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Leg Bone Geometry in Type 2 Diabetes Mellitus

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LEG BONE GEOMETRY IN TYPE 2 DIABETES MELLITUS
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Abstract

The objective was to investigate the effects of type 2 diabetes mellitus (T2DM) on tibial cortical and medullary cross-sectional areas (CSA) using magnetic resonance imaging. Sequential 1mm-thick slice images were acquired of the right leg from the tibial plateau to the talus in 8 individuals with T2DM and 9 age- and sex-matched (32 to 79 y) controls. The CSA (cm^2) was measured at 3 sites, 20%, 50% and 80% of tibial length, by a blinded analyzer. At the 20% site, medullary CSA in T2DM was significantly greater than controls (mean ± SD: 5.9 ± 1.2 vs. 4.8 ± 0.7). No differences were found at the 50% and 80% sites. These preliminary results indicate bone geometry is negatively affected by T2DM at the proximal tibia, due perhaps to decreased muscle tensions and accelerated aging. Lower levels of physical activity, heavier body weights and diminished muscle strength may be other factors influencing T2DM bone geometry.

Keywords

Tibia, Cross-sectional Area, Diabetic Neuropathy, Magnetic Resonance Imaging, Muscle Strength, Bone Quality.

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Chapter 1

1 Brief Literature Review

1.1 Bone

Bone is a tissue of remarkable strength, found throughout the human body. It has vital functions, including structural support, locomotion, protection of organs and calcium storage. Bone is composed of mostly of type 1 collagen, a substance secreted by the osteoblast cells of bone. The inherent strength of bone is derived from the hardening of collagen, completed by the mineral salts (e.g., hydroxyapatite, calcium carbonate) in the bone matrix (area surrounding the cells of the bones) (Bauer and Link, 2009). Bone is porous, with many spaces between its hard, mineralized components. The distribution and size of these spaces has led to two macroscopic classifications of bone: cortical (i.e., compact) and medullary (i.e., spongy, cancellous).

Cortical bone contains fewer and smaller spaces, thus making the harder of the two bone types. It is dense, strong and most resistant to compressive forces. The external layer of all bones, and the outer layer of the shaft of long bones are made of cortical bone. Medullary bone consists of a spongy, latticework, or honeycomb-like trabeculae. This architecture is less dense and lighter than that of cortical bone and centrally located, deep to the cortical bone.

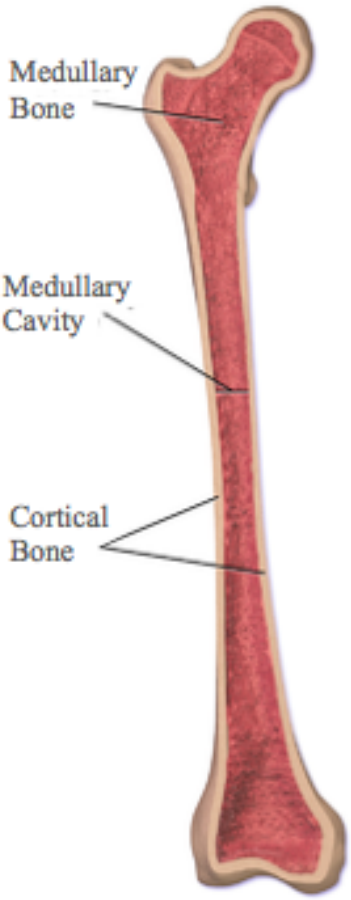

Figure 1 Example of the components of a long bone (femur)

As a highly metabolic and dynamic tissue, bone remodels to adapt to the external forces placed upon it. For example, medullary bone remodels to increase thickness and number of trabeculae, and orient trabeculae axially relative to the stress placed upon the bone (Turner, 1998). This remodeling is performed by osteoclast cells resorbing existing bone and osteoblast cells forming new bone (Novack and Teitelbaum, 2008; Romani et al., 2002). Imbalance in the remodeling process can lead to diseases of the bone, with negative implications for bone health, quality and strength (Novack and Teitelbaum, 2008). The most well-known and prevalent of these diseases is osteoporosis, in which osteoclast activity outpaces that of osteoblasts, leading to progressive bone degeneration (Bauer and Link, 2009; Novack and Teitelbaum, 2008). Throughout this thesis, the

phrase “bone quality” will be used to refer to the mechanical competence of bone and its ability to resist fracture.

1.1.1 Bone Quality and Geometry

Bone quality is affected by multiple factors, including total bone mass, bone geometry (i.e., bone size & dimensions, gross structural geometry, and mass distribution), and the properties of the respective components of the bone (Burghardt et al., 2010; Hamilton et al., 2013; Honda et al., 2014). Clinically, bone quality is essential as it is a predictor of fractures and necessary for independent living (Donnelly, 2011). Fracture occurs when a bone is unable to withstand external forces resulting in failure of integrity (Garg et al., 2012). Multiple factors affect bone quality, including mechanical (e.g., load bearing, muscle contraction, geometry, mass and spatial distribution) and non-mechanical (e.g., hormones, diet, pharmaceutical, genetic) influences.

Bone quality per se, is not measurable. Rather, clinicians and researchers use surrogate measures to assess and infer bone quality. Assessment of bone can be done at various anatomic levels ranging from macroscopic (whole bone) to microscopic (cellular and molecular levels), and using different *in vivo* and *ex vivo* measures.

