et al.*, 2010; Dolbow *et al.*, 2011), however neural signals and hormones have also been suggested (Jiang *et al.*, 2006).

It has been shown that the bone mineral and mass alterations after SCI may differ based on severity of injury. For example, individuals who are ambulatory after SCI will lose less relative bone mass (Garland *et al.*, 2004) and BMD (Singh *et al.*, 2014) than individuals with non-ambulatory SCI (i.e. complete), which has also been shown in a severity based rat model (Voor *et al.*, 2012). Persons with paraplegia have been shown to display similar total bone mass at the arms, trunk, and regions of the femur and tibia when compared to persons with tetraplegia (Biering-Sørensen *et al.*, 1988; Frey-Rindova *et al.*, 2000; Singh *et al.*, 2014), however others have shown that persons with

tetraplegia may have lower BMD and BMC at the distal femur and proximal tibia (Garland *et al.*, 1992). Similar to able-bodied populations, women with SCI generally have lower BMD than men, but the distribution of bone mineral loss is similar in that it is restricted to the lower limbs (Kiratli *et al.*, 2000).

Regional bone mineral alterations after spinal cord injury (SCI)

It is well known that SCI osteoporosis is localized to the lower limbs. Total body BMD losses of 5-15% occur within the first two years after SCI (Gilchrist *et al.*, 2007; Alekna *et al.*, 2008), and progress to bone mineral losses of 15-20% in the chronic stage, which is commonly referred to as more than 1 year post injury (Bauman *et al.*, 1999; Jones *et al.*, 2002). Below the lesion level, it has been shown that there are losses in BMC of 40% in the chronic stage of SCI, but no differences above the lesion when compared to persons who are able-bodied (Dauty *et al.*, 2000). As well, evidence suggests that there are minimal bone mineral alterations at the head, arms, distal radius, and lumbar spine when assessed throughout the first years of injury (Wilmet *et al.*, 1995; Gilchrist *et al.*, 2007; Alekna *et al.*, 2008; Singh *et al.*, 2014), and in the chronic stage (Biering-Sørensen *et al.*, 1988; Goemaere *et al.*, 1994; Bauman *et al.*, 1999; Dauty *et al.*, 2000; Jones *et al.*, 2002; Modlesky *et al.*, 2004).

In the lower body, early studies demonstrated a proximal to distal gradient for loss of bone mass, BMC, and BMD. At the pelvis, bone mineral and mass losses happen rapidly within the first year of injury and stabilize with total loss of 30-50% (Garland *et al.*, 1992; Wilmet *et al.*, 1995; Bauman *et al.*, 1999; Dauty *et al.*, 2000; Alekna *et al.*, 2008). Losses of 10-35% in bone mineral have

been shown within the first two years in the total legs (Wilmet *et al.*, 1995; Gilchrist *et al.*, 2007; Alekna *et al.*, 2008), with up to 50% loss in the chronic stage (Garland *et al.*, 1992; Bauman *et al.*, 1999; Dauty *et al.*, 2000; Jones *et al.*, 2002). At the hip, reductions are generally between 25-40% in the first years of injury and in the chronic stage when comparing the total hip (Goemaere *et al.*, 1994), trochanter (Goemaere *et al.*, 1994; Dauty *et al.*, 2000; Jones *et al.*, 2002), or femur neck (Biering-Sørensen *et al.*, 1988; Goemaere *et al.*, 1994; Dauty *et al.*, 2000; Kiratli *et al.*, 2000; Jones *et al.*, 2002; Singh *et al.*, 2014). At the femoral diaphysis (shaft), bone mineral reductions of 25% have been reported (Biering-Sørensen *et al.*, 1988; Goemaere *et al.*, 1994; Kiratli *et al.*, 2000). At the distal femur, BMD losses of approximately 25% are shown in the first year of SCI (Garland *et al.*, 1992; Wilmet *et al.*, 1995; Kiratli *et al.*, 2000; Edwards *et al.*, 2014a). The loss of BMD at the distal femur progresses and eventually stabilizes within 5 years at losses of up to 70% (Garland *et al.*, 1992; Dauty *et al.*, 2000; Kiratli *et al.*, 2000; Eser *et al.*, 2004a; Eser *et al.*, 2004b; Collins *et al.*, 2010; Dionyssiotis, 2011; Dudley-Javoroski & Shields, 2012). At the proximal tibia, slightly greater losses in bone mineral have been shown at up to 4% per month for trabecular sites within the first years of injury (Garland *et al.*, 2004; Dudley-Javoroski & Shields, 2012; Edwards *et al.*, 2014a, b; Singh *et al.*, 2014). The loss at the proximal tibia has also been shown to progress rapidly, but stabilizes at approximately 3 years post injury at up to 70% bone mineral loss (Biering-Sørensen *et al.*, 1988; Garland *et al.*, 1992; Dauty *et al.*, 2000; Eser *et al.*, 2004a; Modlesky *et al.*, 2004; Dudley-Javoroski & Shields, 2012). At the distal tibia, the

loss in the first year of injury has been shown to be approximately 20% (Frey-Rindova *et al.*, 2000; Singh *et al.*, 2014), which can progress into losses similar to that at the proximal tibia in the chronic stage at approximately 60% (Eser *et al.*, 2004a). Although less often studied, the os calsis (calcaneus) has shown to produce the most rapid declines in BMD with approximately 40% loss within two years of injury (Garland *et al.*, 2004), however this has not been assessed in persons with chronic SCI. In the studies that have examined bone mineral loss at several sites within an individual, it has been shown that bone mineral and mass losses approximate a proximal to distal gradient below the level of the lesion, such that distal bones (e.g. tibia) have lower BMD than more proximal bones (e.g. femur) (Biering-Sørensen *et al.*, 1988; Biering-Sorensen *et al.*, 1990; Garland *et al.*, 2004). It is evident that the greatest bone mineral and mass losses occur at the distal femur, proximal tibia, distal tibia, and calcaneus after SCI, and areas composed of primarily trabecular bone (i.e. epiphyses) lose bone at a more rapid rate and progress into greater losses than areas with a greater composition of cortical bone (i.e. diaphyses).

Alterations in the architectural properties of bone after spinal cord injury (SCI)

Trabecular and cortical bone areas undergo unique architectural changes after SCI that can be assessed using pQCT and approximated with DXA. At the proximal femur, greater trochanter, and femoral neck, Edwards and colleagues (2013 and 2014) found that integral (whole) and trabecular BMD show similar losses of ~3-4%/month, but cortical bone is lost at ≤1%/month in the 8 months

post-injury (Edwards *et al.*, 2013; Edwards *et al.*, 2014c). This loss was accompanied by a reduction in cortical bone volume of ~3%/month at each region, but not for integral bone volume which demonstrates that bone loss after SCI is primarily through endosteal resorption (i.e. cortical bone becomes more thin and trabecular area widens). These findings describe information that is unique and has not been presented before, but build on previous research (Kiratli *et al.*, 2000; Eser *et al.*, 2004a), which showed a similar pattern of increased (27%) inner diameter and reduced (15-35%) cortical area and thickness of the femur and tibia shaft when assessed in the chronic stage of SCI. Edwards and colleagues continued using the same method to analyze the distal femur and proximal tibia (Edwards *et al.*, 2014a). They found that within the first 8 months of SCI the integral and trabecular BMD of the distal femur and proximal tibia are reduced by 3-5%/month, whereas this is <1%/month at cortical sites (Edwards *et al.*, 2014a). Prior studies have also emphasized the preferential loss of trabecular sites at the distal femur, such as that by Eser and colleagues (2004) where they observed losses in trabecular BMD of 55% but only 45% losses for total BMD (Eser *et al.*, 2004a). The results from these studies demonstrate that although a proximal to distal gradient of bone mineral loss exists after SCI, their evidence would suggest a within bone epiphysis to diaphysis gradient and an overall proximal to distal gradient is more descriptive of the clinical findings (Edwards *et al.*, 2014a).

Fracture incidence and prevalence after spinal cord injury (SCI)

After SCI, fractures of the lower limbs are common and frequently occur with minimal impact, therefore are often referred to as fragility fractures (Vestergaard *et al.*, 1998). Recently, Dionyssiotis (2011) cited epidemiologic data from the United States (Model SCI Systems) suggesting that within the 10 years after injury, 28% of individuals will suffer a fracture, with this increasing to 39% within 15 years (Dionyssiotis, 2011). This is similar to values reported in other studies that have shown up to 34% fracture prevalence in persons with chronic SCI when assessed in a chart review (Frisbie, 1997) and cross-sectional study (Lazo *et al.*, 2001) (Table 2). Szollar and colleagues (1998) suggested that 76% of the population with SCI is affected by fractures, but did not provide a specific reference to support that claim (Szollar *et al.*, 1998).

The most commonly reported sites of fracture after SCI are regions of the femur and tibia, in particular the distal femur and proximal tibia (Comarr *et al.*, 1962; Freehafer & Mast, 1965; Ragnarsson & Sell, 1981; Freehafer, 1995; Frisbie, 1997; Vestergaard *et al.*, 1998; Morse *et al.*, 2009a). In relation to this, a BMD (g/cm^3) fracture risk threshold has been suggested, below which fractures were likely to occur from mild trauma in their sample (Eser *et al.*, 2005). Eser and colleagues (2005) identified this in a sample of almost 100 persons with motor complete SCI as 114 mg/cm^3 at the distal femur and 72 mg/cm^3 at the distal tibia (Eser *et al.*, 2005). There is no known fracture risk threshold at the proximal tibia, however using the knee region which includes both the distal femur and proximal tibia, a threshold for risk of 0.78 g/cm^2 has been suggested with a

breakpoint at 0.49 g/cm^2 (Garland *et al.*, 2005). In populations with SCI the secondary consequences of fractures have been shown to occur for up to one month in over 50% of cases, and lead to higher risk of respiratory illness, pressure ulcers, and urinary tract infections when compared to people with SCI who have not suffered a fracture (Morse *et al.*, 2009a; Carbone *et al.*, 2013). In addition, fractures in persons with SCI are a significant burden to the health care system, with average hospital stays being 35 days (5 is normal) (Morse *et al.*, 2009a).

Table 2: Fracture incidence and prevalence in persons with spinal cord injury (SCI).

Study	Time since injury	SCI participants (Female)	Age	Percent
Incidence				
(Zehnder *et al.*, 2004a)		100	18-60	
	< 1 year	16		1%/year
	1-9 years	38		1.3%/year
	10-19 years	31		3.4%/year
	20-29 years	13		4.6%/year
(Vestergaard *et al.*, 1998)	0-61 years	438 (129)	10-80	2%/year
Prevalence				
(Logan Jr *et al.*, 2008)	22±13 years	6132 (23.8%)	52.1±14	24.8%
(Lazo *et al.*, 2001)	1.1-43.1 years	49	27-83	34%
(Frisbie, 1997)	21.1±12.1 years	126	20-77	33%
(Ingram *et al.*, 1989)	> 1 year	526	13-70	5%
(Ragnarsson & Sell, 1981)	~9 years at fracture	578	4-71	4%
		3027 (564)	13-77	1.45%
(Freehafer *et al.*, 1981)		546		3%
(Freehafer & Mast, 1965)		264		<1%
(Eichenholtz, 1963)		700		4.5% total
(Comarr *et al.*, 1962)	0-15 years	1363	18-58	11%
(Carbone *et al.*, 2013)	≥ 2 years	7,590	22-94	14%
(Morse *et al.*, 2009a)	≥1 year	315	54.2±14.1	~9.5%

Abbreviations: SCI, spinal cord injury

Quantification of skeletal properties

In the clinical realm of assessment, radiologic scanning and biochemical markers in blood and urine are used to measure bone status. Radiologic scanning of bone properties in populations with SCI has frequently used DXA and pQCT and there are strengths and limitations of each that are evident after

reading the literature (Table 3). For example, it is often suggested that DXA has improved availability, repeatability, and more standard analysis sites than pQCT. However, pQCT is able to quantify trabecular and cortical BMD individually, determine the architectural and mechanical properties of bone, and analyze sites not possible by DXA with still high repeatability (Engelke *et al.*, 2008).

Table 3: The differences between dual-energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT).

Measurement	DXA	pQCT
Area of Interest	Large	Small
BMC	Yes	Yes
BMD	Areolar	Volumetric
Type of bone	Non-specific	Trabecular and cortical
Geometry of bone	No [a]	Yes
Strength of bone	Implied [a]	Quantified
Fracture risk	Non-specific	Specific
Availability	High	Low
Radiation	Low	Low
Repeatability	High	High
Resistant to body size change	Moderate	Moderate
Analysis	Standard sites	Non-standard sites
Influence of fat content	High	Moderate

BMC, bone mineral content; BMD, bone mineral density; DXA, dual-energy x-ray absorptiometry; pQCT, peripheral quantitative computed tomography
[a] Equations are published to estimate geometric and mechanical properties from DXA (Sievänen *et al.*, 1996).

Biomarkers

Bone turnover markers obtained from the blood and urine can provide identification of the processes behind altered bone turnover, are easily obtained, and can show changes in the early stages of injury that radiologic scanning may not detect (Maïmoun *et al.*, 2002; Qvist *et al.*, 2002; Vasikaran *et al.*, 2011; Chubb, 2012). When collecting blood for analysis of bone turnover markers, it is

important for collection methods, sample handling, and patient preparation to be well regulated and organized to maintain sample integrity (Qvist *et al.*, 2002; Biver, 2012; Chubb, 2012).

The International Osteoporosis Foundation (IOF) suggests that serum procollagen type 1 N propeptide (s-PINP) be used as a biomarker of bone formation and serum C-terminal telopeptide of type 1 collagen (s-CTX) be used to signify bone resorption (Vasikaran *et al.*, 2011). The applicability of this to persons who have SCI is important given that markers of bone resorption rise immediately after injury and peak between 10 to 16 weeks post injury (3 times increase in serum; 5 times increase urine), but increased levels can persist up to 12 months after SCI (Roberts *et al.*, 1998; Maïmoun *et al.*, 2002; Bubbear *et al.*, 2011; Chubb, 2012). A recent review by Garnero (2014) discussed the use of biological markers to identify specific aspects of bone metabolism (Garnero, 2014). For example, results from a recent study were described which demonstrated up-regulation of 9 micro RNA (miRNA) in the serum of able-bodied older adults with fracture (Seeliger *et al.*, 2014). Each miRNA is thought to be involved in the pathways of osteoblast and osteoclast development, therefore may provide specific information about bone metabolism.

