BONE QUALITY IN ADULTS WITH TYPE 2 DIABETES

THE ASSESSMENT OF STRUCTURAL AND MATERIAL BONE QUALITIES IN ADULTS WITH TYPE 2 DIABETES

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To Mom, Dad, Jaclyn and Marsha

With all my love

Descriptive Note

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ABSTRACT

The risk of fracture is higher in adults with type 2 diabetes compared to controls without type 2 diabetes, despite normal or higher than normal bone mineral density (BMD). In addition to BMD, bone strength depends on other factors such as structural and material *bone qualities*, which are not accounted for in BMD measurements. Our objective was to determine whether structural and material *bone qualities* are different in adults with type 2 diabetes compared to controls without type 2 diabetes. First, a cross-sectional study was undertaken using MRI to investigate distal radius trabecular bone microarchitecture, a structural *bone quality.* In women with type 2 diabetes, trabecular bone holes were larger compared to controls, which is important because greater trabecular bone hole size is related to reduced bone strength. Next, a two year prospective study was conducted with the participants involved in the cross-sectional study to determine whether changes in trabecular bone microarchitecture are different in women with type 2 diabetes compared to controls. We found that there was a greater increase in the number of trabecular bone holes in women with type 2 diabetes compared to controls, which provides early evidence of trabecularization of cortical bone in women with type 2 diabetes. In the third study, we used quantitative backscattered electron imaging (qBEI) to derive bone

mineralization density distribution (BMDD) outcomes for bone samples from adults with and without type 2 diabetes to compare material *bone quality.* We show evidence of elevated bone calcium concentration and reduced mineralization heterogeneity in bone samples from adults with type 2 diabetes compared to controls, which may contribute to bone brittleness. In summary, differences in structural and material *bone qualities* identified in this body of work provide explanations for elevated fracture risk in adults with type 2 diabetes.

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LIST OF ABBREVIATIONS and TERMS

2-D	Two dimensional
3-D	Three dimensional
µCT	Micro-computed tomography
µsec	Micro second
aBMD	Areal bone mineral density
AGE	Advanced glycated end-product
Al	Aluminum
BMD	Bone mineral density
BMDD	Bone mineral density distribution
BMI	Body mass index
BVTV	Bone volume fraction
C	Carbon
Ca	Calcium
Ca_{MEAN}	Mean bone calcium concentration
Ca_{WIDTH}	Mineralization heterogeneity
Ca_{PEAK}	Most frequently occurring calcium concentration
CaM*os*	Canadian multicentre osteoporosis study
CI	Confidence interval
CTX	C-terminal cross-linking telopeptide of type 1 collagen
DISH	Diffuse Idiopathic Skeletal Hyperostosis
DM	Diabetes mellitus
DXA	Dual x-ray absorptiometry
EDS	Energy dispersive x-ray spectrometry
FFQ	Food Frequency Questionnaire
FN	Femoral neck
FSE	Fast spin echo
FWHM	Full width at half maximum
GL	Gray level
HbA1c	Glycated hemoglobin
HR-pQCT	High resolution peripheral quantitative computed tomography
ICC	Intra-class correlation coefficient
IGF	Insulin-like growth factor
iPTH	Intermittent parathyroid hormone
Kcal	Kilocalorie
LS	Lumbar spine
MgO	Magnesium oxide
MRI	Magnetic resonance imaging
NHANES	National Health and Nutrition Examination Survey

NS	Not significant
P1NP	Procollagen type 1 N-terminal propeptide
pA	Pico amp
PASE	Physical activity scale for the elderly
PPAR- γ	Peroxisome proliferator-activated receptor-gamma
pQCT	Peripheral quantitative computed tomography
qBEI	Quantitative backscattered electron imaging
QCT	Quantitative computed tomography
RANK	Receptor activator of nuclear factor kappa B
rhPTH	Recombinant human parathyroid hormone
RMSCV	Root mean square coefficient of variation
ROS	Reactive oxygen species
RR	Relative risk
SD	Standard deviation
SEM	Scanning electron microscope
SRCT	Synchrotron radiation computed tomography
T2D	Type 2 diabetes
Tb.N	Trabecular number
Tb.Sp	Trabecular separation
Tb.Th	Trabecular thickness
TUG	Timed-Up-And-Go test
TZD	Thiazolidinedione
WMGL	Weighted mean gray level
Wt % Ca	Weight percent calcium (calcium concentration)

CHAPTER ONE

INTRODUCTION

Currently, over 5 million Canadians are over the age of 65 years, making up nearly 15% of the entire population (Statistics Canada, 2012). The aging population in Canada will have a profound impact on chronic disease prevalence, particularly osteoporosis and type 2 diabetes.