1.1.2 Assessment of Bone Quality

When attempting to quantify bone quality, researchers and clinicians most frequently use dual-energy X-ray absorptiometry (DXA). This 2-dimensional, macroscopic, *in vivo* assessment sends X-rays from two energies through the body and measures the attenuation of these X-rays. From this, measurement of bone mineral content (grams) and bone mineral density (BMD, g/cm^2) can be calculated (Bouxsein, 2008; Cummins et al., 2002; Donnelly, 2011). DXA has many advantages, as it is cost-effective, non-invasive, low radiation exposure, high precision and short measurement times. It thus lends itself most readily to clinical screening and research. The measure of BMD allows for clinical predictions of bone quality. DXA is currently the gold standard imaging modality for prediction of fracture risk and low BMD is considered a risk factor for fracture (Bouxsein, 2008). Furthermore, BMD is a surrogate measure of strength and the mechanical properties of bone (Bauer and Link, 2009; Beck, 2003; Bonnick, 2007;

Burghardt et al., 2010; Garg et al., 2012). Despite the associations between BMD and fracture risk, BMD is not always indicative of the degree of bone strength, nor the risk of fracture (Beck, 2003). One-half of post-menopausal women with an incident of low energy bone fracture (i.e., fall from standing) are above the World Health Organization BMD osteoporosis threshold (defined at ≥ 2.5 standard deviations below the average value for young, healthy women) (Bauer and Link, 2009; Bouxsein, 2008). However, BMD is believed to contribute 70-75% of the variance in bone strength (Burghardt et al., 2011). Furthermore, despite having the same BMD, bones with different geometries can have very different strengths (Hamilton et al., 2013). More sensitive measures of bone quality are required, which can assess and predict fracture risk with increased accuracy, and potentially determine the underlying causes of bone fragility.

Recognizing the limitations of DXA, researchers and clinicians have turned to computer tomography (CT) to assess bone quality. Quantitative CT (QCT) rotates X-rays around the body to create 3-dimensional (3D) images of bone geometry, measured as volumetric BMD (Donnelly, 2011). This method allows for measurement of bone density separate from its size (unlike BMD), differentiation of the cortical and medullary components of bone, and assessment of more in depth bone quality measures, including geometric and microarchitectural (e.g., trabecular orientation and spacing, cortical porosity) features (Burghardt et al., 2011). QCT has short scanning times and increased quality of assessment of geometry and volumetric bone density, but the limitations include patient exposure to ionizing radiation, increased cost and decreased availability (Donnelly, 2011). High-resolution peripheral QCT (HR-qCT) allows for extremely detailed, *in vivo* 3D imaging of trabecular bone, but due to large doses of radiation to internal organs it is limited to distal extremity (the forearm and leg) sites (Burghardt et al., 2010). This eliminates assessment of vertebral bodies and the hip (sites which are frequently fractured) (Burghardt et al., 2011). Additionally, specialized scanners are required, decreasing accessibility, and longer scanning times increase motion artifact (Bouxsein, 2008).

Quantitative ultrasound is a useful modality in the assessment of bone *in vivo*. With portable machines, no ionizing radiation and low cost, it was believed to be a promising

method of evaluating bone strength (Laugier, 2006). Measurement of speed of sound (m/s) and broadband attenuation (dB/MHz) as the sound waves travel through the bone are used to estimate BMD. However, the International Society of Clinical Densitometry only approves the calcaneous as a site for measurement of bone health (Krieg et al., 2008). Ultrasound has shown to be approximately equal to DXA for prediction of fracture risk (see Chin and Ima-Nirwana, 2013 for review). Therefore with its low cost and portability, it is of increasing popularity in developing countries, and in countries where DXA is established, it is utilized almost exclusively in research settings (Laugier, 2006).

Use of magnetic resonance imaging (MRI) allows for generation of 3D images with no ionizing radiation and therefore without the associated risk of CT. Strong magnetic fields can be coupled with radiofrequency pulse sequences to allow imaging of moving molecules within the body (typically from water and fat). Thus, MRI is becoming a more popular mode of assessment of bone cross-sectional area (CSA) (Bouxsein, 2008), but is still limited by its increased expense and limited availability compared to DXA. Bone imaging through MRI has been validated with comparison to cadaver CSA (Gomberg et al., 2005) and was found to highly correlate with CSA measures using QCT ($r^2 = 0.98$) (Hong et al., 2000). Additionally, MRI has shown to have a high intra- and interobserver repeatability for all parameters (CV<0.8%) (Woodhead et al., 2001).

MRI has a distinct advantage over DXA by allowing for simultaneous muscle assessment. The largest stresses placed upon bone (excepting trauma) are forces due to muscle contraction (Frost, 1997). For example, the abductor muscle group in the hip exerts forces several times larger than body weight in order to maintain a level pelvis in a one-legged stance (Burr, 1997). Wolff's Law, penned by Julius Wolff in 1892, states the mechanical load placed upon the bone adapts its remodeling process (Bauer and Link, 2009; Frost, 2004). It is well known and continually recognized that bone mass is linked to muscle strength; as muscle strength increases or decreases through changes in physical activity levels, bone mass follows (Burr, 1997). Using MRI facilitates investigation of the relationship between muscle mass and bone quality (Frost, 1997; McNeil et al., 2009).

1.1.3 Use of MRI to Assess Bone Geometry

The body of literature using MRI to analyze bone quality is less well developed than that of CT or DXA. Specifically, geometric and architectural features assessed by exploring areas and volumes of gross anatomical bone structure using MRI is a relatively new topic. Because of this, there is understandably a lack of consistency with regards to methods on how to assess CSA with MRI. Several studies have quantified bone CSA by analyzing the middle third of the femur (Duncan et al., 2002; Högler et al., 2003; Kato et al., 2014; Woodhead et al., 2001), but bone heterogeneity or alterations of specific features, such as cortical and medullary architecture assessed by CSA or volume, exists throughout the length of a limb bone. Thus it is prudent, and beneficial, to compare multiple sites within the same bone to explore regional alterations in relation to potential bone remodeling due to disease or activity (Cole and van der Meulen, 2011; McNeil et al., 2009). Additionally, averaging multiple MRI slices at the various main regions (e.g., proximal, middle and distal) is considered more representative of bone geometry than a single slice (Klein et al., 2002). Populations which have been studied using MRI CSA include the elderly (Allen et al., 2012, 2011; Klein et al., 2002; McNeil et al., 2009), osteoporotic (Shen et al., 2013), elite athletes versus controls (Duncan et al., 2002; Honda et al., 2014; Kato et al., 2014; Nikander et al., 2009, 2007), peripubertal females (Högler et al., 2008, 2003; Maïmoun et al., 2013) and contra-lateral differences in one-sided dominant, competitive athletes (Ducher et al., 2011).