Computed tomography (CT) scanning

pQCT is a non-projection technique, allows for 3D analysis, separates cortical and trabecular bone, and is resistant to errors due to degenerative changes in segments such as the lumbar spine (Engelke *et al.*, 2008). However, the International Society for Clinical Densitometry (ISCD) position statement on

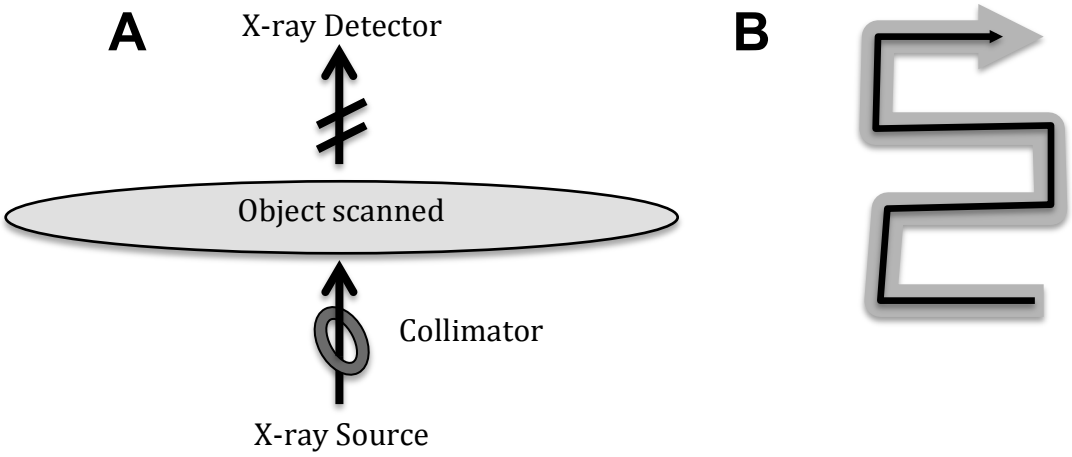
pQCT recognizes that DXA is the more widely used method and offers better precision (Engelke *et al.*, 2008). Initially, pQCT scanners analyzed a single slice from a segment (e.g. L1), but now use a spiral method that enables multiple-slices for each segment (e.g. 64 slices of L1). Further, there are now areal detectors so no rotation or movement of the person is needed and reduces scan time. pQCT is calibrated against a volume of water, which means that machines can be compared and used interchangeably. This information shows that pQCT has advantages over DXA, unfortunately scanners are not widely available and are only used by a few North American research groups.

Dual-energy x-ray absorptiometry (DXA) scanning

DXA scanning has become the most available and frequently used method to assess bone health in the last 20 years (Mazess *et al.*, 1990). In general, a DXA scanner has an x-ray source housed within the frame of the scanner bed, but in some models this is located above (Figure 1A). The x-ray source emits two photon energies, whose particles act as the carrier of the radiation emitted. The particles then pass through a collimator, and are directed to the object being scanned in either a pencil beam or a wider fan beam mode (Figure 1B). The fan beam mode of scanning is the most often used method of DXA today as it reduces the time of the scan, but it may increase radiation exposure during the scan. However, it has been shown that DXA scans deliver a radiation dose of less than 0.01 millisieverts (mSv), which is quite small when compared to the average yearly exposure of 2.4 mSv from natural background radiation (Damilakis *et al.*, 2010). The object being scanned, which was positioned by a given rater, absorbs

the x-ray photons (i.e. attenuation) and the remainder is detected. In humans, soft tissue (i.e. fat and muscle) has a lower density than bone, which means it does not attenuate the x-ray beam as much as in bone. This enables the calculation of the amount of bone mineral as calculation of soft tissue absorption involves both energies, whereas only the high energy is absorbed by bone. All of these variables contribute to the error of the DXA machine, and can be subdivided into error of the device and error of the object or rater.

Figure 1: Principles of dual-energy x-ray absorptiometry (DXA). A) Pathway of DXA x-rays from source to detection. B) Image representing the mode of DXA scanning as pencil beam (black arrow) and fan beam (gray arrow).



The standard assessment of age related osteoporosis in men and women is through BMD measurement via DXA at the lumbar spine, total hip, femoral neck, or occasionally the distal 1/3 radius. According to the ISCD position statement (Schousboe *et al.*, 2013), BMD measurement should be performed on:

- Women age 65 or older.
- In women younger than 65 or in a menopausal transition, if there is a history of low body weight, fracture, high medication use, or disease associated with bone loss.
- Men 70 and older.
- In men younger than 70, if there is a history of low body weight, fracture, high medication use, or disease associated with bone loss.
- Adults considered for pharmacologic therapy, being treated to monitor effect, or individuals who may be considered open to treatment if bone loss was present.

The measured BMD values are compared to the reference mean of a 20-29 year old Caucasian female from the United States National Health and Nutrition Examination Survey (NHANES) III database. Persons are given a T-score, which is the number of standard deviations away from the reference mean (Schousboe *et al.*, 2013). If the T-score is less than or equal to 2.5 standard deviations below the reference mean (T score ≤ 2.5) the individual is classified as having osteoporosis, and if the T-score is between 1 and 2.5 standard deviations below the reference mean they are classified as having osteopenia. However, males younger than 50 and pre-menopausal females are compared to a reference mean of similar ethnicity and BMD is considered normal if it falls within 2 standard deviations of average (known as a Z-score), whereas if below this level further testing is required as the BMD is considered below normal for a given age. Currently, DXA scanning is not part of standard of care for persons with SCI and as such there are no accepted reference standards for BMD.

The importance of dual-energy x-ray absorptiometry (DXA) precision studies

Determining the precision of DXA is one of the first steps needed when designing a clinical research study and is also required when using a new or modified scanner, new technologist, or new protocol. There are published guidelines available to conduct a precision analysis [see Steps of a precision analysis in Appendix] (Glüer *et al.*, 1995; Bonnick *et al.*, 2001). For a precision analysis, a sample of participant(s) must be scanned on multiple occasions, with a total of at least 30 degrees of freedom using the formula:

$$degrees\ of\ freedom = (number\ of\ measurements - 1)\ x\ (n)$$

where, *n* is the number of participants in the study (Glüer *et al.*, 1995; Bonnick *et al.*, 2001; Baim *et al.*, 2005). Within an individual, the standard deviation in g/cm^2 (SD) or coefficient of variation in % (CV) in BMD is calculated between multiple scans. The values for each person are pooled across the sample and described as the absolute precision error as root mean square standard deviation (RMS-SD in g/cm^2) or relative precision error as root mean square coefficient of variation (RMS-CV in %). This allows for calculation of a final value known as the least significant change (LSC) in g/cm^2 or percent. The LSC is determined by the formula:

$$LSC = Z'(\mathrm{Pr})\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

where, Z' is the value based on the given confidence level (e.g. 95% confidence level = 1.96), *Pr* is the precision error as RMS-SD or RMS-CV, and *n* is the number of scans at baseline (n_1) and follow-up (n_2). The LSC is the value that must be surpassed to determine true biologic change in follow-up scans. This is then used to determine the approximate length of follow-up needed between DXA scans given the formula:

$$Length\ of\ follow-up\ required = \frac{LSC}{Expected\ rate\ of\ change/year}$$

where, *LSC* is the calculated least significant change and the expected rate of change/year is derived from previous studies. If an initial precision analysis is not completed, any BMD change observed in a clinical study cannot be viewed as

a true biologic change as it may unknowingly represent the error in the method (Bonnick *et al.*, 2001).

The precision of DXA at standard sites such as the lumbar spine, total hip, and femoral neck is well known. In most of the studies published, the average precision error (CV) is between 0.5-3 % (Leslie & Moayyeri, 2006; Tothill & Hannan, 2007; Hind *et al.*, 2010; Edwards *et al.*, 2014c). Supporting this are results from a 2005 meta-analysis of precision studies by the ISCD, which found that the median precision values expressed as CV (%) were between 1-2% at the lumbar spine (1.1%), total hip (1.2%), and femoral neck (1.85 %) (Shepherd *et al.*, 2006b). Further, the ISCD published guidelines that state the minimum acceptable precision for a DXA technologist is a LSC of 5% at the total hip, 5.3% at the lumbar spine, and 6.9% at the femoral neck (Shepherd *et al.*, 2006b).

The precision error of DXA derived BMD is influenced by the DXA scanner, the position and structural/anatomical variability of the object being scanned, the operator, and the analysis of the scan (El Maghraoui & Roux, 2008). The error associated with the DXA scanner itself, such as differences in x-ray signal or detection is likely very small, but would be present in all studies and is very difficult to quantify. Baim and colleagues (2005) suggested that one of the most influential variables on DXA precision error is positioning (Baim *et al.*, 2005). It has been shown that when rotation of the hip is not well controlled by the operator, the precision error of DXA derived BMD can increase (Penny *et al.*, 2010), which may due to anatomical variability (El Maghraoui & Roux, 2008). The size and distribution of soft tissue around the region being scanned may also

contribute to the error of DXA. Within a region of interest (ROI), it has been shown that there is a positive relationship between BMI and relative precision error at the lumbar spine, femoral neck, and total body (Knapp *et al.*, 2012; Knapp *et al.*, 2014). However, in other studies this relationship was not present at the spine, femoral neck, and total hip (Rajamanohara *et al.*, 2011), or lumbar spine alone (Hopkins *et al.*, 2014). As BMI is known to be associated with increases in BMD (Castro *et al.*, 2005; Asomaning *et al.*, 2006), increases in BMD may also increase precision error. However, it has been shown that the CV is actually quite stable over a range of BMD, but the SD would be expected to increase with increasing BMD (Kiebzak *et al.*, 2012). When analyzing a scan, small variations in setting the analysis ROI also influence the precision error. This is difficult to account for in serial BMD measurements, but can be approximated when a test for intra-rater reliability is performed with repeat analyses of the same scan (Bakkum *et al.*, 2013). In addition, it has been suggested that a smaller ROI may increase precision error (Lodder *et al.*, 2004; El Maghraoui & Roux, 2008). When designing a clinical study, all of these factors must be taken into consideration as they can unknowingly influence the precision error of DXA.

Dual-energy x-ray absorptiometry (DXA) scanning at clinically relevant sites in persons with spinal cord injury (SCI)

The standard methods of DXA scanning at the lumbar spine, total hip, and proximal femur are not clinically relevant in persons with SCI. DXA scanning at the distal femur, proximal tibia, and calcaneus are clinically relevant sites for

BMD measurement after SCI due to fracture risk (distal femur and proximal tibia) and BMD loss (calcaneus). Currently, there are no formal methods to measure these sites and although some studies have published protocols (Table 4), the precision of the methods are not well known.

There are minimal studies that have completed precision analyses of DXA at clinically relevant sites of the distal femur and proximal tibia for persons with SCI. Morse and colleagues (2009) showed that in 20 persons with SCI the precision of DXA at the distal femur was slightly better (RMS-CV = 3.01%, RMS-SD = 0.025 g/cm^2, BMD range = 0.342 g/cm^2 to 1.028 g/cm^2) than at the proximal tibia (RMS-CV = 5.91%, RMS-SD = 0.030 g/cm^2, BMD range = 0.115 g/cm^2 to 1.192 g/cm^2) (Morse *et al.*, 2009b). These results support earlier work from Shields and colleagues (2005) who showed an improved intraclass correlation coefficient (ICC), which is not a measure of precision but is a measure of agreement, between 4 raters at the distal femur (all >0.97) when compared to the proximal tibia (all > 0.87) (Shields *et al.*, 2005). Second, Haddaway and colleagues (2013) compared the precision of 6 regions of the lower body, which were composed of integral (whole) bone measuring the entire femur and tibia (i.e. three regions per bone longitudinally), in 30 persons who are able-bodied (Haddaway *et al.*, 2013). They found a large range in precision error (as RMS-SD) for these 6 regions (0.023 to 0.048 g/cm^2), which was reflected in the corresponding LSC values that were between 4.6 and 12.4%. The best precision error in their study was for a region at the mid-shaft of the femur (LSC, 4.6%), but is an area that experiences minimal BMD loss after SCI therefore is not as

clinically relevant. McPherson and colleagues (2014) assessed three regions of the distal femur and proximal tibia that are similar to the regions used by Haddaway and colleagues, but did so in persons with acute and chronic SCI (McPherson *et al.*, 2014). They found that the precision error of DXA ranged from 1.39 – 1.70% in persons with acute SCI and between 3.12 – 4.70% in persons with chronic SCI.

At the calcaneus, DXA scanning has rarely been used to assess BMD in persons with SCI, and in the published literature there are only three such studies (Warden *et al.*, 2001; Warden *et al.*, 2002; Garland *et al.*, 2004). The region used by Warden and colleagues was based on a prior study using DPA (Szücs *et al.*, 1992), and Garland and colleagues (2004) did not provide information regarding the specific location of their calcaneus assessment. Szücs and colleagues (1992) first demonstrated that a homogenous bone mineral region exists 3.6 cm from the heel and 4.0 cm from the sole in able-bodied cadavers. At this region, they found an average precision error (CV) of 2.8% when using DPA in 6 persons who are able-bodied that were scanned a total of 6 times each. Warden and colleagues reported the precision error in their study to be 1.0% when using DXA to assess approximately the same region in 5 persons who are able-bodied over 5 scans (Warden *et al.*, 2002). To date, there is no study that has assessed the precision of DXA at the distal femur, proximal tibia, and calcaneus in persons with SCI compared to persons who are able-bodied. Therefore, it is not well known as to how the change in BMD from normal to

osteoporotic after SCI, and how paralysis of the lower body, influence the precision error of DXA.

Table 4: Precision and reliability studies of dual-energy x-ray absorptiometry (DXA) scanning at site-specific regions of the lower limb.

Study	Population	Scan type	Regions	Outcome measures
(Shields et al., 2005)	SCI (n=11) and matched persons who are able-bodied	Spine	Distal femur and proximal tibia	ICC for inter-rater reliability.
(Morse et al., 2009b)	SCI (n=20)	N/A	Distal femur and proximal tibia	RMS-CV and RMS-SD.
(Haddaway et al., 2013)	Able bodied (n=30)	Whole Body	Femur and Tibia	CV and LSC; intra-operator and inter-operator errors.
(Gaspar *et al.*, 2012)	SCI (n=20)	N/A	Distal femur	CV
(Bakkum et al., 2013)	Able bodied (n=10)	Forearm	Distal femur and proximal tibia	ICC, standard error of the mean, smallest detectable difference
(McPherson et al., 2014)	SCI (n=12 acute, n=34 chronic)	Forearm	Distal femur and proximal tibia	RMS-CV, RMS-SD, and ICC

Abbreviations: CV, coefficient of variation; ICC, intraclass correlation coefficient; LSC, least significant change; RMS-CV, root mean squared – coefficient of variation; RMS-SD, root mean squared – standard deviation; SCI, spinal cord injury

Chapter 2: Study design and methods

Statement of problem

Bone loss and osteoporosis after SCI is of significant concern given the increased risk of fracture. Standard BMD measurement by DXA is not specific for the regions of highest fracture risk and greatest loss after SCI, namely the distal femur, proximal tibia, and calcaneus. In order to evaluate potential treatments to prevent SCI osteoporosis, clinically relevant and reproducible methods to assess BMD and fracture risk are required. Previous studies have used site-specific DXA scanning to measure BMD, but these studies did not compare a group of persons with SCI (expected low BMD) to a group of persons who are able-bodied (expected normal BMD), therefore it is not known how SCI and low BMD influence precision error results.

Rationale

This study is an attempt at reducing these prior pitfalls and combining three regions that are clinically relevant after SCI to assess the reproducibility of site-specific DXA scanning in persons with chronic SCI (expected low BMD) and persons who are able-bodied (expected normal BMD), and to do so in accordance with the guidelines of the ISCD.