1.1 Epidemiology and Diagnosis of Osteoporosis

Osteoporosis is a chronic disease that is common among Canadian older adults (Tenenhouse et al., 2000). In men and women, fractures due to osteoporosis include non-traumatic fractures of the hip, vertebrae, wrist, proximal humerus, rib, pelvis, clavicle, scapula and sternum (Kanis et al., 2001). Fractures of the tibia and fibula are considered to be osteoporotic fractures in women only (Kanis et al., 2001). The major osteoporotic fractures, hip, vertebral, wrist and proximal humerus fractures, afflict 1 in 3 women and 1 in 5 men in their lifetime (Kanis et al., 2000; Leslie, O'Donnell, et al., 2010; Melton, Chrischilles, Cooper, Lane, & Riggs, 1992). Wrist fractures are the most common type of major osteoporotic fracture. An individual who has a history of a wrist or proximal humerus fracture is at greater risk for experiencing a recurrent osteoporotic fracture (Hodsman, Leslie, Tsang, & Gamble, 2008). While wrist and proximal humerus fractures are burdensome, hip and vertebral fractures are the more serious consequences of osteoporosis because the cost per care of hip and vertebral fracture is higher (Kaffashian et al., 2011; Tarride et al., 2012) and hip

and vertebral fractures decrease quality of life and increase the risk of death (Ioannidis et al., 2009; Papaioannou et al., 2009; Sawka et al., 2005). Overall, annual healthcare expenditures related to the treatment of osteoporotic fractures are estimated to be over $2.3 billion, with estimates closer to $3 billion when long-term care related costs due to osteoporosis are considered (Tarride et al., 2012).

Osteoporosis is most often defined as a disease of low bone mineral density (BMD) and bone microarchitectural deterioration resulting in an increased risk of fracture (National Institutes of Health, 1993). Dual x-ray absorptiometry (DXA) is the most widely used method for measuring BMD of the lumbar spine (L1-L4), proximal femur (femoral neck, greater trochanter, intertrochanteric region, total hip), distal radius and whole body (Kanis, Oden, Johnell, Jonsson, de Laet, & Dawson, 2001; Marshall, Johnell, & Wedel, 1996). DXA measures the attenuation of x-rays, providing an indirect measure of amount of bone mineral content in a projected area (g/cm^2) (Pacifici et al., 1988). DXA is useful for measuring BMD in a clinical setting because of the low radiation exposure associated with the scan, short scan time, low operating costs, and ease of use. Lumbar spine and femoral neck BMD measurements are valid (Ho, Kim, Schaffler, & Sartoris, 1990; Johanson et al., 1993) and highly reproducible (Lilley, Walters, Heath, & Drolc, 1991; Orwoll & Oviatt, 1991). The usefulness of BMD in assessing skeletal health lies in the strong relationship between BMD and fracture risk (Marshall et al., 1996). Although various anatomical sites can be

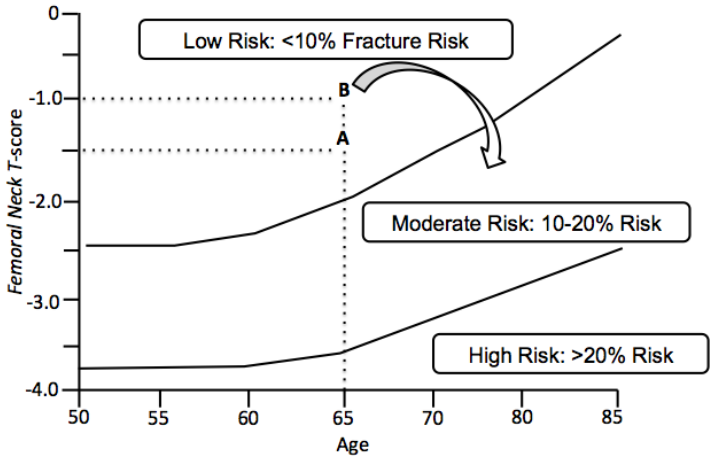
measured with DXA, femoral neck BMD is the strongest predictor of hip fracture compared to the other sites (Cummings et al., 1993; Marshall et al.,1996).