Although there have been studies completed in the thigh and upper limb (see above), only two studies have assessed bone CSA using MRI in the leg. McNeil et al. (2009) completed CSA measures in the leg in males, comparing young adults and older adults (61 – 91 years). Although they found total CSA (tCSA) consistent at both proximal and distal sites of the tibia, the medullary CSA (mCSA) was greater and conversely, cortical CSA (cCSA) was lower in the elderly. In a similar study conducted with young adult compared with older adult women (61 – 80 years), Allen et al. (2011) found tibial cortical volumes (calculated as: cCSA x slice thickness) were lower in the proximal section, but not in the distal section in the older women.

1.2 Diabetes

Diabetes has become the fastest growing chronic disease, affecting nearly every country, including Canada (*Diabetes in Canada*, 2011; Shaw et al., 2010). Canada has the third highest prevalence rate of diabetes (at just over 9%) of countries from Europe, North America and Oceania (*Diabetes in Canada*, 2011). There are two types of diabetes. Type 1 diabetes mellitus affects only 5-10% of those with diabetes and is typically diagnosed in childhood or early adulthood. It is the result of pancreatic dysfunction leading to insulin not being secreted by the beta cells; blood glucose levels are controlled with pharmacological insulin supplementation.

Type 2 diabetes mellitus (T2DM) is the more prevalent version of diabetes; >90% of those with diabetes are classified as T2DM. It stems from insulin insensitivity – the inability of tissue to use insulin for the uptake of blood glucose (*Diabetes in Canada*, 2011). With an increasingly passive lifestyle and its associated features, T2DM prevalence rates continue to rise across all age groups and races (Shaw et al., 2010; Sicree and Shaw, 2007). Chronic hyperglycemia leads to a multitude of secondary complications, including cardiovascular disease, stroke, renal failure, neuropathy, retinopathy and changes in bone quality (Kannel and McGee, 1979). These complications make diabetes a major cause of morbidity and mortality in North American and European countries (de Waard et al., 2014).

1.2.1 T2DM and Bone

Bone quality in the T2DM population has been extensively investigated. There is an increased risk of low-trauma hip fracture (Gorman et al., 2011), estimated to be 1.4 times higher than otherwise similar and healthy controls (Schwartz and Sellmeyer, 2007). There are two main factors causing this increased fracture risk: increased falls and decreased bone quality (Vestergaard, 2007). Many of the secondary complications associated with T2DM – retinopathies, neuropathies, muscular weakness, vestibular disorders, fainting due to hypoglycemia or orthostatic posture, and polypharmacy – increase the risk of falls (de Waard et al., 2014; Gower and Casazza, 2013; Vestergaard, 2007). However, when bone quality in those with T2DM has been explored using

imaging modalities, expected results have not been forthcoming. Despite an increased fracture risk, DXA, the most frequently used modality for assessing bone health in T2DM, shows similar, if not increased BMD in the T2DM population (Gorman et al., 2011; Schwartz and Sellmeyer, 2007; Vestergaard, 2007). This creates a T2DM and BMD paradox; T2DM illustrated similar or increased BMD, yet decreased bone quality (i.e., higher risk of fracture). This raises the concerns that 1) due to bone microarchitecture and structural geometry, T2DM bone may be more susceptible to fracture than bone of equal BMD in healthy controls (Hamilton et al., 2013; Schwartz and Sellmeyer, 2007) and 2) DXA is not a sufficiently sensitive method to assess fracture risk (Gorman et al., 2011). Although frequently used in clinical practice, DXA has proven to be unhelpful in the T2DM population (Garg et al., 2012). It does not explain the increased fracture risk in this population, and ultimately is not a measurement which can determine bone strength (Garg et al., 2012). The more in depth and accurate HR-qCT has been used to further investigate whether changes in bone microarchitecture are responsible for the increased fragility in diabetic bone. Cortical porosity, an important factor in bone quality (Sundh et al., 2015), has been found to be significantly higher in T2DM individuals compared to controls (Burghardt et al. 2010) and in T2DM with a history of a fragility fracture compared to T2DM without this history (Patsch et al., 2013). Petit et al. (2010) found an increase in trabecular density in the distal radius and tibia and Burghardt et al. (2010) noted a significantly increased trabecular thickness in the distal tibia. These factors all indicate a redistribution of bone mass in T2DM, supporting the idea that diabetes reduces the ability of bone to respond to mechanical stimuli or alters the normal balance between bone accretion and resorption (Adami, 2009; Garg et al., 2012). Ultrasound used to estimate BMD showed a decrease in speed of sound in T2DM and this result is more indicative of the increased risk of fracture, and thus some consider ultrasound a more useful tool for assessing bone health in those with diabetes (Tao et al., 2008). Bone quality in those with diabetes assessed by MRI is limited, thus creating the purpose for this study.

1.2.2 T2DM & Bone CSA

Studies assessing long bone CSA with MRI in T2DM population is limited. Pritchard et al. (2012 & 2013) have completed two studies to assess radius bone quality in T2DM by means of MRI. The first of these was completed in post-menopausal women and was designed to assess bone microarchitecture. They did however also measure endosteal area (mCSA) at the distal aspect of the radius and found no difference between the T2DM and control groups (Pritchard et al., 2012). The second study was a follow-up assessment of this same population, 2 years later, to longitudinally assess bone health. Again, mCSA at the distal radius was not significantly different between groups (Pritchard et al., 2013).