Hypotheses

1. At every ROI, the BMD will be lower in persons with SCI when compared to persons who are able-bodied.

This is hypothesized because it has been previously shown that rapid BMD loss at the distal femur, proximal tibia, and calcaneus occurs after SCI (Biering-Sorensen *et al.*, 1990; Garland *et al.*, 2004).

2. At each ROI, the relative precision error of DXA derived BMD will be higher (less precise) in the group of persons with SCI.

This was hypothesized because factors that have been shown to increase precision error such as higher percent fat content, reductions and/or alterations in the distribution of soft tissue, and difficulties in positioning are more likely to be present in persons with SCI.

Participants

We recruited persons with SCI and persons who are able-bodied from the local community via word of mouth and advertisements at the SCI outpatient clinic of the Health Sciences Centre Manitoba. All participants were required to be fracture free in the leg scanned, not pregnant, pre-menopausal, and without a prior history of bone disease. In addition, persons with SCI were required to have a motor complete SCI of at least 2 years duration, be able to transfer independently to and from the scanning bed, and have minimal issues with spasticity and extension of the lower limbs. All participants provided their written informed consent, were fully disclosed as to the process of the study, and were provided with modest monetary compensation ($40) for their time. The University of Manitoba Research Ethics Board approved all study procedures.

In the group of persons with SCI, injury level, completeness, and injury duration (years) were determined from self-report or through verbal questions

according to the American Spinal Injury Association guidelines (Maynard *et al.*, 1997; Marino *et al.*, 2003). We recorded sex, age (years), height (cm), body mass (kg), and femur length (cm) for all participants. Femur length (cm) was measured with the knee bent at 90° while seated in either a wheelchair or standard chair, and was taken as the distance from the inguinal ligament (just distal to the palpated anterior superior iliac spine) to the proximal patella as per standard protocols (Timothy G Lohman *et al.*, 1988).

Dual-energy X-ray Absorptiometry

DXA scans were completed at the Manitoba Institute of Child Health on two separate visits, which were between 5 – 20 days (mean 8.85) apart for all participants. All scans were completed by the same certified DXA technologist using a Hologic QDR 4500A pediatric scanner in forearm mode, and all analyses were conducted using the installed subregion forearm analysis application. To accommodate barriers associated with DXA scanning for persons with SCI, a ramp, platform, and headrest were fabricated for the specifications of the room (Figure 2A and B). All scans were completed on the dominant leg unless previously fractured. A daily DXA quality control procedure was performed using a lumbar spine phantom. During the course of this study, the mean CV for BMD of the L1-L4 spine phantom was 0.26%, with a range of 0.45-0.65% for individual vertebrae.

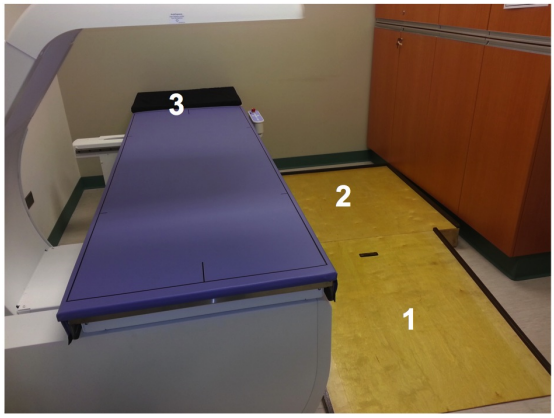
Once an individual had successfully transferred to the scanning bed, a single research assistant positioned the participant and secured the limb in the appropriate brace (Figure 2C-E). We did not have participant's transfer on and

off the scanning bed, but used a rotating scan order (knee, ankle, knee, ankle) to ensure positioning was new for each subsequent scan. For knee scans, which contained the distal femur and proximal tibia ROIs, the participant was positioned supine, with feet oriented anteriorly, and the legs together in the sagittal plane. In persons who are able-bodied, a custom 90° brace was used (Figure 2C). In persons with SCI, we used a standard Hologic hip brace (Figure 2D), which minimized overlap of the tibia and fibula and provided an image that was similar to the persons who are able-bodied. For the calcaneus scan, participants were positioned on the side of interest, and the leg not being scanned was placed on top of the leg being scanned to improve participant comfort (Figure 2E). One 90° brace was used for all participants (Figure 2E), which oriented the leg to the long axis of the scanning bed, while keeping the external aspect of the brace flat. If required, additional weights, foam blocks, and strapping were used to obtain the desired position for both knee and calcaneus scans. A total of four scans were completed over 2 visits for each participant (2 scans each of the knee and calcaneus per visit). Therefore, we had 30 degrees of freedom in each group [degrees of freedom = (number of scans per person – 1) x n], which satisfies the guidelines stated by the International Society for Clinical Densitometry (Bonnick *et al.*, 2001). We chose to complete scans over two visits as it mimics a clinical scenario when participants would have a DXA scan at baseline and then again at follow-up. Further, it is important to complete a precision analysis over multiples visits as others have suggested that conducting

a precision analysis on a single day will lead to lower precision errors that when conducted over multiple days (Bonnick *et al.*, 2001; Leslie & Moayyeri, 2006).

Figure 2: Representative image of the dual-energy x-ray absorptiometry (DXA) scanner and braces used. A) Image of the DXA scanning room showing the 1) ramp, 2) platform, and 3) headrest. B) Table listing the dimensions of the DXA scanner, room, and fabricated platforms from A). C) Image of the set-up for the knee scans in the group of participants who are able-bodied. D) Image of the set-up for the knee scans in the group of participants who have SCI. E) Image of the set-up for the calcaneus scans in both participants who are able-bodied and those with SCI. Abbreviations: H, height; L, length; W, width. Permission was granted from the individual in this photo to be included in the thesis.

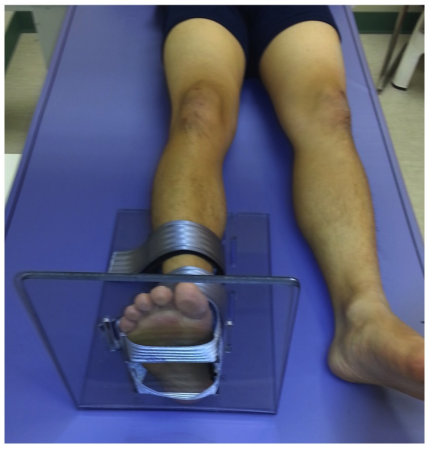
A



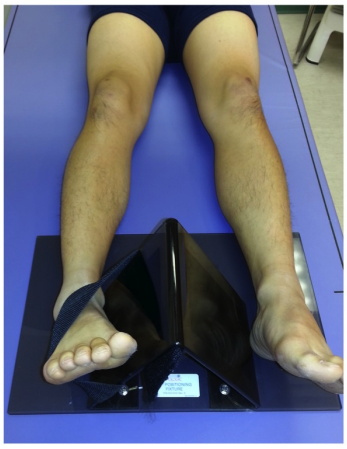
B

Object	L (cm)	W (cm)	H (cm)
DXA	202	70	71.5
Room	406.5	283	X
Door	X	91.4	X
Ramp	106	91	12.7
Platform	106	106	12.7
Headrest	56	71	71

C



D



E

For scan analysis we chose a total of 7 ROIs at the distal femur, proximal tibia, and calcaneus (Figure 3 and Figure 4). One person, who designed and had significant practice with the protocol, completed all of the measurements in this study but was not blinded to the status of each participant. At each ROI, we determined the area (cm^2), BMC (g), and BMD (g/cm^2). At the distal femur, a proximal border of 20% femur length was used, as measured from the most distal aspect of the lateral condyle, which is similar to that done previously (Shields *et al.*, 2005; Morse *et al.*, 2009b). The distal border of the ROI was set at 15% femur length measured from the distal aspect of the lateral condyle, as we found that using the 13% femur length of Shields and colleagues included the patella in some scans, which has shown to increase BMD (Bakkum *et al.*, 2013). Further, using 15% femur length as the distal border circumvented the need to rely on the patella for setting the border as done by Morse and colleagues. One ROI box (ROI # 1) was then centered within the visible cortical borders, which was arbitrarily defined as 70% of the bone width at the proximal border and was maintained at that width for all four scans of an individual, while a second ROI box (ROI # 2) was positioned just outside the whole bone margins. At the proximal tibia, we created three ROI boxes to analyze both the epiphysis and metaphysis. The epiphyseal proximal tibia ROI (ROI # 3) was selected as the same size as the distal femur ROI # 1. The proximal border was placed at the most proximal point of contact between the tibia and fibula and was centered within the tibia epiphysis in an area known to be predominantly trabecular bone (Clarke, 2008), which is consistent to that done previously (Shields *et al.*, 2005;

Morse *et al.*, 2009b). At the proximal tibia metaphysis, the proximal border was set at 2 pixels below the distal contact of the tibia and fibula, with extraneous bone removed from the interosseous membrane, and the height of the box equal to that of 5% femur length. One region (ROI # 4) took the entire bone width, while the other (ROI # 5) was set and maintained for all analyses of an individual at 50% of the bone width at the distal border within the visible cortical boundaries, which is a ROI that is similar to other studies (Vazquez *et al.*, 2004). At the calcaneus one ROI (ROI # 6) was selected as a region corresponding to the coordinates outlined by Szücs and colleagues. This ROI had a size of 26 x 12 pixels (1.32 cm^2) in all participants and was visually centered in the posterior calcaneal body using the anatomical landmark of the calcaneal tuberosity, similar to that done previously (Szücs *et al.*, 1992; Warden *et al.*, 2001; Warden *et al.*, 2002). A second ROI at the calcaneus consisted of the entire posterior calcaneal body (ROI # 7), which may act to limit any minor inconsistencies in bone mineral of the calcaneus and is similar to prior studies (Sievanen *et al.*, 1992).

Figure 3: Image of a knee scan in an able-bodied participant (A) and a participant with spinal cord injury (SCI) (B). Outlined in the scan are the 5 regions of interest (ROI) obtained. R1 = ROI # 1; R2 = ROI # 2; R3 = ROI # 3; R4 = ROI # 4; R5 = ROI # 5. Note: images were obtained using the same contrast.

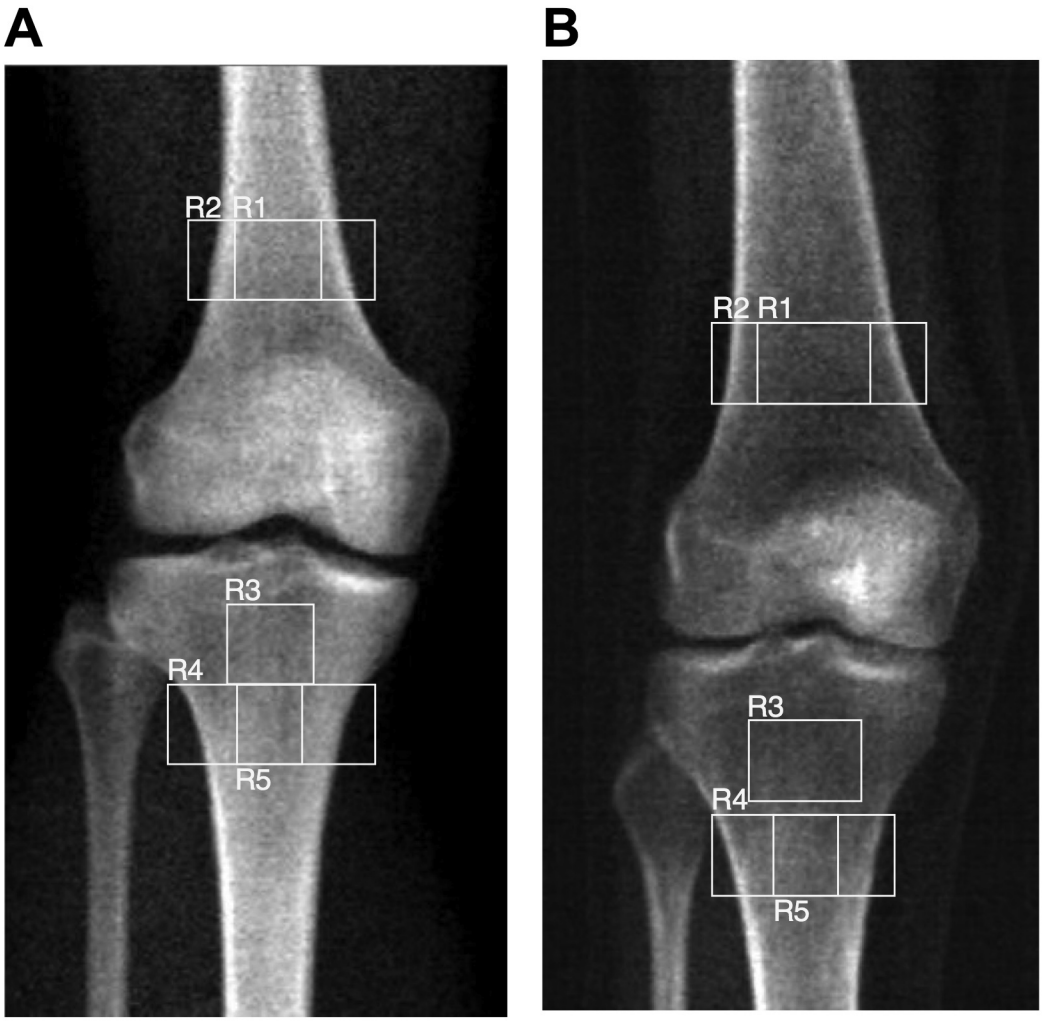
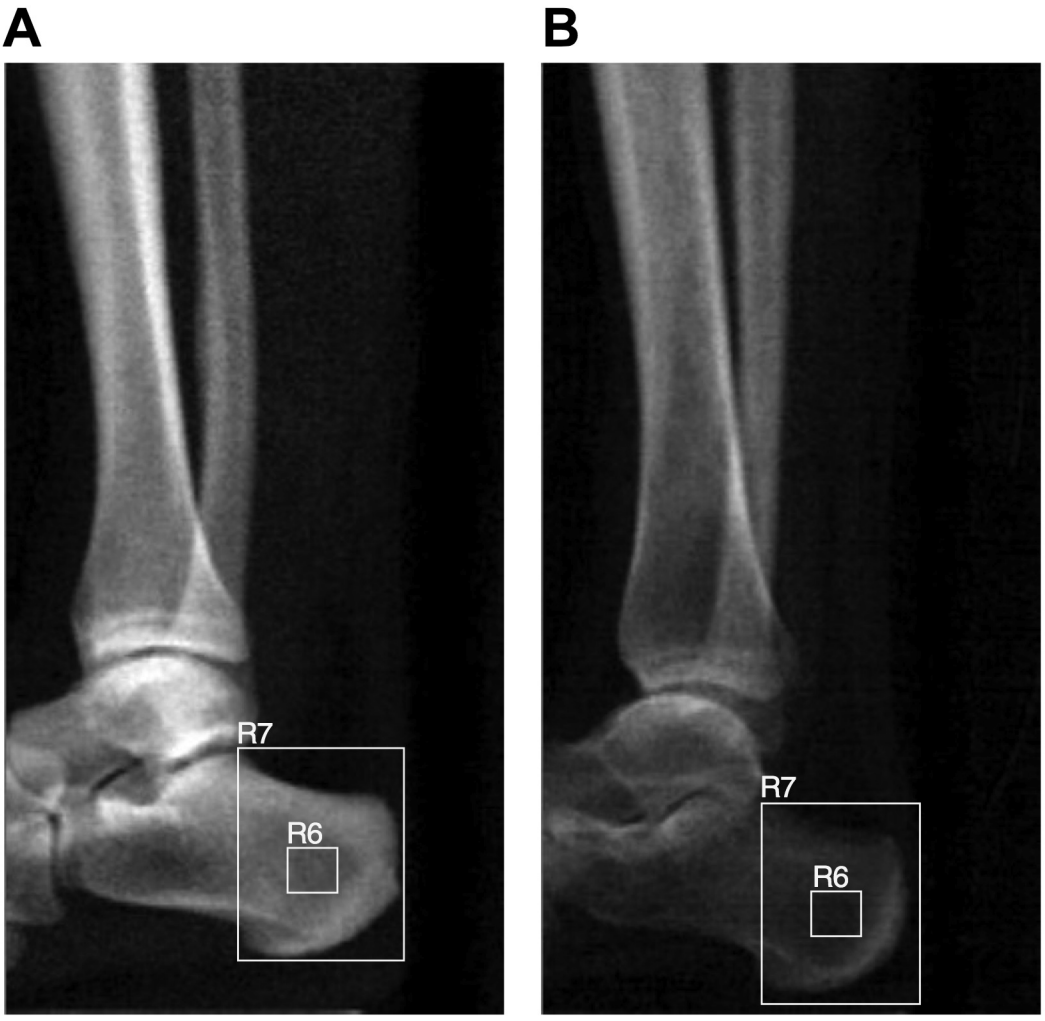


Figure 4: Image of a calcaneus scan in an able-bodied participant (A) and a participant with spinal cord injury (SCI) (B). Outlined in the scan are the 2 regions of interest (ROI). R6 = ROI # 6; R7 = ROI # 7. Note: images were obtained using the same contrast.