A number of international organizations have recommended that osteoporosis be diagnosed based on femoral neck BMD (Binkley & Bilezikian, 2006; Kanis, Melton, Christiansen, Johnston, & Khaltaev, 1994; Kanis & Gluer, 2000). Using the Third National Health and Nutrition Examination Survey (NHANES III), a reference database for femoral neck BMD in young adults aged 20-29 years was established (Looker et al., 1998). Based on this reference database, *T*-scores for postmenopausal women and men over age 50 years are computed. Osteoporosis is diagnosed if an adult's femoral neck *T*-score is less than or equal to 2.5 standard deviations below peak BMD for the reference population (Binkley & Bilezikian, 2006; Kanis et al., 1994; Kanis & Gluer, 2000). This diagnostic criterion was useful for setting intervention thresholds and recruitment criteria for epidemiologic studies. However, there are limitations associated with DXA-derived BMD measurements. BMD is based on a two dimensional projected image and can over-estimate BMD of bone that is of greater volume (Yu, Gluer, Fuerst, et al., 1995). BMD does not completely explain variations in bone strength, evidenced by the overlap in *T*-scores in adults with and without fracture history (Cranney, Jamal, Tsang, Josse, & Leslie, 2007; Wainwright et al., 2005). Furthermore, very small improvements in BMD (~4%) with osteoporosis-related treatment do not fully explain the large reductions in fracture risk (~50%) that are associated with treatment (Cummings et al., 2002).

Therefore, a new paradigm that includes clinical risk factors for fracture risk prediction has been implemented for the assessment of osteoporosis (Papaioannou et al., 2010).

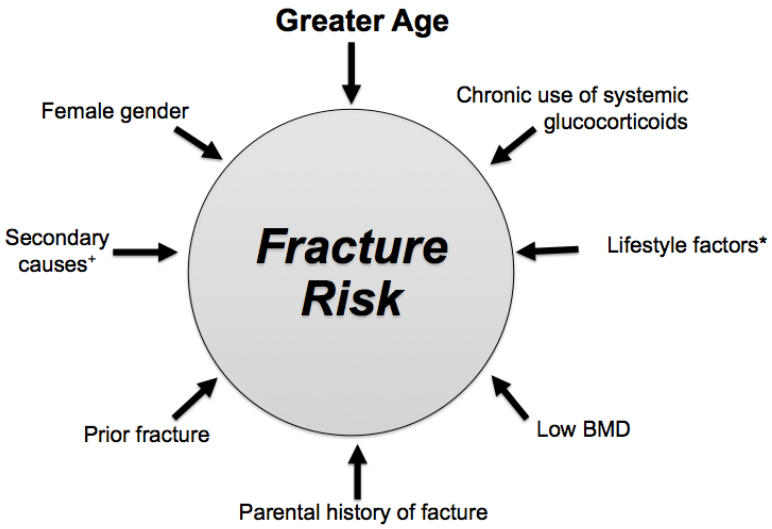
The Canadian Association of Radiologists and Osteoporosis Canada (CAROC) developed a tool for assessing fracture risk in Canadians (Leslie, Berger et al., 2010; Siminoski et al., 2005). This gender-specific tool uses femoral neck *T*-score, age, history of prior fracture and chronic systemic glucocorticoid use to provide a 10-year major fracture risk prediction (Figure 1.1). An individual over the age of 50 with a history of a hip or vertebral fracture or two or more fractures is automatically considered to be at high risk for subsequent fracture (Kanis et al., 2004; Papaioannou et al., 2010)

Figure 1.1 Canadian Association of Radiologists and Osteoporosis Canada (CAROC) Fracture Risk Assessment tool and modification of risk zone with major risk factors. Patient A is a 65 year old woman with a femoral neck *T*-score of -1.5. Her predicted risk of major osteoporotic fracture in the next 10 years is low. Patient B is a 65 year old woman with a femoral next *T*-score of -1.0. She experienced a wrist fracture at age 50. This increases her predicted fracture risk from low to moderate.