There is a similar, but slightly better established, body of literature using QCT or HR-qCT to assess bone CSA in T2DM. In a population of postmenopausal women with T2DM, Burghardt et al. (2010) found no difference in tCSA, cCSA or mCSA in the distal aspects of either the radius or tibia compared to controls. In males, Petit et al. (2010) found lower tCSA values for the tibia and radius in T2DM compared to controls after adjusting for age, race, clinic site, bone length and body weight. When only the first 4 variables were accounted for (excluding body weight), no difference was found between the two groups (Petit et al., 2010). Melton et al. (2008) analyzed CSA of the distal forearm, comparing T2DM males and females with age- and sex-matched individuals, and adjusting for BMI. At the radius, both the tCSA and mCSA were significantly smaller in T2DM than controls when analyzing both sexes as a combined group. However separately, only the tCSA in males was significantly smaller. Although not a direct measure of CSA, cortical thickness (mm) was measured and not found to be significantly different (Melton et al., 2008). Farr et al. (2014) also assessed cortical thickness and found it significantly lower in both the distal tibia and radius of T2DM females compared to age- and sex- matched controls. This difference was no longer apparent after adjusting for BMI (Farr et al., 2014). Thus, from the above studies, it is not clear whether individuals with T2DM exhibit differences in bone geometry, and if so, in what measures, compared to controls.

1.2.3 Indirect Effects on Bone Quality in T2DM

The many complications of T2DM include both direct and indirect effects on bone quality. Charcot neuroarthropathy is a complication of T2DM characterized by damage to bones and joints (typically in the ankle and foot), but as a secondary complication to neural dysfunction (i.e., diabetic neuropathy) (Low and Peh, 2015). It typically leads to multiple fractures of the metatarsals and potential foot amputations. The neurovascular theory of this cascade of events suggests denervation of the sympathetic nervous system leads to opening of the vasculature inside the bone, through reduced sympathetic tone (Mascarenhas and Jude, 2014). This has two negative effects on bone strength. First, through up-regulation of osteoclast cells and second, by quenching minerals (Mascarenhas and Jude, 2014; Varma, 2013). Barwick et al. (2014) published a meta-analysis assessing foot bone health in those with T2DM. No significant difference in the calcaneus bone was found between diabetics with neuropathy and diabetics without neuropathy through assessment with ultrasound, X-ray and DXA (Barwick et al., 2014).

Chapter 2

2 Introduction

Diabetes is a growing concern in the Canadian health care system. Canada has the third highest prevalence rate of countries in North America, Europe and Oceania, with 1 in 11 adults diagnosed with the disease (*Diabetes in Canada*, 2011). Of individuals with diabetes, 90-95% have type 2 diabetes mellitus (T2DM) (i.e., non-insulin dependent diabetes mellitus) (*Diabetes in Canada*, 2011). T2DM is known to lead to a multitude of secondary complications, including retinopathies, neuropathies, cardiovascular disease and alterations in bone quality. Bone quality in T2DM has been studied extensively with dual-energy x-ray absorptiometry, the current gold standard for assessment of bone fracture-risk and osteoporosis. In the T2DM population, a higher fracture risk and incidence rate is noted, but these individuals also have a higher bone mineral density (BMD) than their non-T2DM counterparts (Gorman et al., 2011; Schwartz and Sellmeyer, 2007; Vestergaard, 2007). This has created a T2DM and BMD paradox. The increased fracture risk is partly explained by increased risk and frequency of falls, but after eliminating these factors T2DM bone is still at higher risk of fracture than a non-T2DM bone of equal BMD (Schwartz and Sellmeyer, 2007).

The relationship between T2DM and bone quality has been explored to lesser extents using more in-depth and detailed imaging modalities, including quantitative computed tomography (QCT) and magnetic resonance imaging (MRI). Long bone cross-sectional area (CSA) has been assessed through QCT, with no consensus regarding whether changes are present in T2DM compared to controls. Burghardt et al. (2010) found no difference in total (tCSA), cortical (cCSA) or medullary (mCSA) CSAs of the distal radius or tibia in postmenopausal females compared between individuals with T2DM and those without. In older males with T2DM, Petit et al. (2010) found a lower tCSA in the radius and tibia compared to controls, after adjusting for body weight. Melton et al. (2008) used a sample of both males and females, assessing distal forearm CSA. When both sexes were combined, a significant decrease in tCSA and mCSA was detected; when assessing the sexes separately only tCSA in males was significantly decreased compared

to controls. Cortical bone was measured as thickness (mm) (rather than CSA) and no difference was found between the two groups by Melton et al. (2008) nor Farr et al. (2014). Finally, Pritchard et al. (2013 & 2012) completed a longitudinal study analyzing radius bone health in postmenopausal T2DM women via MRI. At both the first time point and the follow up visit 2 years later, no differences were found in mCSA (the only CSA measured) at the most distal aspect of the radius. Thus from these relatively few studies of mixed designs, it is unclear why bone quality is lessened in those with diabetes, and whether these negative alterations can be assessed via bone CSA measurements. Some of the limitations of these previous studies include measurement of CSA at only one point throughout the length of the bone, and assessment of only one of the three potential CSA measures (total, cortical or medullary). The current study is being conducted to determine whether the lesser bone quality observed in those with T2DM will be reflected through tibial CSA and volumetric measures. Previous studies have not assessed the three CSA measures at three different points throughout the length of the tibia, as well as cortical and medullary volumes. This comprehensive set of measures will allow for a more thorough investigation of bone geometry to determine differences which may occur in the T2DM individuals compared to controls. MRI is the imaging modality chosen for this study due to its relative patient safety, accurate measurements and novelty in the diabetic population, as only one other study has assessed T2DM long bone CSA with MRI (Pritchard et al., 2013, 2012). The ankle shows a higher relative risk of fracture than other sites in the body (Schwartz et al., 2001) and thus the tibia was selected as an important bone in the ankle joint. It has only been studied in the T2DM population a limited number of times with CSA measurements (Burghardt et al., 2010; Petit et al., 2010), and not with MRI. Based on the results of similar CSA assessment studies in individuals with T2DM (Melton et al., 2008; Petit et al., 2010), it was hypothesized that in the tibiae of a group of participants with T2DM there will be a lower tCSA compared to an age- and sex-matched control group. This smaller tCSA will be explained by a matching smaller mCSA and no change in cCSA.