Statistics

The group of persons with SCI and the group of persons who are able-bodied were not matched for age, sex, or body mass. Physical characteristics of height, age, body mass, and BMI were compared between groups using unpaired t-tests. BMD was used for statistical analysis as it is calculated via BMD = BMC/area, therefore takes the size of bone into account. At each ROI, an unpaired t-test was used to compare the BMD between the group of persons with SCI and the group of persons who are able-bodied, which was calculated as the average BMD for each participant from the 4 scans. For each ROI, Bland-Altman plots were used to determine if BMD (i.e. high or low) had a systematic effect on the error between days (i.e. negative or positive) (Bland & Altman, 1986), and paired t-tests were used to compare the mean BMD between visits. Pearson or Spearman correlations were used to determine the relationship between BMD and BMI at each ROI.

To determine the precision error of DXA derived BMD values at each ROI (Bonnick *et al.*, 2001), we used the ISCD precision calculator (http://www.iscd.org/resources/calculators/precision-calculator/faq/). For each participant, the average BMD, CV, and SD across the 4 scans was calculated at each ROI. RMS-CV, RMS-SD, and LSC were calculated for each ROI in both groups of participants. To calculate LSC, we used the formula:

$$LSC = Z'(Pr)\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

where, Z' is the desired confidence level (i.e. 95% = 1.96), *Pr* is the calculated RMS-SD or RMS-CV, n_1 is the number of scans at baseline, and n_2 is the number of scans at follow up (Bonnick *et al.*, 2001). Confidence intervals (95%) were calculated for RMS-SD and RMS-CV using the formula:

$$\sqrt{\frac{df}{\chi^2_{\frac{\alpha}{2},df}}}SD^2 < \sigma^2 < \sqrt{\frac{df}{\chi^2_{1-\frac{\alpha}{2},df}}}SD^2$$

which was developed by Glüer and colleagues (1995) (Glüer *et al.*, 1995), and has since been used in a number of precision studies (Leslie & Moayyeri, 2006; Penny *et al.*, 2010; Knapp *et al.*, 2012; Krueger *et al.*, 2013). To compare the precision error between the group of persons with SCI and the group of persons who are able-bodied, we used a variance ratio F-test. Precision errors were transformed to a logarithmic scale, which reduced the skewness and allowed data to pass the assumption of normality. A p-value <0.05 was considered statistically significant, however when multiple statistical tests were completed a Bonferroni correction was used (e.g. p=0.05 / 7, therefore the p value was equal to 0.007). All data is presented as mean ± standard deviation unless otherwise indicated. All statistical procedures were completed using STATA for Mac version 12 (StataCorp, College Station, TX) and GraphPad Prism 6.2 for Mac (Graph Pad Software, La Jolla CA).

Chapter 3: Results

Participants

We enrolled 10 persons with SCI (C6-L2, AIS A-B) and 10 persons who are able-bodied (Table 5). All of the participants completed both visits. In total, we had 4 females in the group of persons who are able-bodied and 2 females in the group of persons with SCI, which is representative of the demographics of the population with SCI. We did not obtain background information regarding lifestyle factors such as smoking or physical activity for participants. The group of persons with SCI included 8 individuals with tetraplegia (2 paraplegia) and had an injury duration of 22.7±9.37 years (range: 4–37). The group of persons with SCI was significantly older (44.50±11.57 years) than the group of persons who are able-bodied (29.10±8.16 years) ($p<0.01$). There were no significant differences in height, mass, or BMI between the two groups.

Table 5: Participant Characteristics.

Subject	Sex	Age (years) †	Height (cm)	Mass (Kg)	Level of SCI	AIS	Years post SCI
Group of persons who are able-bodied							
1	M	38	168	80	X	X	X
2	M	21	180	74	X	X	X
3	M	30	185	100	X	X	X
4	F	24	160	54	X	X	X
5	M	23	175	77	X	X	X
6	M	24	170	71	X	X	X
7	F	28	175	70	X	X	X
8	F	28	163	54	X	X	X
9	F	27	155	54	X	X	X
10	M	48	180	92	X	X	X
Group of persons with spinal cord injury (SCI)							
1	M	56	165	82	C6	A	34
2	M	29	196	86	C6	A	14
3	F	46	170	58	C8	B	25
4	M	58	187.5	100	(L) L2; (R) T8	B	37
5	M	54	172.5	96	C6/7	B	25
6	M	39	177.5	95	T6/7	A	19
7	M	41	170	67	C6-7	A	21
8	F	47	170	58	C8	B	25
9	M	23	177.5	62	C6/7	B	4
10	M	51	180	75	C6	B	23

Abbreviations: F, female; M, male

AIS, American Spinal Injury Association impairment scale (Maynard *et al.*, 1997; Marino *et al.*, 2003).

† The group of persons with SCI were significantly older (44.50±11.57) than the group of persons who are able-bodied (29.10±8.16) ($p<0.01$).

Bone mineral density results

There were no systematic differences in the average BMD between visits as indicated by Bland-Altman plots, and none of the differences between visits were significant when compared using paired t-tests with a Bonferroni correction (p value range, 0.01 – 0.77) (Figure 5). At every ROI, the group of persons with SCI had a lower BMD than the group of persons who are able-bodied ($p<0.0001$) (Table 6 and Figure 6). The BMD values in the group of persons with SCI were between 33 – 56% of that for the group of persons who are able-bodied (Table 6 and Figure 6). The greatest difference was at ROI # 6, where the group of persons with SCI had a BMD that was 33% of the group of persons who are able-bodied (SCI, 0.22±0.06 g/cm^2; able-bodied, 0.66±0.12 g/cm^2). In the group of persons who are able-bodied, we observed positive correlations for BMD with BMI at ROI # 6 ($r = 0.80$, $p=0.005$) and ROI # 7 ($r = 0.81$, $p=0.004$) of the calcaneus, which is an area of loading in persons who are able-bodied (Table 7). There was no consistent relationship in persons with SCI.

Figure 5: Evaluation of bone mineral density (BMD) repeatability across multiple visits for all regions of interest (ROIs). Bland-Altman plots of the difference between days (visit 2 – visit 1) on the y-axis against the average BMD on the x-axis. There were no significant differences in the BMD between days at any ROI, which was assessed by paired t-tests with a Bonferroni correction (p value range, 0.01 – 0.77).

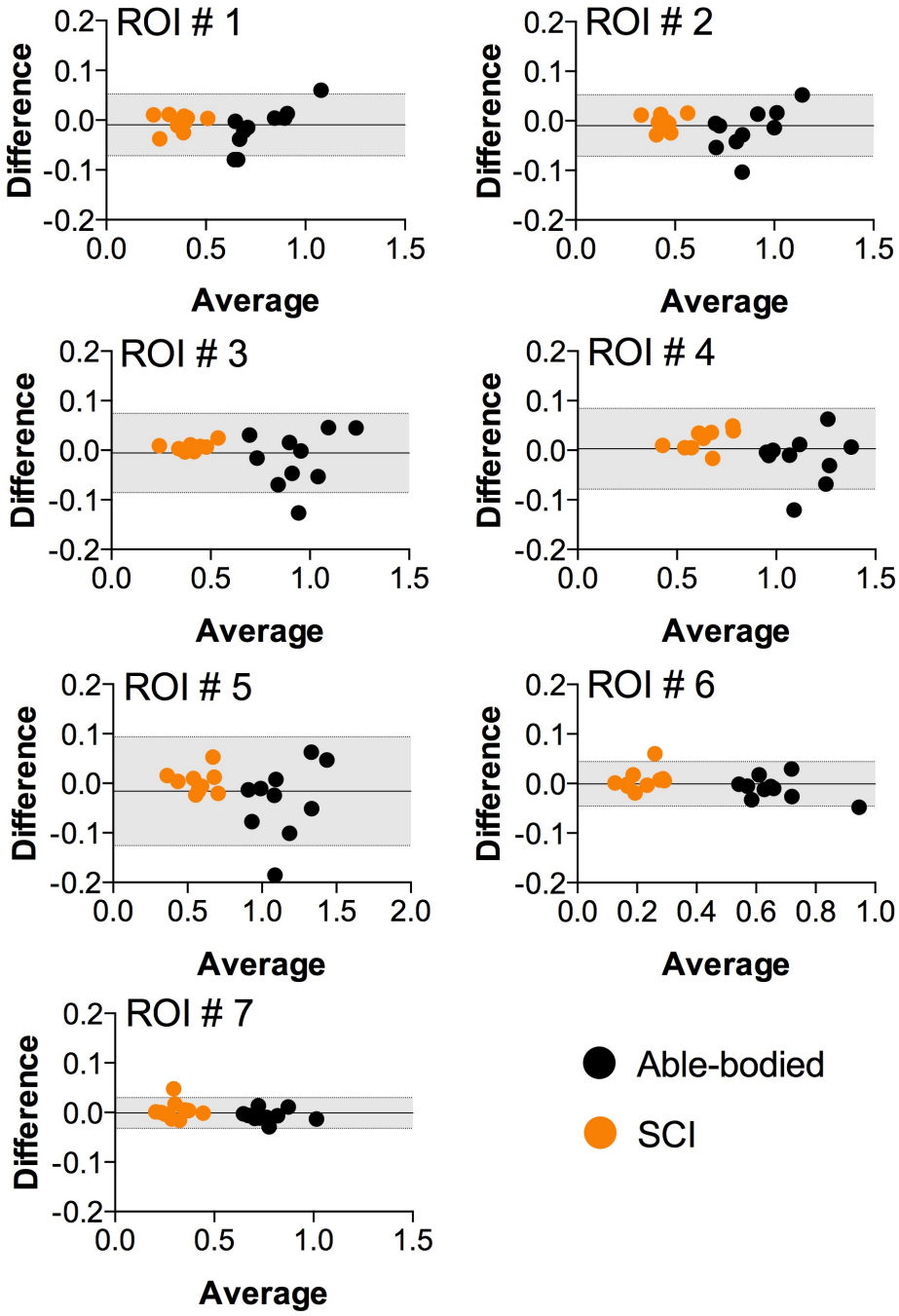


Table 6: Bone mineral density (BMD) at each region of interest (ROI) in the group of persons who are able-bodied and the group of persons with spinal cord injury (SCI).

	Bone mineral density (BMD) for each scan				Average results	
	Visit 1		Visit 2			
	Scan 1	Scan 2	Scan 3	Scan 4		
	Mean g/cm^2 (SD)	Mean g/cm^2 (SD)	Mean g/cm^2 (SD)	Mean g/cm^2 (SD))	Mean g/cm^2 (SD)	Percent of AB group
Group of persons who are able-bodied						
ROI 1	.78 (.15)	.78 (.12)	.77 (.18)	.76 (.16)	.78 (.15)	-
ROI 2	.87 (.14)	.88 (.14)	.87 (.17)	.85 (.16)	.87 (.15)	-
ROI 3	.95 (.18)	.94 (.14)	.93 (.17)	.93 (.17)	.94 (.16)	-
ROI 4	1.14 (.15)	1.14 (.15)	1.12 (.15)	1.13 (.16)	1.13 (.15)	-
ROI 5	1.15 (.17)	1.16 (.18)	1.11 (.20)	1.13 (.19)	1.14 (.18)	-
ROI 6	.67 (1.3)	.67 (.11)	.66 (.11)	.66 (.12)	.66 (.12)	-
ROI 7	.77 (.11)	.78 (.11)	.77 (.11)	.76 (.11)	.77 (.11)	-
Group of persons with spinal cord injury (SCI)						
ROI 1	.38 (.08)	.36 (.09)	.37 (.08)	.36 (.09)	.37 (.08)	47.07§
ROI 2	.44 (.07)	.43 (.06)	.43 (.06)	.43 (.07)	.44 (.06)	51.03§
ROI 3	.41 (.09)	.40 (.07)	.41 (.09)	.42 (.08)	.40 (.09)	42.66§
ROI 4	.62 (.10)	.62 (.11)	.64 (.12)	.64 (.11)	.63 (.11)	55.69§
ROI 5	.58 (.11)	.56 (.11)	.57 (.11)	.57 (.11)	.57 (.11)	50.10§
ROI 6	.22 (.06)	.21 (.06)	.22 (.06)	.22 (.06)	.22 (.06)	33.08§
ROI 7	.30 (.07)	.30 (.07)	.31 (.07)	.31 (.07)	.31 (.07)	39.68§

Abbreviations: AB, able-bodied; ROI, region of interest. Values are mean ± standard deviation. The group of persons with SCI had a lower BMD at every ROI as indicated in the table (§$p<0.0001$).

Figure 6: Graphical display of bone mineral density (BMD) at each region of interest (ROI) in the group of persons who are able-bodied and the group of persons with spinal cord injury (SCI). At every ROI, the BMD was lower in persons with SCI when compared to persons who are able-bodied (p<0.0001).

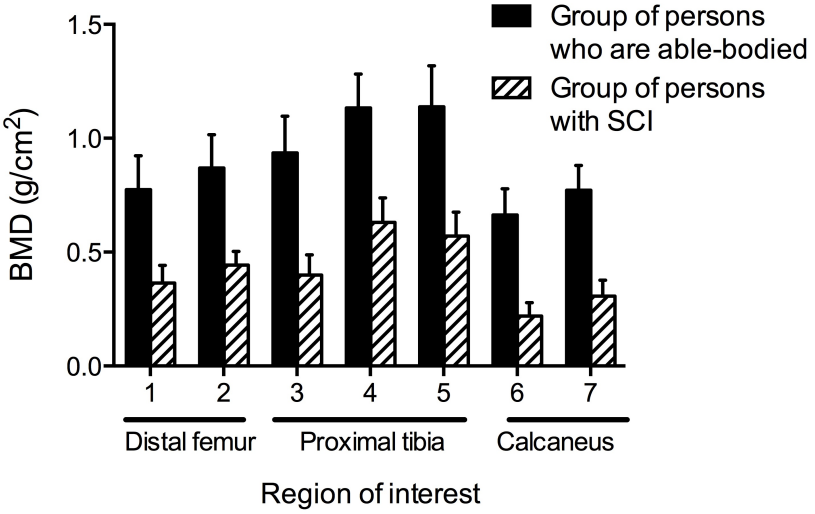


Table 7: Correlation of bone mineral density (BMD) with body mass index (BMI)

		Bone mineral density (BMD) at each ROI						
		1	2	3	4	5	6	7
Group of persons who are able-bodied								
BMI	Correlation coefficient (r)	0.19	0.45	0.08	0.61	0.38	0.80†	0.81†
Group of persons with spinal cord injury (SCI)								
BMI	Correlation coefficient (r)	-0.70	-0.56	-0.15	-0.44	-0.40	0.15	0.09

[a] Data were obtained via self-report
†p<0.01.

Precision errors

For each participant, the 4 BMD values at each ROI were used to calculate the precision error. There was a trend for greater variance of the relative precision in persons with SCI at ROI # 1 in comparison to the group of persons who are able-bodied (p=0.018) (Figure 7 and Table 8). This resulted in a RMS-CV (95% CI) precision error of 8.40% (6.71-11.16) in persons with SCI, which was greater than the 5.63% (4.50-7.49) for the group of persons who are able-bodied. There was also a trend (p=0.051) for greater relative precision error at ROI # 7 in persons with SCI (Figure 7 and Table 8). At this ROI, the group of persons with SCI had a RMS-CV (95% CI) precision error of 3.52% (2.81-4.71) compared to 1.78% (1.42-2.38) in the group of persons who are able-bodied. In persons who are able-bodied, the ROI with the best precision error appeared to be ROI # 7, which had a LSC of 4.94%. None of these differences were statistically significant once a Bonferroni correction for multiple comparisons was made. There was no relationship of precision error with BMI in the group of persons who are able-bodied or in the group of persons with SCI (Table 9).

Figure 7: Graphical display of precision error in persons who are able-bodied and persons with spinal cord injury (SCI) at each region of interest (ROI). A) Relative precision error as coefficient of variation (CV, %), displayed as mean±standard deviation. B) Absolute precision error as standard deviation (SD, g/cm^2), displayed as mean±standard deviation.

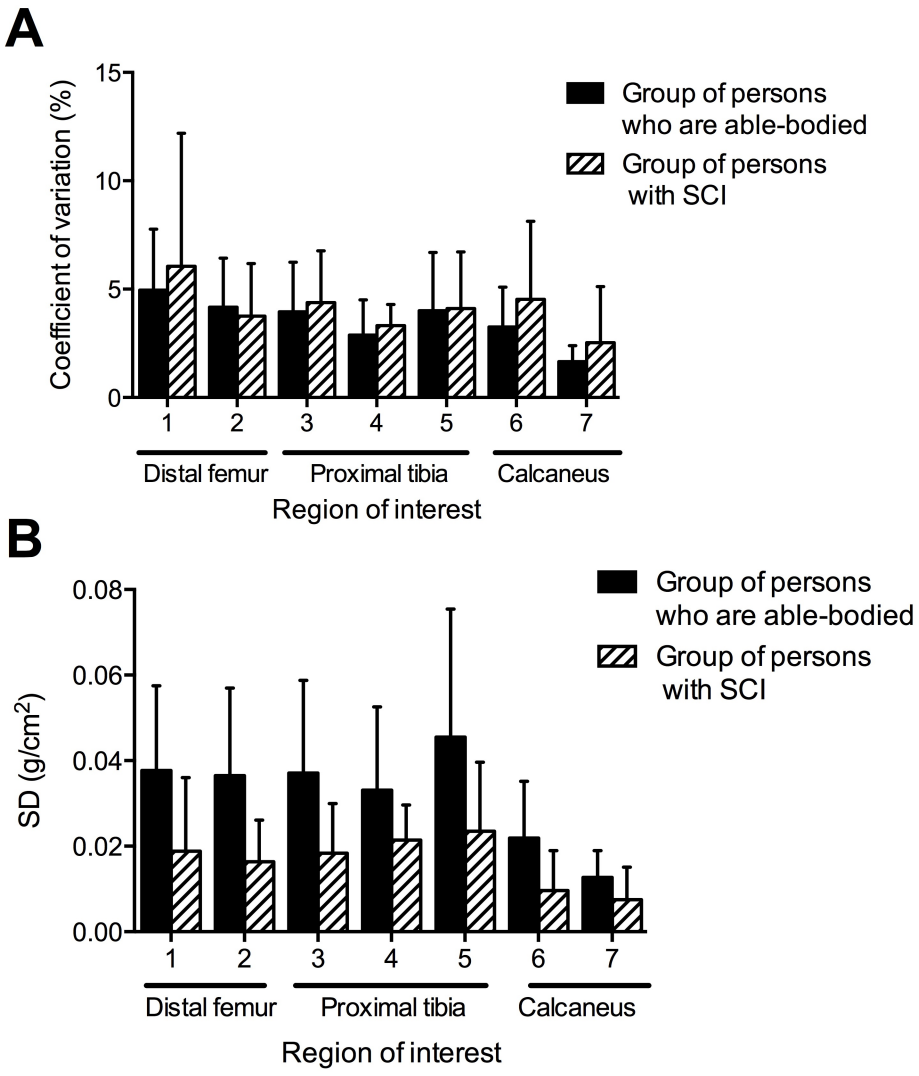


Table 8: Precision error and least significant change (LSC) calculated at a 95% confidence interval with one baseline and one follow-up scan.

	Individual precision error		Precision expressed as RMS-SD and RMS-CV		Least significant change (LSC)	
	SD	CV	RMS-SD as g/cm^2 (95% CI)	RMS-CV as % (95% CI)	RMS-SD as g/cm^2	RMS-CV as %
Group of persons who are able-bodied						
ROI 1	.038	4.96	.042 (.034-.056)	5.63 (4.50-7.49)	.116	15.60
ROI 2	.037	4.17	.041 (.033-.055)	4.68 (3.74-6.26)	.114	12.97
ROI 3	.037	3.96	.042 (.034-.057)	4.51 (3.60-6.02)	.118	12.48
ROI 4	.033	2.88	.038 (.030-.051)	3.26 (2.60-4.36)	.106	9.03
ROI 5	.046	4.00	.054 (.043-.072)	4.73 (3.78-6.33)	.149	13.11
ROI 6	.022	3.26	.025 (.020-.034)	3.68 (2.94-4.92)	.070	10.19
ROI 7	.013	1.65	.014 (.011-.019)	1.78 (1.42-2.38)	.039	4.94
Group of persons with spinal cord injury (SCI)						
ROI 1	.019	6.06	.025 (.020-.033)	8.40 (6.71-11.16)	.069	23.26
ROI 2	.016	3.76	.019 (.015-.025)	4.41 (3.53-5.90)	.052	12.22
ROI 3	.018	4.38	.021 (.017-.029)	4.94 (3.95-6.60)	.059	13.68
ROI 4	.021	3.32	.023 (.018-.030)	3.44 (2.75-4.60)	.063	9.53
ROI 5	.024	4.10	.028 (.022-.037)	4.78 (3.82-6.39)	.078	13.23
ROI 6	.010	4.53	.013 (.011-.018)	5.66 (4.52-7.56)	.037	15.67
ROI 7	.008	2.53	.010 (.008-.014)	3.52 (2.81-4.71)	.029	9.75

Abbreviations: CI, confidence interval; CV, coefficient of variation (%); LSC, least significant change; RMS-CV, root mean squared coefficient of variation; RMS-SD, root mean squared standard deviation; ROI, region of interest; SCI, spinal cord injury; SD, standard deviation (g/cm^2).

LSC was calculated using the formula

$$LSC = Z'(Pr)\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

which was derived previously (Bonnick *et al.*, 2001).

Confidence intervals were calculated based on the formula

$$\sqrt{\frac{df}{\chi^2_{\frac{\alpha}{2},df}}}SD^2 < \sigma^2 < \sqrt{\frac{df}{\chi^2_{1-\frac{\alpha}{2},df}}}SD^2$$

which was derived by Glüer et al., (1995) (Glüer *et al.*, 1995).

Table 9: Correlation of precision error with body mass index (BMI)

		Precision error (CV) for each ROI:						
		1	2	3	4	5	6	7
Group of persons who are able-bodied								
BMI	Correlation coefficient (r)	-0.16	0.13	-0.03	0.15	0.03	-0.01	0.26
Group of persons with spinal cord injury (SCI)								
BMI	Correlation coefficient (r)	0.10	0.27	-0.09	-0.19	-0.19	0.10	0.45

Abbreviations: BMI, body mass index; CV, coefficient of variation; ROI, region of interest.

Chapter 4: Discussion

Rationale

The objective of this study was to assess the precision error of DXA derived BMD at the distal femur, proximal tibia, and calcaneus, and to determine the difference in precision error between persons with chronic SCI and persons who are able-bodied. Despite large differences in BMD between groups, the relative precision error was similar between persons with SCI and persons who are able-bodied. To our knowledge this is the first study to assess the precision error of DXA at these sites in persons who are able-bodied (expected normal BMD) in comparison to persons with chronic SCI (expected low BMD). Comparing these two groups of persons has important clinical applications when designing a study that will follow persons from the time of acute SCI to chronic SCI. We chose to assess these sites as they are regions of bone that are commonly fractured (i.e. distal femur and proximal tibia) (Comarr *et al.*, 1962; Ragnarsson & Sell, 1981; Vestergaard *et al.*, 1998) and experience rapid BMD loss after SCI (i.e. calcaneus) (Garland *et al.*, 2004). We enrolled 10 participants in each group and over the two visits we completed a total of 4 scans at each ROI for every participant. Therefore, we had a degrees of freedom of 30 in each group, which meets the requirements for precision analyses set by the ISCD (Bonnick *et al.*, 2001), and surpasses the 27 degrees of freedom suggested previously (Glüer *et al.*, 1995).

Bone mineral density results

To evaluate the first hypothesis, we compared the BMD at every ROI between persons with SCI and persons who are able-bodied. It was hypothesized that the BMD will be lower in persons with SCI at every ROI when compared to persons who are able-bodied, which was what we found. The greatest BMD differences were shown at the calcaneus (ROI # 6 = ~32% of able-bodied; ROI # 7 = ~ 40% of able-bodied). These differences are much greater than the 38% loss previously reported after two years of SCI (Garland *et al.*, 2004), which suggests that the calcaneus may experience the most rapid and profound declines in BMD after SCI. One confounding variable that may influence our results is the older age of the participants with SCI, however it is unlikely that this would have contributed significantly to the lower BMD values. The best support for this would be a study by Bauman and colleagues (1999), who demonstrated that in 8 pairs of twins (one with SCI and one without), the twin with SCI had a significantly lower BMD than the twin who was able-bodied, regardless of age (Bauman *et al.*, 1999).

The individuals with SCI in our study did not have any fractures in the leg scanned, but the majority had BMD values at the distal femur and proximal tibia that were at or below 0.49 g/cm^2, which is the fracture break point at the whole knee suggested previously (Garland *et al.*, 2005). At the distal femur alone, Eser and colleagues proposed a fracture risk threshold of 114 mg/cm^3 using peripheral quantitative computed tomography (pQCT) (Eser *et al.*, 2005). The majority of the persons with SCI in our study would have BMD values that were

also lower than this, which was approximated in g/cm^2 by previously derived equations (McPherson *et al.*, 2014). The fracture threshold value derived by Eser and colleagues uses 4% of femur length whereas we used 15 – 20% femur length, and that by Garland and colleagues is based on the whole knee, therefore these values can only provide an indirect estimate of fracture risk in our study. The distal femur epiphyseal region demonstrated in the study by McPherson and colleagues appears to be derived from a 0-10% femur length measurement (Edwards *et al.*, 2014a), which would be more comparable to the ROI for the fracture threshold of Eser and colleagues (2005) and would put some of the individuals with SCI in their study below the fracture threshold, however they did not report any evidence of fracture in their study. Even with the variability in ROIs between studies, the values we report for BMD in both the persons with SCI and the persons who are able-bodied are similar to those reported previously at the distal femur and proximal tibia (Shields *et al.*, 2005; Morse *et al.*, 2009b; McPherson *et al.*, 2014), as well as at the calcaneus (Warden *et al.*, 2002; Garland *et al.*, 2004).

In the group of persons who are able-bodied, there was a positive correlation of BMD with BMI at the calcaneus, which is similar to that described in post-menopausal women at standard sites (Castro *et al.*, 2005; Asomaning *et al.*, 2006). In the group of persons with SCI, this same relationship was not found, which supports the notion that unloading is a cause of SCI osteoporosis (Dauty *et al.*, 2000; Qin *et al.*, 2010; Dolbow *et al.*, 2011). It is known that a low BMD in persons who are able-bodied is predictive of hip fracture and that increases in

BMI of only one unit can reduce the relative risk (De Laet *et al.*, 2005). In their meta-analysis, De Laet and colleagues demonstrated that a BMI of 20 kg/m^2 was associated with almost twice the fracture risk when compared to a BMI of 25 kg/m^2. In another study, it was shown that for every unit increase in BMI there was a decrease in risk of low BMD at the lumbar spine in persons who are able-bodied (Castro *et al.*, 2005). However, given that the body composition of people with SCI is significantly altered in the chronic stage (Laughton *et al.*, 2009), this would likely influence the relationship of BMD and BMI, and may explain why the relationship was more variable in this group.

Precision errors

To evaluate the second hypothesis, we compared the precision error of DXA derived BMD at each ROI between the group of persons with SCI and the group of persons who are able-bodied. It was hypothesized that the group of persons with SCI would have a greater precision error than the group of persons who are able-bodied. This was hypothesized because factors that have been shown to increase precision error such as higher percent fat content, reductions and/or alterations in the distribution of soft tissue, and difficulties in positioning are more likely to be present in persons with SCI. Overall, the statistical analysis of the results from our study do not support the hypothesis that precision error is greater in the group of persons with SCI. However, at ROI # 1 there was a trend for greater precision error in the group of persons with SCI as the RMS-CV equalled 8.40%, but was only 5.63% in the group of persons who are able-bodied ($p=0.018$). Additionally at ROI # 7 of the calcaneus, the group of persons

with SCI had a RMS-CV of 3.41% and the group of persons who are able-bodied had a RMS-CV of 1.78% (p=0.051).