Chapter 3

3 Methods

3.1 Participants

Individuals were recruited for a larger study focusing on the neuromuscular properties of individuals diagnosed with a diabetic neuropathy. Individuals were included based on the presence of T2DM, with clinical and electrophysiologoical features confirming the diagnosis of diabetic neuropathy. Clinical neurological and electrophysiological testing eliminated other possible conditions. The diabetic neuropathy group included 8 individuals (32-79 years; 3 females); the control group included 9 age- and sex-matched individuals (4 females). All were recruited from the community and screened for exclusion criteria. The local university's Research Ethics Board approved the study and all individuals gave informed oral and written consent.

3.2 Imaging

Magnetic resonance images of the leg were acquired in all individuals. Participants were supine and entered the magnet feet first. Feet and knees were strapped together with inelastic Velcro straps to minimize potential movement of the lower limb. Both legs were imaged, including the knee and ankle joints using serial axial plane imaging in a 3.0-T magnet (Verio MRI, Siemens, Erlangen, Germany). The following parameters were used for proton density acquisition: 9.57 ms repetition time (TR), 2.46 ms echo time (TE), 240 x 320 mm field of view, and 1 mm slice thickness.

3.3 Image Analysis

CSA analysis of the leg was completed off-line, using stored images (see Figure 2), by one trained and blinded analyzer using OsiriX image processing software (version 4.0, Geneva, Switzerland). tCSA of the tibia was measured beginning at the first slide distal to the tibial articular surface of the knee joint and continued until the articular surface for the talus. The closed polygon tool was used to create a region of interest (ROI), isolating the tibia from the remainder of the leg. This was repeated every fifth slice; OsiriX

automatically interpolated an ROI for the missing intermediate slides. Every slide was visually inspected by the analyzer and any errors produced through automatic ROI generation were manually corrected. All pixels outside the ROI were set to white; pixels within the ROI were differentiated by density to determine cortical bone from the medullary area. The software calculated the respective CSA of the entire ROI (tCSA), cCSA and mCSA regions. This method has been shown to have a high intra- and interrater reliabilities (Berger et al., 2012). Proximal, middle and distal CSAs were calculated by averaging two adjacent slices at 20% (proximal site), 50% (middle site), and 80% (distal site) of tibial length, measured from tibial plateau to talus. Volume of the entire tibia was calculated by OsiriX, using the respective CSA slices and multiplying by the slice thickness, creating total volume (TV), cortical volume (CV) and medullary volume (MV) (measured in cm^3). CV and MV were analyzed as a percentage of TV.

3.4 Statistical Analysis

Means were compared between the control and DN groups using an independent t-test, using SPSS (Version 22, IBM, Chicago, IL). Values in tables and figures are expressed as mean ± standard deviation; significance was given if $p \leq 0.05$.