In a recent study by McPherson and colleagues (2014) (McPherson *et al.*, 2014), it was demonstrated that persons with chronic SCI had approximately twice the precision error when comapred to persons with acute SCI at the distal femur metaphysis, distal femur epiphysis, and proximal tibia epiphysis, although this was not evaluated statistically. The largest difference they found was at the distal femur metaphysis (RMS-CV of 1.39% vs. 4.70%), which is similar to ROI # 2 in our study. We did not find statistically significant differences between groups at this ROI, although our precision errors were similar to their group of persons with chronic SCI (our able-bodied group, 4.68%; our SCI group, 4.41%). Our results are similar to another study that compared the pQCT derived BMD at the distal tibia and shaft between persons who are able-bodied and persons with chronic SCI (Giangregorio *et al.*, 2013). In this study, they found minimal difference in precision error between the group of persons who are able-bodied and the group of persons with chronic SCI for trabecular BMD, total density, and total area. In each of these studies, although some differences in precision error have been observed between persons who are able-bodied and persons with SCI, no statistically significant differences have been reported. The difference in precision error values between studies may be due to the different ROIs used, differences in methods of positioning between studies, or may reflect differences due to sampling from the larger population (Leslie & Moayyeri, 2006).

Even though the differences in precision error between groups in our study were not statistically significant, the minor differences observed at ROI # 1 and ROI # 7 were likely due to large individual precision errors in some participants. At ROI # 1, in the group of persons with SCI there were three persons with large precision errors (10.5, 14.7, and 17.5%), whereas there was only 1 individual in the group of persons who are able-bodied that had a similar error (11.2%). At ROI # 7 the precision error for all participants was ≤3.5%, except for one participant in the group of persons with SCI who had a precision error of 9.3%. Given that the formula to calculate precision is sensitive to large precision errors, especially if the sample size is low, it was likely that this contributed to the difference in the size of the precision error between groups. Some of the high individual precision errors in our study, and those in a previous study by Morse and colleagues, were caused by one BMD value that could be considered an outlier in a set of scans for an individual (Morse *et al.*, 2009b). For example, one participant in our study had a precision error (CV) of 14.7% at ROI # 1 with a BMD for the first scan of 0.326 g/cm^2, whereas the last three scans had BMD values that were very close together (0.247, 0.257, 0.241 g/cm^2). If this was to occur in a clinical setting you would certainly consider a decrease in BMD of ~25% to be clinically significant, however since three of the four scans were very similar, the first was probably due to an unknown measurement error and indicates that caution should be used when interpreting only two scans from an individual.

The reason why the group of persons with SCI had large individual precision errors more often that the group of persons who are able-bodied, and by extension slightly greater precision error, is not clear from our study. It has been suggested that alterations in positioning is the most likely factor contributing to BMD differences between scans (Baim *et al.*, 2005), and in support of this small rotational differences have been shown to increase precision error at the hip (Penny *et al.*, 2010). Other potential factors that could contribute to these errors are artifacts present in the scans, changes in clothing, or the variability in the distribution of soft tissue around the scan region, which would likely influence the BMD value due to inconsistent x-ray attenuation. For example, it has been shown that adding layers of fat around the scan region can increase the BMD value leading to increased precision error of the lumbar spine and hip (Yu *et al.*, 2012), which is supported by evidence suggesting that there is greater precision error for total body, lumbar spine, femur neck, and total hip in persons with high BMI (Knapp *et al.*, 2012; Knapp *et al.*, 2014). However, others have suggested that there is no significant relationship between BMI and relative precision error at these standard sites (Rajamanohara *et al.*, 2011; Hopkins *et al.*, 2014). In our study, we did not find a correlation between BMI and precision error at any ROI in either group. This would suggest that either there is not a relationship at the sites we measured, the relatively limited range of BMI in our study may limit our comparisons, or we had a sample size that was too small to detect any relationship. Given that there is increased percent body fat in persons with SCI for a given BMI (Spungen *et al.*, 2003), it is possible that the higher

percent body fat in those with SCI may lead to greater precision error. However, we did not specifically test this hypothesis in our current study.

In our study all of these sources of error were each likely to influence the BMD values and precision error we found. Variations in the distribution and location of soft tissue, but not necessarily the amount of soft tissue, around the distal femur may have contributed to the large individual precision error for some persons at ROI # 1. Given the amount of soft tissue above the knee, and the lack of muscle tone in persons with SCI, differences in the distribution and location of this soft tissue between scans may have occurred in persons with SCI and may explain both the large individual precision errors and the resultant small differences between groups at the distal femur. In the study by Morse and colleagues (2009) they also found large individual precision errors, but at the proximal tibia and not distal femur, which they suggested was a due to the spatial geometry of the tibia (Morse *et al.*, 2009b). Given that both studies used similar ROIs and a group of persons with similar duration of SCI, the reason why we found large precision errors at the distal femur and they found large errors at the proximal tibia is unknown. At ROI # 7, the explanation for the high precision error that was found for one person with SCI in our study seems to be more clear. It is likely not due to soft tissue as there is minimal soft tissue around the calcaneus, but due to positioning differences between scans or an artificat in one of the scans. To account for these sources of error in future studies is difficult, but likely could be reduced with strict positioning of all lower body segments.

Additionally, we compared the precision error between ROIs within each group of participants. The precision error at the calcaneus is reported to be similar to standard sites (Sievanen *et al.*, 1992), whereas the precision error at the distal femur and proximal tibia has been reported as higher than standard sites (Morse *et al.*, 2009b; McPherson *et al.*, 2014). In our study, the group of persons who are able-bodied had a precision error that was quite low at ROI # 7 with a RMS-CV of 1.78%, whereas the precision error at the distal femur and proximal tibia was slightly greater (RMS-CV range, 3.26 – 5.63%). On the contrary, in the group of participants with SCI, the precision error at ROI # 7 (RMS-CV, 3.52%) was not lower than the other ROIs (RMS-CV range, 3.44 – 8.40%). One participant in the group of persons with SCI had a CV of 9.3% at ROI # 7, whereas the other participants had errors between .05 – 3.5%, which likely prevented a similar finding as the group of persons who are able-bodied. Anatomically, the calcaneus is not surrounded by as much soft tissue as the knee, which may have led to the slightly lower precision error at this site when compared to the distal femur and proximal tibia. As ROI # 7 consists of the majority of the posterior calcaneus body, this may also act to limit the influence of the asymmetrically distributed bone mineral at the calcaneus that others have suggested (Sievanen *et al.*, 1992), and would then be less influenced by positioning differences between scans. However, we did not have sufficient numbers to test this question statistically in our study, but it would be of interest to assess this in a larger sample.

When comparing the precision error between the distal femur and proximal tibia we did not observe a similar trend to Morse and colleagues (2009), who found that the precision error (RMS-CV) in a sample of persons with SCI was 3.01% at the distal femur, which was better than the 5.91% observed at the proximal tibia (Morse *et al.*, 2009b). This may be due to the slightly different ROIs used between the studies, or may reflect true differences in the samples (Leslie & Moayyeri, 2006). In contrast to the results of Morse and colleagues, other studies (Bakkum *et al.*, 2013; McPherson *et al.*, 2014), have found that the distal femur is not as precise as the proximal tibia, which together would suggest that both the distal femur and proximal tibia may be prone to rotational errors. We did find that within each bone the ROI composed of integral (whole) bone had lower precision error than the ROI within the visible cortical border (e.g. ROI # 5 vs. ROI # 4), which is similar to what others have found when using ROI of different sizes (i.e. larger is more precise) (Penny *et al.*, 2010). This may also be due to the percentage of cortical and trabecular bone within each ROI, as it has been shown that the precision error of cortical BMD is better than trabecular BMD, suggesting that a ROI of integral bone may have a lower precision error due to inclusion of a greater percentage of cortical bone (Giangregorio *et al.*, 2013). Despite these differences, the precision errors in our study are similar to other studies that have presented BMD precision error at non-standard DXA sites (Sievanen *et al.*, 1992; Vazquez *et al.*, 2004; Morse *et al.*, 2009b; Bakkum *et al.*, 2013; Giangregorio *et al.*, 2013; Haddaway *et al.*, 2013). One consistent finding between all of the non-standard sites described in these studies is that

the precision error is higher than standard sites of the lumbar spine and hip, which is often reported as <2% (Takada *et al.*, 1997; Shepherd *et al.*, 2006a; Kiebzak *et al.*, 2012; Knapp *et al.*, 2012).

The ROIs that were used in this study are similar to other studies of DXA, but also resemble areas of analysis that have previously been used for BMD and architectural measurement by pQCT. McPherson and colleagues (2014) demonstrated that DXA and pQCT derived BMD of similar regions at the distal femur and proximal tibia were well correlated (Pearson correlation coefficient range, 0.934 – 0.955) in persons with chronic and acute SCI (McPherson *et al.*, 2014). However, they did not compare the DXA derived BMD with other variables that have been analyzed with pQCT. Recently, Edwards and colleagues demonstrated that along with reductions in bone mineral of ~4%/month at the distal femur and proximal tibia, reductions in cortical and trabecular strength indices of up to 6%/month are also possible within the first year of SCI (Edwards *et al.*, 2014a). Further, they showed in the same persons that cortical bone volume decreased by up to 5%/month, but cross sectional area and integral bone volume did not decline. In future investigations, it would be valuable to determine if DXA derived BMD can estimate the reductions in strength as identified by pQCT.

Future clinical implications of this project

To properly design a study measuring BMD after SCI, an appropriate follow-up period is required to allow for identification of true biologic change. Using the findings from our study, we can estimate the time required between

DXA scans in the acute stage of SCI (time interval = LSC / expected rate of change per year) (Bonnick *et al.*, 2001). Using the LSC values from the group of persons who are able-bodied, a minimum follow-up interval after acute SCI is approximately 10 months at the distal femur, 7 months at the proximal tibia, and 4 months at the calcaneus given the annualized rate of loss presented by Garland and colleagues (Garland *et al.*, 2004). The values in persons with SCI would be slightly longer, however at the time of SCI the able-bodied group would be more comparable to the population. The LSC values in our study are likely not able to be used in persons with chronic SCI as they have reached the steady state of BMD loss (Biering-Sorensen *et al.*, 1990; Eser *et al.*, 2004b), therefore any change would likely be within the error of our method. These results would then question the time frame for follow-up used in some intervention studies. In one example, Singh and colleagues (2014) followed 106 people with SCI from onset to one year and completed DXA scans and blood biochemical analyses at baseline, 3 months, 6 months, and 12 months (Singh *et al.*, 2014). At one year after motor complete SCI they found 30.8% BMD reductions at the proximal tibia, which given our LSC values is likely a true biologic change, however our results would suggest that the losses reported at 3 months (5%) and 6 months (12%) were not true biological changes.

The LSC values and calculated follow up time from our study, can be used to design interventions aimed to prevent BMD loss after SCI. Passive standing represents a means to counteract unloading of the lower limbs after SCI, and it has been shown that people with acute SCI who stood for ≥ 5 hours/week

displayed attenuation in loss of bone density at the tibia when assessed 6 months after SCI (De Bruin *et al.*, 1999), and similar attenuations in BMD after 24 months of SCI have been described for two participants who reported standing for ≥ 4 hours/week in a prospective study (Frey-Rindova *et al.*, 2000). Other studies have used various research designs, but not a randomized controlled trial, to investigate the effect of passive standing on bone and have not found consistent results [reviewed in (Biering-Sørensen *et al.*, 2009b)], which may be due to a variety of factors including an insufficient follow-up time. Bisphosphonates, which act to decrease osteoclast formation and activation, have been shown to attenuate declines in lower limb BMD after SCI when administered as 10 mg oral alendronate per day (Sniger & Garshick, 2002; Zehnder *et al.*, 2004b; Moran de Brito *et al.*, 2005; Gilchrist *et al.*, 2007) or 4-5 mg intravenous zoledronic acid once per year (Shapiro *et al.*, 2007; Bubbear *et al.*, 2011), but others have not shown any positive effects (Bauman *et al.*, 2005). Pro-bone formation drugs such as testosterone (Yarrow *et al.*, 2014) and nandrolone (an anabolic steroid) (Sun *et al.*, 2013), have shown to prevent bone health declines at the distal femur and proximal tibia in a rat model of SCI, but can lead to the deleterious side effect of prostate enlargement. Recently, using sclerostin knock out mice, which is a known negative regulator of bone formation and mediator of bone response to load (Tu *et al.*, 2012), Morse and colleagues demonstrated that mice exposed to mechanical load can still have an anabolic bone response (Morse *et al.*, 2014). This evidence suggests that an intervention combining passive standing with an antibody against sclerostin

may have the potential to prevent bone loss after SCI. The troubling aspect of pharmacologic agents is that they act body wide, therefore may have deleterious side effects above the level of the lesion and may lead to unwanted effects on other pathways in the body, but as of now these are not often reported in the literature. Regardless of the type of intervention planned, appropriate follow-up times and estimates of expected changes in BMD need to be calculated to ensure that any difference observed reflects a true biological change.

Study limitations

This study was limited by a few factors. First, although we used braces to secure persons limbs into position (Figure 2C-E), we only used visual identification of the limb via the DXA image and did not quantify the actual position of the limb. Therefore, minor variations in variables, such as rotation of the limb or distance from the scanning bed, may influence our results. Second, to improve the clinical application of our method we did not have participants dismount from the scanning bed after each scan on a single visit but instead used a rotating scan order (knee, ankle, knee, ankle), which involved significant full body rotation and may minimize this potential problem. As well, we allowed participants to keep their clothes on, which we viewed was as a more practical option for persons with SCI. Third, we chose each group of participants as a convenience sample and did not have set criteria for participant characteristics. However, we did restrict the age of persons to those who were older than 18 but premenopausal, as within this age range the BMD would be expected to be normal. Finally, although our sample size of 10 participants in each group is

adequate for the minimum 30 degrees of freedom suggested by the International Society for Clinical Densitometry (Bonnick *et al.*, 2001) and surpasses the 27 suggested previously (Glüer *et al.*, 1995), others have shown that using this sample size to identify precision error and LSC values could over or under predict the percent of persons with true biologic change at follow-up by up to 10% when compared to a larger sample size of 198 (Leslie & Moayyeri, 2006).

Conclusion

In this study we have demonstrated that DXA derived BMD can be reliably assessed at 7 ROIs at the distal femur, proximal tibia, and calcaneus and the precision error is similar in persons who are able-bodied and persons with chronic SCI. We present evidence to show that DXA derived BMD of the calcaneus body (ROI # 7) may be a more precise ROI when compared to regions of the distal femur and proximal tibia in persons who are able-bodied. The next step is to use this protocol to assess BMD changes over time in persons with acute SCI, and to apply an intervention to prevent BMD loss. Given the LSC values we report, a follow-up interval of at least 10 months from onset of SCI would be a time frame where someone can be reasonably certain that a true change in BMD would be likely to occur at the distal femur, proximal tibia, and calcaneus if no intervention was applied. The LSC values at the ROI we tested, with the exception of ROI # 7 at the calcaneus body, may prevent using this protocol in persons with chronic SCI who have reached a steady state of BMD loss, as any change in BMD is likely to be within the error of the method.

Appendix

Informed Consent

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RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

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Sponsor: University of Manitoba, University Research Grants Program

You are being asked to participate in a research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this study and you may discuss it with your friends, family or your doctor before you make your decision. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand.

PARTICIPANT INITIALS_____

Purpose of Study

Osteoporosis occurs in the legs of all persons paralyzed due to spinal cord injury and results in an increased risk of fracture. Currently, there is no 'standard of care' method to prevent the bone mineral loss that occurs in everyone paralyzed due to spinal cord injury. In order to identify methods that may help treat or prevent bone density loss, a reliable means to measure bone mineral density at the sites that are affected is needed.

This research study is being conducted to find out how accurate repeated DEXA (Dual-energy X-ray absorptiometry) scans are for measuring bone density at the knee and heel, so that we may then use this method to test the effect of interventions aimed at reducing the bone mineral density loss that occurs after spinal cord injury. To test this, two groups of participants will be recruited for this study: those with expected normal bone density at the knee and heel (able-bodied participants) and those with expected low bone density (persons living with a motor complete spinal cord injury for more than 2 two years).

Up to 20 participants will participate in this study.

Study procedures

Once you decide to participate, you will be asked to attend the Manitoba Institute of Child Health on two occasions. Each visit should take approximately 30 to 45 minutes.

If you take part in this study, you will have the following procedures:

On the first visit, a Dual-energy X-ray absorptiometry (DEXA) scan will be made of your dominant knee and ankle. In order to make these scans we will need you to get on the scanner bed and lie on your back and we will position your leg with supports and Velcro straps with your leg out straight and then ask you to lie without moving for the duration of the scan. We will then ask you to lie on your side and re-position your leg and do a scan of your heel, also using supports and straps to keep your foot and leg in position. We will then repeat this process in order to take another scan of your knee and heel.

You will then be asked to return to the Institute for a second visit, at least one week later to have the same procedure repeated.

For this study some volunteers will have had a paralyzing spinal cord injury and other volunteers will be able-bodied with no history of disease that would be expected to reduce bone mineral density or osteoporosis.

Participation in the study will be for two visits each requiring about 30 - 45 minutes.

The researcher may decide to take you off this study if you experience any adverse responses such as undue discomfort while the scan is performed.

You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study staff first.

A few weeks after testing is completed you will be able to review your own results from the tests you participated in.

Risks and Discomforts

There are no significant risks anticipated from participation in this study. A DEXA scan is a radiologic scan of the body, but the exposure to radiation is considered to be minimal and non-harmful. Procedures will be in put in place to limit scans to areas of interest. Testing and transferring to the scanner bed will occur in a private laboratory and only you and the study staff will be present during testing. If you wish, someone may accompany you to this testing.

Benefits

There may or may not be a direct benefit to you from participating in this study. We hope the information learned from this study will benefit other people with spinal cord injury as we try to identify treatments that will reduce the bone mineral density loss that occurs after paralyzing spinal cord injury.

Costs

All the procedures, which will be performed as part of this study, are provided at no cost to you.

Payment for participation

You will be given $20 per completed study visit to a maximum of $40 upon termination of your participation in this research study.

Confidentiality

Information gathered in this research study may be published or presented in public forums, however your name and other identifying information will not be used or revealed. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law.

All study-related documents will bear only your Participant ID number and any reports summarizing the study data will not contain any identifying information (other than duration and level of injury as relevant to the study).

Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba.

The University of Manitoba Health Research Ethics Board may review records related to the study for quality assurance purposes.

All records will be kept in a locked secure area and only those persons identified will have access to these records. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No information revealing any personal information such as your name, address or telephone number will leave *the University of Manitoba*.

Voluntary Participation/Withdrawal from the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision not to participate or to withdraw from the study will not affect your current medical care or academic endeavors. If the study staff feel that it is in your best interest to withdraw you from the study, they will remove you without your consent.

We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

Medical Care for Injury Related to the Study

In the case of injury or illness resulting from this study, necessary medical treatment will be available at no additional cost to you.

You are not waiving any of your legal rights by signing this consent form nor releasing the investigator(s) or the sponsor(s) from their legal and professional responsibilities.

Questions

You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study or if you have a research-related injury, contact the study doctor and the study staff:

Kristine Cowley – (204) 789-3305; [redacted]

For questions about your rights as a research participant, you may contact The University of Manitoba, Bannatyne Campus Research Ethics Board Office at (204) 789-3389

Do not sign this consent form unless you have had a chance to ask questions

PARTICIPANT INITIALS_____

and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with *Dr. Kristine Cowley* and or her study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statements or implied statements. Any relationship (such as employer, supervisor or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of any of my records that relate to this study by The University of Manitoba Research Ethics Board, for quality assurance purposes.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

I agree to be contacted for future follow-up in relation to this study,
Yes _____ No _____

Participant signature _____Date _____
(day/month/year)

Participant printed name: _____

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: _____Date _____
(day/month/year)

Signature: _____

Role in the study: _____

Relationship (if any) to study team members:_____

Advertisement

UNIVERSITY OF MANITOBA

Department of Physiology
405 – 745 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0J9
Telephone (204) 789-3305
kris@scrc.umanitoba.ca

Ever wondered about your bone density?

If you have a motor complete spinal cord injury you may be eligible to participate …

If you require the use of a wheelchair because of motor paralysis from a spinal cord injury you may be eligible to participate in this study.

We need up to 10 persons with motor complete spinal cord injury to help us.

As you may know, osteoporosis occurs to all persons paralyzed due to spinal cord injury. As a result, people living with longstanding spinal cord injury are at increased risk of bone fracture.

We are interested in figuring out ways to reduce the rate of bone mineral loss that occurs after spinal cord injury. In order to do so, we need a reliable means to measure bone density over time, particularly at sites prone to fracture (e.g. the knee). We can then test various strategies for preserving bone density in those with spinal cord injury.

So, in order to figure this out, we need volunteers to visit a DEXA scanning site to receive bone scans of the knee and heel and then to have these scans repeated at a later date.

Each participant will receive a report on his or her own knee and heel bone density. An honorarium will be provided.

If you are interested in learning more about this study and whether you are eligible to volunteer please contact Kristine Cowley or Will Peppler.

Kristine Cowley, PhD and Barbara Shay, PhD are researchers at the University of Manitoba and are working with Karen Ethans, MD and Rudy Niebuhr (PT) from the Health Sciences Centre to investigate questions that aim to improve the health of those living with a spinal cord injury.

Kris Cowley - [redacted]789-3305
kris@scrc.umanitoba.ca

Will Peppler – 789-3305
umpepple@cc.umanitoba.ca

Copy of Ethics Approval

UNIVERSITY OF MANITOBA | BANNATYNE CAMPUS
Research Ethics Boards

P126 - 770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Telephone 204-789-3255
Fax 204-789-3414

HEALTH RESEARCH ETHICS BOARD (HREB)
CERTIFICATE OF FINAL APPROVAL FOR NEW STUDIES
Full Board Review

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. K. Cowley	UofM / Department of Physiology	HS16277 (H2013:174)
HREB MEETING DATE:	**APPROVAL DATE:**	**EXPIRY DATE:**
April 22, 2013	July 16, 2013	April 22, 2014
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE:
NA	Passive standing to prevent bone density loss after spinal cord injury
SPONSORING AGENCIES AND/OR COORDINATING GROUPS:	
UofM Internal Funds	

Submission Date(s) of Investigator Documents:	REB Receipt Date(s) of Documents:
April 5 and July 10, 2013	April 8 and July 15, 2013

THE FOLLOWING ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date

Protocol:

Protocol received April 8 and clarification received July 15, 2013

Consent and Assent Form(s):

Research Participant Information and Consent Form - Project 1	6-28-2013
Research Participant Information and Consent Form - Project 2	6-28-2013

Other:

Revised Advertisement received July 15, 2013
Data Collection Form received April 8, 2013

CERTIFICATION
The University of Manitoba (UM) Health Research Board (HREB) has reviewed the research study/project named on this ***Certificate of Final Approval*** at the ***full board meeting*** date noted above and was found to be acceptable on ethical grounds for research involving human participants. The study/project and documents listed above was granted final approval by the Chair or Acting Chair, UM HREB.

HREB ATTESTATION
The University of Manitoba (UM) Health Research Board (HREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the HREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

QUALITY ASSURANCE
The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

CONDITIONS OF APPROVAL:

1. The study is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of approval. A Bannatyne Campus Annual Study Status Report** must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the HREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM HREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

Please quote the above Human Ethics Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255/ **Fax:** (204) 789-3414

Steps of a precision analysis

1. Perform and analyze scan based on standard operating procedures.
2. Calculate the mean ($\bar{x}$) value at a give region of interest for each individual.

$$\bar{X}i \quad = \frac{\sum x_i}{n}$$

3. Calculate how much each scan's value differs from the overall (ROI specific) mean.

$$Difference = x_i \; - \; \bar{X}_i$$

4. Calculate the variance.

$$SD_i = \sqrt{\frac{\sum_{i=1}^{n_i}(x_i - \bar{X}_i)^2}{n_i - 1}}$$

5. Determine the CV as a percentage.

$$CV = \left(\frac{SD}{\bar{X}_i}\right) * 100$$

6. If completing a short-term precision analysis, calculate the average value, SD, CV for each of the participants. It is recommended that a degrees of freedom [number of participants * (number of scans – 1)], which would ensure that the upper limit of a 95% confidence interval based on the precision value is no more than 34% greater than the precision value.
7. To complete this analysis, calculate root-mean-square standard deviation or root-mean-square coefficient variation. If a long-term precision study was completed, the standard error of the estimate would be used.

$$SD_{RMS} \; = \sqrt{\frac{\sum_{i=1}^{m}(SD^2)}{m}}$$

$$CV_{RMS} \; = \sqrt{\frac{\sum_{i=1}^{m}(CV^2)}{m}}$$

8. Calculate the LSC value.

$$LSC = Z'(\mathrm{Pr})\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

9. Determine length of follow up.

$$Time\ of\ follow\ up = \frac{LSC}{Rate\ of change\ per\ year}$$

International Society for Clinical Densitometry Precision Calculator

ISCD Advanced Precision Calculating Tool

This calculator is intended for use by advanced bone densitometritsts only.
It may be considered for special clinical practice situations and for clinical research.
Please note that the ISCD recommends expressing precision as RMS SD, and LSC at the the 95% confidence level.
Using this calculator, you may:

1. ***Calculate precision error with as many as 50 patients.***
2. ***Express precision error as RMS SD (absolute value in g/cm^2), CV, or %CV.***
3. ***Express LSC (Least Significant Change) with a choice of confidence levels.***

Instructions: Enter BMD measurements to 3 decimal places for at least 15 patients scanned 3 times each, or 30 patients scanned 2 times each. Precision and LSC must be calculated separately for each skeletal site and ROI (L1-L4, total proximal femur, femoral neck, etc.). BMD results from as many as 50 patients may be entered. The calculator does the rest.

Patient	Scan 1	Scan 2	Scan 3	SD	SD sq	CV	CV sq
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
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42							
43							
44							
45							
46							
47							
48							
49							
50							

Skeletal Site / ROI Tested:

n =	0	(Number of Patients)
Sum =		(Sum of SD sq)
Sum / n =		(Sum of SD sq / n)
SqRT =		(Square Root of above)
RMS SD =	g/cm^2	(Root Mean Square SD)
CV =		(Coefficient of Variation)
%CV =	%	(% Coefficient of Variation)

LSC based on at least 15 patients with triplicate scans:

	LSC with Different Levels of Confidence				
Precision	**95%**	**90%**	**85%**	**80%**	**Units**
RMS SD					**g/cm^2**
CV					
%CV					**%**

LSC based on at least 30 patients with duplicate scans:

	LSC with Different Levels of Confidence				
Precision	**95%**	**90%**	**85%**	**80%**	**Units**
RMS SD					**g/cm^2**
CV					
%CV					**%**

DEVELOPED BY: E. Michael Lewiecki, M.D., FACP, CCD for ISCD

Dual-energy X-ray Absorptiometry (DXA) Protocol

Dr. Kris Cowley's Laboratory
Edition # 1
2014

This protocol is designed to work with a Hologic QDR4500A Pediatric DXA scanner. This specific protocol was developed for use at the Manitoba Institute of Child Health (5th floor John Buhler Research Center, University of Manitoba)

Contact information

For use of protocol:
Kristine Cowley – (204) 789-3305; kris@scrc.umanitoba.ca; 405 BMSB
Will Peppler – (204) 789-3305; umpepple@myumanitoba.ca; 405 BMSB
For DXA specific information:
Jeannine Schellenberg RN, CDT – (204) 789-3206; JSchellenberg@mich.ca; 539 JBRC.
Debbie Korpesho – (204)789-3447; dkorpesho@mich.ca; front desk MICH

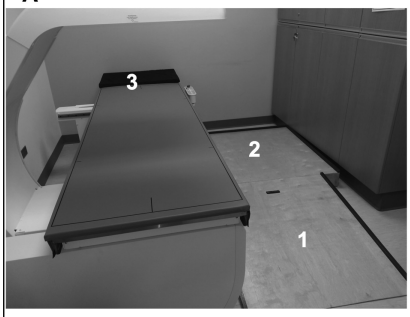
Materials:
Braces and straps – room 405 BMSB, University of Manitoba
Ramp, platform, headrest – room 559 MICH
Cheat sheet:

- Knee scans are completed in left forearm mode, regardless of knee scanned.
- Ankle scans are completed in right forearm mode, regardless of ankle scanned.
- If you click bone map it erases the previous work you did with bone map, so it is easiest to do this last (i.e. right before you click results).

Room set-up and preparation

*Make sure you have booked a scan with the DXA technologist Jeannine Schellenberg via email or phone call *
Note: These DXA scans can take up to 30 minutes to complete. In addition, you must plan for up to 15 minutes of set-up and take down if a ramp is required. The ramp, platform, and head rest are located in room 559 at the MICH and you can obtain the key from the front desk or the Clinical Research Unit. You must set them up as shown in Figure 1A.