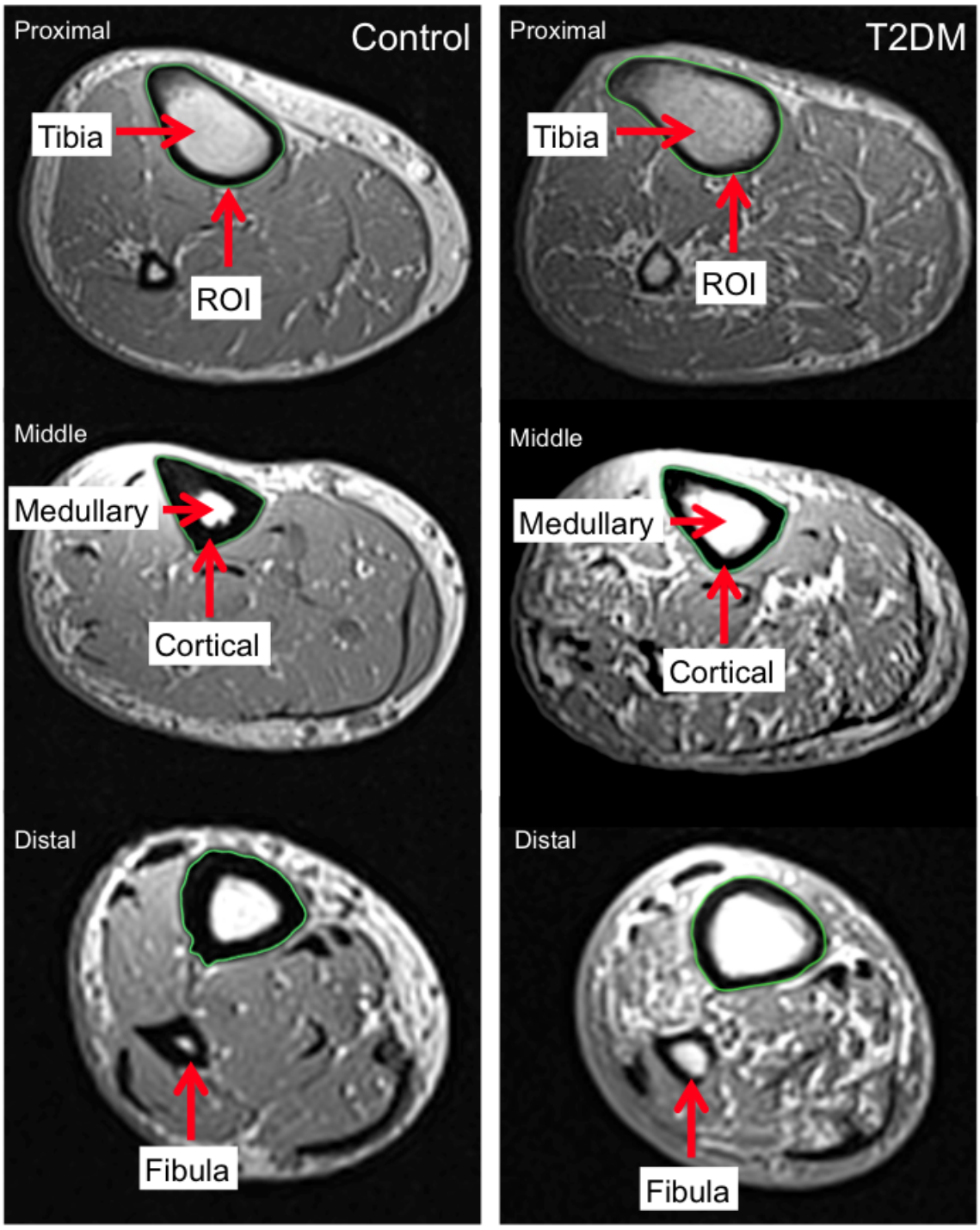

Figure 2 Control and T2DM tibiae at proximal, middle and distal sites

The control individual on the left is a 65 year-old male; the T2DM individual on the right is a 79 year-old male with T2DM for 35 years (22 years of diabetic neuropathy) showing

extreme changes in bone geometry. T2DM, type 2 diabetes mellitus; ROI, region of interest.

Chapter 4

4 Results

4.1 Participant Characteristics

The diabetic sample (n=8) of participants had an average age of 60.4 ± 15.0 years, height of 169.0 ± 7.4 cm, weight of 83.7 ± 8.2 kg and body mass index (BMI) of 29.4 ± 2.8 kg/m^2. The average duration of T2DM was 14.4 ± 10.2 years and the average duration of diabetic neuropathy was 8.8 ± 6.2 years. The control group (n=9) had an average age of 57.3 ± 19.0 years, height of 174.5 ± 8.0 cm, weight of 77.2 ± 9.0 kg and BMI of 25.3 ± 1.4 kg/m^2. Only BMI was statistically different between the two groups, being greater in the T2DM sample. Participant characteristics are shown in Table 1.

4.2 Tibial CSA & Volume

The tCSA, cCSA and mCSA (measured in cm^2) were analyzed at each respective leg length site and compared between the control and T2DM group. At the proximal site, mCSA was significantly greater (p<0.05) in the T2DM group than controls (5.9 ± 1.2 vs. 4.8 ± 0.7). However, as a percent of tCSA, the T2DM group did not have significantly more mCSA. No other significant results were found at any site of the tibia.

As a percentage of TV, neither CV nor MV were significantly different between the control and T2DM groups. Table 2 includes all values, including CSA, expressed as mean ± SD.

The above CSA and volume results were correlated to duration of T2DM and duration of diabetic neuropathy. No significant correlations were found.

Table 1 Participant characteristics

	Age (y)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Length of T2DM (y)	Length of DN (y)
Control (n=9, 5 males)	57.3 ± 19.0	174.5 ± 8.0	77.2 ± 9.0	25.3 ± 1.4	-----	-----
T2DM (n=8, 5 males)	60.4 ± 15.0	169.0 ± 7.4	83.7 ± 8.2	29.4 ± 2.8*	14.4 ± 10.2	8.8 ± 6.2

Values are mean ± SD. BMI, body mass index; T2DM, type 2 diabetes mellitus; DN, diabetic neuropathy.

*Significant difference between groups ($p \leq 0.05$)

Table 2 Geometry of the tibia

	Control	**T2DM**
Tibia Proximal Site		
tCSA (cm^2)	7.7± 1.0	8.9 ± 1.5
cCSA (cm^2)	2.8 ± 0.6	2.8 ± 0.6
mCSA (cm^2)	4.8 ± 0.7	5.9 ± 1.2*
Relative cortical area (%)	37.0 ± 5.0	32.3 ± 5.9
Relative medullary area (%)	63.0 ± 5.0	66.5 ± 5.2
Tibia Middle Site		
tCSA (cm^2)	4.3 ± 0.8	3.1 ± 0.8
cCSA (cm^2)	3.1 ± 0.8	3.2 ± 0.6
mCSA (cm^2)	1.2 ± 0.4	1.3 ± 0.4
Relative cortical area (%)	72.8 ± 8.9	70.7 ± 8.1
Relative medullary area (%)	27.3 ± 8.9	29.4 ± 8.1
Tibia Distal Site		
tCSA (cm^2)	4.3 ± 1.2	4.0 ± 0.6
cCSA (cm^2)	1.9 ± 0.3	2.1 ± 0.5
mCSA (cm^2)	2.4 ± 0.9	2.0 ± 0.5
Relative cortical area (%)	45.9 ± 6.6	51.1 ± 7.5
Relative medullary area (%)	54.1 ± 7.5	48.5 ± 9.8
Tibial Volume		
Relative cortical volume (%)	36.1 ± 8.4	31.4 ± 5.6
Relative medullary volume (%)	63.9 ± 8.4	68.6 ± 5.6

Values are mean ± SD. T2DM, type 2 diabetes mellitus; tCSA, total cross-sectional area; cCSA, cortical cross-sectional area; mCSA, medullary cross-sectional area.