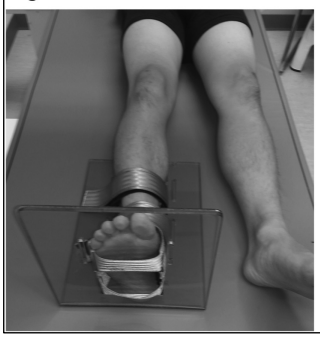
A



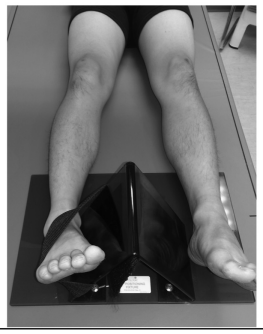
B

Object	L (cm)	W (cm)	H (cm)
DXA	202	70	71.5
Room	406.5	283	X
Door	X	91.4	X
Ramp	106	91	12.7
Platform	106	106	12.7
Headrest	56	71	71

C



D



E

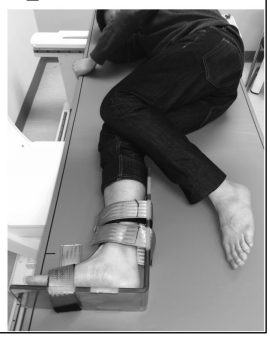


Figure 1: DXA room and participant set-up. A) The set-up of the DXA room with 1) ramp, 2) platform, and 3) headrest. B) Table of the measurements of the DXA room. C and D) Braces required for knee scanning in able-bodied C) and SCI groups D). E) Image of the calcaneus scan set-up.

STEP 1: Initial requirements

1. Materials required:
    a. Knee and Ankle frames with attached Velcro strapping
    b. Additional Velcro strapping
    c. Goniometer
    d. Notepad
    e. Tape measure
2. The participant is not required to *prepare* in advance of the scan. If possible, instruct the participant to come to the appointment wearing loose clothing and not jeans [this is generally done via email when booking the participant for the scan]. All participants must remove jewelry and metal objects from around the scans sites.
3. Ensure that the participant is aware of the proceedings and have signed the consent form if required.

STEP 2: Preparing for the scan

1. Turn on the computer for the DXA scan and open up the Hologic software.
    a. Note: The DXA technologist will control <u>everything</u> from this point on regarding the computer and operation of the scanner. The research assistant's role is to prepare the participant to be scanned, position the scan, and ensure that the participant is safe and comfortable throughout.
2. Have the participant come into the room and on the platform that you have set up.
3. Have the participant remove all jewelry and metal objects from pockets or around the scan regions – pants do not need to be removed, but best to be rolled up.
4. Measure the length of the participant's femur – this is the most important step for setting up a proper and accurate analysis, so take your time to measure the femur properly and repeat the measurement to get an accurate reading.
    a. The participant should be seated in either a wheelchair or regular chair, with leg bent as close to 90 degrees as possible.
    b. Palate the anterior superior iliac spine (ASIS), which is on the front part of the hip. Then, locate and palpate the inguinal ligament, which is just distal to the ASIS and will be tender when pressed in participants who are able-bodied. Place the proximal end of the tape measure on the inguinal ligament.
    c. The distal boundary of the tape is placed just proximal to the patella, on the on the anterior portion of the knee [there should be a 'crevice' just proximal to the patella that will be palpable].
    d. Record to the nearest 0.1 cm.
5. Have the participant prepare for transfer onto the scanning bed. The participant should be aware of how they usually transfer themselves and if they require additional assistance. This involves them getting onto the platform and centered.
    a. Note: Use the controls on the participant side of the DXA scanner to move the bed laterally and closer to the participant to ease transfer (Figure 2).
6. Have the subject transfer onto the scanning bed.
    a. Note: the subject's head <u>must</u> be on the side furthest from the door.
7. Once the subject has successfully transferred complete the preparations.

For each scan, the area being scanned must be above a mark on the DXA scanner located about 1/3 of the length of the bed. This mark is visible on the right side of Figure 1D and the left side of Figure 1E (in this image, the participant would need to move up to complete the scan since they are below the line)

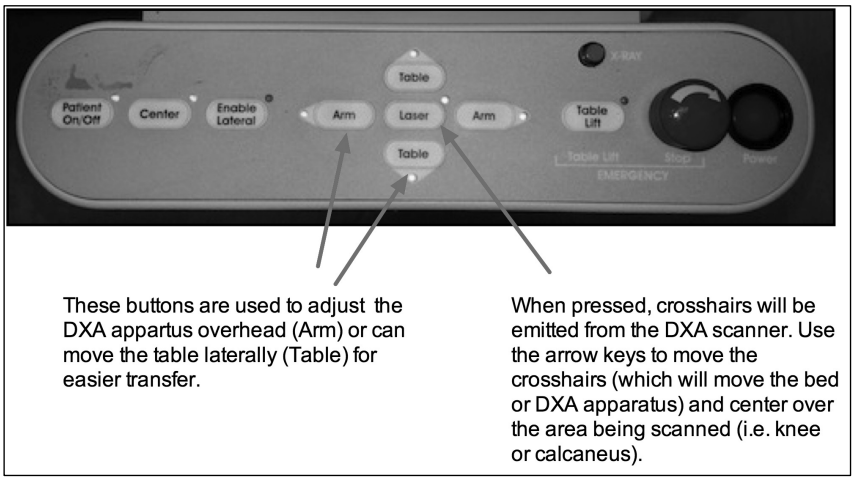


Figure 2: DXA control panel. To center the knee crosshairs, use the patella as a landmark and for the calcaneus use the posterior-inferior aspect. If the calcaneus crosshair is too low, the DXA machine picks up the brace first and will alter the contrast of the image.

STEP 3: Completing the scan

There are two specific protocols that must be followed.

Scan 1: Knee

1. Participants will be positioned supine, with feet oriented anteriorly and the legs extended and together in the sagittal plane. *The legs should be positioned so that they are running parallel to the length of the bed!*
2. Position the brace as shown in Figure 1C and D, using either the 90-degee brace in persons who are able-bodied (Figure 1C) or the standard Hologic hip brace for persons who have SCI to maximize the gap between the tibia and fibula (Figure 1D).
    - When securing the leg into the brace, be careful not to turn the leg too much.
3. Once the positioning is complete, the scan can be completed using **LEFT** forearm mode. To "set" the DXA to the appropriate position, use the crosshairs tool to have it centered just below the area of interest (i.e. the patella). To set cross hairs press "laser" on the control panel and use the table or laser arrow keys to adjust the cross hairs. This scan is completed using the maximum scan width. A scout scan should

show an image similar to that below, but without the boxes. Repositioning may be necessary.

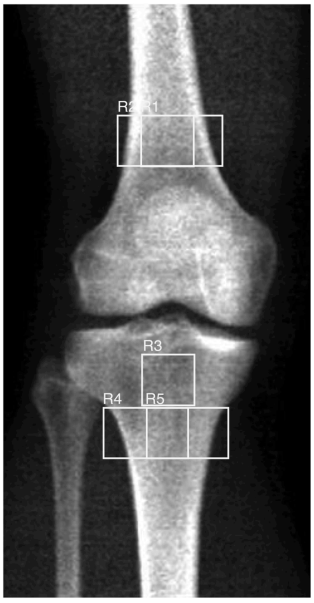


Figure 3: A representative image of the knee scan. Note: the scan will not include the ROIs until after you analyze the image. A key point in this scan is to ensure that the leg is parallel to the bed and there is minimal overlap between the tibia and fibula. The ROIs will be placed after you analyze the scan.

Scan 2: Calcaneus

1. The participant must lie on the side of interest and the brace must be removed and changed for the smaller. In Figure 1E we are scanning the right ankle and this person is on the right side. A modified fetal position with the opposite leg placed over the leg being scanned seems to work best. If needed, assist with movement of persons with SCI as to avoid unnecessary shaking of the bed and their comfort. *The leg should run parallel to the length of the bed with the ankle as close to 90 degrees as possible!*
2. Once positioned, place the brace on the leg and secure the straps as shown in Figure 1E. There is no specific direction to this brace, but in some participants with larger calves putting the side with two Velcro straps (shorter side) on the foot avoided unnecessary calcaneus contact.

3. Once the set up in correct and the participant is comfortable, center the crosshairs just below the region of interest and then complete the scan using **RIGHT** forearm mode.
    - The scout scan should show that the person as in the figure below.

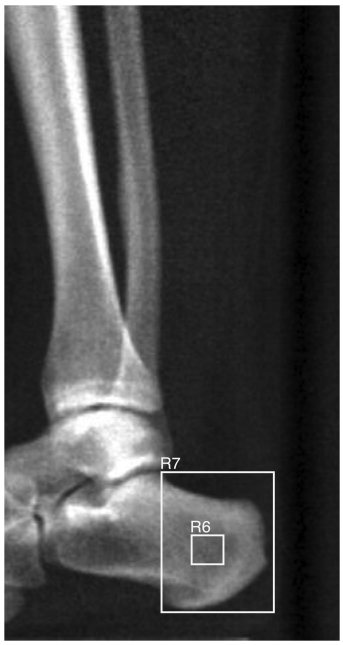


Figure 4: A representative image of a calcaneus scan. A key point in this scan is to maintain the leg as close to parallel to the scanning bed as possible.

Step 4: Selecting the regions of interest

*For further reading, see (Morse et al., 2009; Shields et al., 2005; Szücs, Jonson, Granhed, Hansson, & Szucs, 1992).

Scan analysis does not need to be completed on the same day as a scan. Each scan is archived under a participant ID, so you can return to the DXA room when not in use to analyze the scan.

How to begin?

- Turn on computer in DXA room
- Log on to QDR – no log in information is required

- Click on the application “APEX” on the desktop, this should bring up a screen showing Discovery QDR Series, and then choose “ANALYZE SCAN” from the menu.
- In ANALYZE SCAN, click ALL SCANS and search for the scan you are required to analyze via participant name, id, or date. The ID or name of the participant will change given the study, but can be typed into the search box.
- The scans for each person will be archived by date and find the correct scan as either a knee (L. forearm) or calcaneus (R. forearm) scan.
- Click next.
- Ensure that choose analysis method box is checked as subregion forearm.
- Click next.
- Now, you are ready to begin the scan.
  Note: if the subregion forearm button does not appear on the DXA machine, either the DXA scan was not completed in forearm mode, or this software is not installed on the machine Contact the DXA technologist if this occurs.

Scan 1: Knee

- You should be starting with the blank image in Figure 3 and adding the boxes to it.
- The scan should begin in GLOBAL ROI.
- Now, click BONE MAP and make sure that there is no erroneous bone. If there are areas with no bone, for example at the distal femur, go to the bone map toolbox, in the edit state, click add bone, and use the cursors to adjust the size of the addition. If you make a mistake, press undo.

Marking the distal and proximal borders from the femur length measurement

- Click SUBREGION → [+] → in WHOLE mode, move box with mouse so that the distal border is set on the most caudal portion of the lateral condyle → Click LINE → move the proximal border of the ROI box to the 20% femur length (i.e. if femur length = 39.5 cm, then 20% = 7.9 cm or 79 pixels) in the vertical direction and will read 17 x 79 or so. The pixels are displayed just on the bottom of the image.
- Switch to WHOLE mode → click [+} → line up distal border with the most caudal portion of the lateral condyle, again → now, use LINE mode to move the upper border to 15% of femur length (i.e. if femur length = 39.5 cm, then 15% = 5.9 cm or 59 pixels) in the vertical direction and will read 17 x 59.

Distal Femur

ROI # 1 – Distal Femur, trabecular

- In WHOLE mode, click on the ROI you just created and click [+]. Move the proximal border of this ROI to the proximal border on the 20% line representing 20% femur length from above, use LINE mode to move the distal border to the 15% mark. Now, the height of this box should line up with the two created. You can now delete, using [-] in WHOLE mode, the two marker boxes for 20% and 15% that you just created.
- In WHOLE mode, click the ROI you just created. Using LINE mode, measure the width of the distal femur (should be ~60-90 px) at the proximal edge of this border. Now, in LINE mode, make the new width of this box 70% of this value by adjusting the right border, so in this case it will be 56 x 21. Center this box in the distal femur by moving the box with the arrow keys, it is easiest to have the box the width of the

femur, the figure out 70%, move one side, and center by moving the entire box over half this value. This is now ROI # 1.

ROI # 2 – Distal femur, cortical

- Still in subregion using WHOLE, click on the ROI # 1 and click the [+]. A new ROI should be created.
- Line this ROI up with ROI # 1.
- Using LINE mode, adjust the left and right borders to that its boundaries are outside of the width of the distal femur, but just by 1 or 2 pixels. This is now ROI # 2.

Proximal Tibia

ROI # 3 – Proximal Tibia, epiphysis

- In WHOLE mode, select ROI # 1 and click [+]. Move this new box down to the proximal femur to become ROI # 3.
- Using the mouse, align the distal border of this box with the proximal point of contact between the tibia and fibula. To get a good view, you may need to adjust the light (use the sun and moon in the bottom left). Now, move this toward the center of the tibia using the arrow keys. This ROI should look like ROI # 3.

ROI # 4 – Proximal Tibia, metaphysis cortical – this box will extend outside the bone boundary

- Create, using [+], a new ROI in WHOLE mode.
- Line up the proximal border of the ROI with the distal most point of contact between the tibia and the fibula and move 2 px below using the arrow keys.
- Using the BONE MAP, add or remove necessary bone from the interosseous membrane.
- In WHOLE mode, select the ROI just created and then switch to LINE mode and adjust the lateral borders to be just outside the width of the proximal tibia at this location. This ROI should look like ROI # 4.

ROI # 5 – Proximal Tibia, metaphysis trabecular

- In WHOLE mode, select the ROI # 4 and click [+]. This should create a new ROI. Using the mouse overlay this box on that of ROI # 4.
- In LINE mode, take this box and move the lateral borders so they touch the boundaries of the tibia on the distal aspect of this box. This will allow you to measure the width of the tibia at the distal border of this box. Now, take 50% of this width (e.g. if the width is 80 px, take as 40 px) and move the right lateral border in LINE mode to this. Now, center this box in the tibia metaphysis. This box should be within the visible cortical boundaries and should resemble that of ROI # 5.

Scan 2: Calcaneus

For reference:

- Find the scan that you are going to analyze. Ensure that the scan is using right forearm mode and you are using subregion analysis method.
- You should be starting with the blank image in Figure 4 and adding the boxes to it.
- Note: This does not need to be completed immediately after the scan, but at a later date.
- The scan should begin in GLOBAL ROI.

- Now, click BONE MAP and make sure that there is no erroneous bone. If there are areas with no bone, for example at the distal femur, go to the bone map toolbox, click the edit state, add bone, and use the cursors to adjust the size of the addition. If you make a mistake, press undo.

Calcaneus

ROI # 6 – Calcaneus, trabecular

- Click on SUBREGIONS and [+].
- This ROI corresponds to that from Szücs and colleagues (1992) who found a homogenous region of bone located 4.0 cm from the sole and 3.6 cm from the heel.
- To gather this ROI, set the size of the box in LINE mode to 26 x 12 pixels. The most reliable way is to line the box up in reference to the protrusion of the posterior calcaneal body. Then, line the box up at the center of the entire calcaneal body height and reference it to a diagonal line between the superior/posterior calcaneal eminence and the inferior/anterior eminence.
- Once set, this ROI should look like that in ROI # 6.

ROI # 7, Calcaneus, cortical

- In WHOLE mode, click [+] to create a new ROI box. This ROI will take the entire calcaneus posterior body.
- To set this ROI, you must first set the anterior border which will be closest to the toes in the image. For this, place the corner at the notch of the talocrural joint. Then, in LINE mode, adjust the posterior and caudal borders so that the entire calcaneal body is selected.
- The ROI should look like that in ROI # 7.

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