*Significant differences between groups ($p \leq 0.05$)

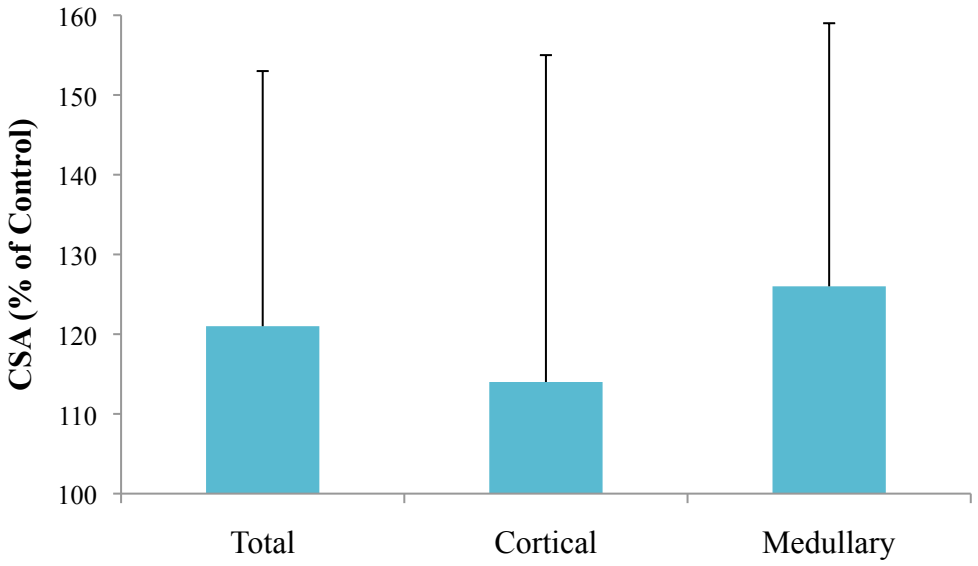

Figure 3 T2DM respective CSAs as percent of control CSAs at proximal site

Values are mean ± SD. CSA, cross-sectional area; T2DM, type 2 diabetes mellitus.

Chapter 5

5 Discussion

5.1 General Discussion

This study was conducted to determine whether bone structural geometry differed between individuals with T2DM and sex- and age-matched controls through assessment with MRI. Except for a significantly larger mCSA at the proximal tibia in T2DM compared with the control group, there were no other differences detected in the various bone parameters measured. This result is not in agreement with the hypothesis, in which it was expected that the tCSA and mCSA would be lower in the T2DM group compared to controls.

Although the sample size was not large, the groups were well matched, with the average control age of 57.3 ± 19.0 years and T2DM age of 60.4 ± 15.0 years with an average duration of T2DM of 14.4 ± 10.2 years. The T2DM tended to have a larger body mass and shorter stature, thus leading to a significantly larger BMI (29.4 ± 2.8 vs. 25.3 ± 1.4 kg/m^2). Despite this difference in BMI, overall bone CSA was not significantly different between these groups, and only one of the submeasures (proximal mCSA) was significantly different.

Although larger BMI is positively correlated to higher BMD, there are still a significant number of fractures in the obese population (Ong et al., 2014). A larger BMI places more mechanical load upon bones, stimulating bone remodeling, but excess adipose tissue negatively affects bone metabolism (Gower and Casazza, 2013). In the present study, the larger BMI of T2DM group was reflected in a greater amount of non-muscle tissue of the leg muscles (see Figure 2). This difference was quantified in a companion study by (Allen et al., 2014). Thus, the amount of viable muscle tissue in the leg of the T2DM group was not larger despite heavier body weight. It appears the effects of excess adipose tissue at a cellular level offset any stimulation for bone remodeling at a gross anatomical level, due to heavier body mass. Furthermore, it is unknown how the interactions in quality or relative quantities of these tissues are affected by physical

activity in this population. Muscle pull and weight bearing are the largest drivers of bone remodeling (Burr, 1997). Two of the subjects used mobility aids, one was wheelchair bound and, in general, individuals with T2DM do not exercise regularly (Krug et al., 1991). Therefore it is challenging to distinguish changes in bone health due to physical inactivity or diabetes. Park (2006) assessed muscle quality (muscle strength per unit regional muscle mass) in diabetics compared to controls, and found it was significantly decreased in diabetics, despite having an increase in actual muscle mass. This may mean that those with T2DM are placing less load upon their bones due to muscular contraction than controls, and therefore may help to explain the significantly larger mCSA in the proximal section of the tibia. Many muscles groups of the thigh and leg have attachments on the proximal tibia; reduced muscle actions on the bone may be causing a decrease in bone quality in this region. This may be related to what occurs with normal adult aging in long bones in which there is endosteal resorption (i.e., osteoclasts resorbing cortical bone by working superficially, or outwards, from the deep surface of cortical bone, bordering the medullary cavity) (Seeman, 2001). This decreases the cCSA and increases the mCSA, with no change in tCSA (Frost, 1997). The larger mCSA observed at the proximal site in the T2DM participants may be indicative of early stages of bone aging, accelerated in those with T2DM. This can be paralleled to the research by Andreassen et al., (2009) documenting accelerating muscle atrophy (i.e., aging) in the leg of individuals with diabetic neuropathy. Further studies are needed to determine the relationship between aging and bone in the T2DM population. Indeed, correlations between bone CSA, and length of T2DM and diabetic neuropathy were not significant in this relatively small sample.

Burghardt et al. (2010) assessed cross-sectional geometry using HR-qCT and found no differences between T2DM and controls in tCSA, cCSA or mCSA at the most distal aspects of the radius or tibia. In this study, scans more proximal than Burghardt et al. (2010) were analyzed, including mid-diaphysis, for a better representation of cortical bone, which might be important when assessing a weight bearing bone. Petit et al. (2010) found a decrease in tCSA in T2DM men compared to controls at both 4% and 66% of tibial length (measured from the distal end) with peripheral QCT, but only after controlling for body weight. The lower tCSA helps explain the higher BMD measured

with DXA, as BMD is a measurement of bone mineral content per area (cm^2). Decreases in the area of a bone with no changes in mineral content will increase BMD (Beck, 2003; Petit et al., 2010; Strotmeyer et al., 2004). Noting the changes reported by Petit et al. (2010), the results of this study were normalized to body weight to explore whether tCSA might be smaller in T2DM compared to controls once body weight was controlled, but no significant differences were found between the T2DM and control participants.

None of the participants in the current study had neuropathic issues severe enough to lead to Charcot neuroarthropathy, yet these underlying issues related to bone integrity as a consequence of diabetic neuropathy cannot be ignored. In a review published by Barwick et al. (2014) no difference was found in calcaneal bone health between diabetics with and without neuropathy through assessment with ultrasound, X-ray and DXA. This is of interest here, as it implies the inclusion criteria of diabetic neuropathy does not limit the results of the present study to those with diabetic neuropathy. Neuropathy affects individuals in a distal to proximal fashion (Barwick et al., 2014). As such, the tibia may only display changes due to neuropathy after an extended length of time with the disease. With this in mind, duration of diabetic neuropathy was correlated with the CSA measures and percent MV and CV, but no statistically significant relationships were found. For a variety of potential reasons, including a more proximal site in the body and a larger bone, the tibia appears to not be affected by diabetic neuropathy, at least overtly using the MRI CSA assessment method and with the current sample size.

From the results of the present study, increased fracture risk in T2DM may not be evident in overall geometry. Nevertheless bone quality may be affected by poorer structural integrity of the bone tissue itself that is not reflected in gross bone architecture. For example, greater cortical porosity has been found in T2DM who have suffered a fragility fracture compared to T2DM without a fragility fracture history (Patsch et al., 2013). Cortical porosity was not increased in T2DM without a fragility fracture history compared to controls, thus it cannot be assumed that increased cortical porosity is characteristic of T2DM (Patsch et al., 2013). Trabecular spacing has also been found to be greater in T2DM than controls (Pritchard et al., 2012). These microarchitectural

factors, which are not apparent with gross imaging, will affect the ability of bone to withstand forces, and thus its susceptibility to fracture.

Furthermore, hormonal, pharmaceutical or nutritional factors are also known to affect bone quality. For example, the thiazolidinedione (TZD) class of antidiabetic drug and chronic hyperglycermia levels both stimulate differentiation of mesenchymal cells into adipocytes at the expense of osteoblasts (Bazelier et al., 2012). Conversely, metformin, an insulin sensitizer anti-diabetic drug not in the TZD class, works in the opposite direction, decreasing risk of bone fracture through promotion of mesenchymal stem cells into osteoblasts rather than adipocytes (Yan and Li, 2013). Chronic hyperglycemia also leads to increased calcium excretion by the kidneys and decrease calcium absorption in the gut (McNair et al., 1979) and decreased renal function, a typical complication of T2DM, also alters bone structure (Jokihaara et al., 2006). These factors have not been measured in the present study but may impact bone health before overall geometric changes are detectable.

5.2 Summary and Conclusion

In conclusion, the current study found changes in T2DM bone quality compared to controls, assessed through CSA and volume in the tibia, only in the proximal mCSA. The greater mCSA in the T2DM compared with controls may be caused by a decrease in muscle pull due to decreased muscle quality in T2DM. The proximal tibia is the attachment point for the major muscles of the thigh and leg, and thus decreases in muscle quality will have the largest effect at this site. Additionally, a larger mCSA is an alteration typically seen in adult long bone aging and the results from this study results may indicate bone aging is accelerated in those with T2DM. Furthermore, negative alterations in bone microstructure that are not apparent with measures of gross geometry may affect bone quality and ultimately help explain the increased fracture risk in those with T2DM.

5.3 Limitations & Future Directions

There are several limitations to this study. These include a small sample size and not controlling for BMI and physical activity levels between the two groups. Increasing sample size to analyze the sexes both individually and separately (and controlling for menopausal status of female participants) would also help eliminate confounding factors. MRI assessment of two diabetic groups – one with a known fragility fracture history and one without this history – also may help elucidate changes in bone geometry. Similar to cortical porosity, changes in bone geometry might only be evident in individuals with a history of fragility fractures. It would also be interesting to analyze tibial CSA in comparison to other long bones, both weight bearing (e.g., femur) and non-weight bearing (e.g., fibula, radius), to determine whether differences occur between bones of the appendicular skeleton. Finally, using more detailed, *in vivo* imaging modalities, such as HR-qCT, to assess both CSA and microarchitectural features may help determine the degree of relation between these variables and their combined influence on bone quality in T2DM.

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Appendix

Appendix A Ethics Approval Notice

Research Western

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Tim Doherty
Review Number: 18141
Review Level: Full Board
Approved Local Adult Participants: 60
Approved Local Minor Participants: 0
Protocol Title: Impacts of Diabetes on the Neuromuscular System in Humans
Department & Institution: Clinical Neurological Sciences,London Health Sciences Centre
Sponsor:
Ethics Approval Date: August 17, 2011 **Expiry Date:** May 31, 2015

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Letter of Information		2011/07/28
UWO Protocol	Including all instruments listed in section 8.1	
Letter of Information & Consent	Healthy volunteers	2011/07/28

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The UWO HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Signature

Ethics Officer to Contact for Further Information

Janice Sutherland (jsuther1@uwo.ca)	Grace Kelly (grace.kelly@uwo.ca)	Shantel Walcott (swalcot@uwo.ca)

This is an official document. Please retain the original in your files.

Curriculum Vitae

Name:	Helen Frances Honig
Post-secondary Education and Degrees:	McMaster University Hamilton, Ontario, Canada 2008-2012 B.Sc.
	The University of Western Ontario London, Ontario, Canada 2013-2015 M.Sc.
Honours and Awards:	Ontario Graduate Scholarship 2012-2013, 2013-2014, 2014-2015
	Western Research Graduate Scholarship 2013-2014, 2014-2015
	Canada Graduate Scholarship, Masters (NSERC) 2014-2015
Related Work Experience	Teaching Assistant The University of Western Ontario 2013-2015

Conference Presentations:

Honig, H.F., Allen, M.D., Allman B.L., Rice, C.L., 2015. Tibial Bone Geometry in Human Diabetic Neuropathy. American Association of Anatomists (Experimental Biology) Conference. Boston, USA. (Poster).

Honig, H.F., Allen, M.D., Allman B.L., Rice, C.L., 2014. Tibial Bone Geometry in Human Diabetic Neuropathy. Anatomy & Cell Biology Research Day. The University of Western Ontario, London, Canada. (Poster).