The World Health Organization's Fracture Risk Assessment tool (FRAX®) has also been validated using Canadian data (Leslie, Lix, et al., 2010). Along with femoral neck *T*-score, age, gender, prior fracture, and use of systemic glucocorticoids, additional risk factors influence the risk of fracture and are factored into this tool's algorithm (Figure 1.2) (Kanis et al., 2007).

Figure 1.2 Risk factors identified for the development of osteoporosis. + Secondary causes include type 1 diabetes, osteogenesis imperfecta, untreated long-standing hyperthyroidism, hypogonadism, premature menopause, chronic malnutrition, malabsorption disease, chronic liver disease. * Lifestyle factors include smoking status, alcohol intake.



Type 1 diabetes is consistently associated with increased fracture risk and low BMD (Janghorbani, Van Dam, Willett, & Hu, 2007; Vestergaard, 2007). In this Fracture Risk Assessment tool, type 1 diabetes is recognized as a secondary cause of osteoporosis and increases the probability of fracture (when a BMD measurement is not entered into the algorithm) (Kanis, Johnell, Oden, Johansson, & McCloskey, 2008). A diagnosis of type 2 diabetes is not factored

into the Fracture Risk Assessment tool algorithm. The 10-year fracture risk prediction obtained from either tool can be used as a basis for treatment decisions, as it is recommended that an adult with a high risk of fracture be considered for pharmacologic therapy (Papaioannou et al., 2010).

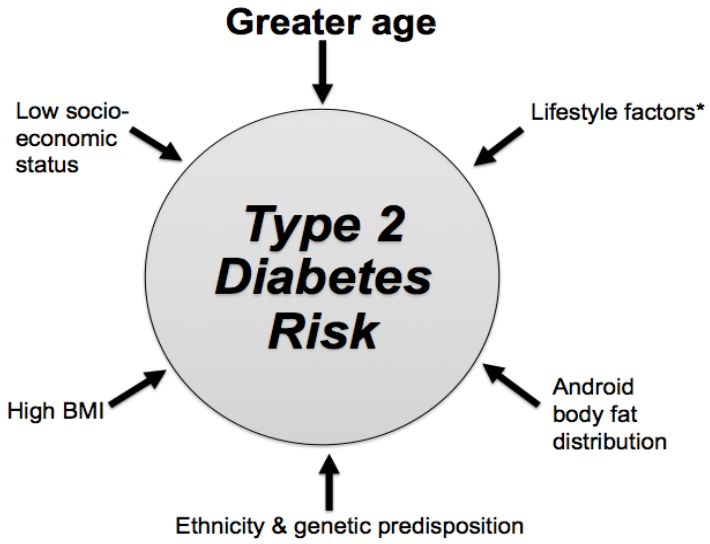
1.2 Epidemiology and Diagnosis of Type 2 Diabetes

Type 2 diabetes is a devastating chronic disease that affects 2.4 million Canadians (Public Health Agency of Canada [PHAC], 2011). In 2009, the prevalence of type 2 diabetes was more than four-fold higher in individuals over the age of 65 years compared to middle-aged individuals (PHAC, 2011). Quality of life is dramatically reduced with type 2 diabetes due to complications associated with the disease, such as peripheral neuropathy, nephropathy, retinopathy and vascular disease (Chyun et al., 2006; Reenders, de Nobel, van den Hoogen, Rutten, & van Weel, 1993; Diabetes Control and Complications Trial, 1995). Annual costs to the Canadian Health Care system due to type 2 diabetes are estimated to be over $8 billion (Ohinmaa, Jacobs, Simpson, & Johnson, 2004; Statistics Canada, 2012).

Type 2 diabetes is hallmarked by hyperglycemia caused by non-autoimmune defects in insulin secretion from the pancreas, and/or impaired insulin action at the level of the muscle, liver and adipose tissue (insulin resistance) (Muoio & Newgard, 2008). There are many risk factors, from lifestyle to genetic factors (Agardh, Hellgren, & Bengtsson, 2011; Chao et al., 2011; Cho

et al., 2009; Edelstein et al., 1997; Ohlson et al., 1985; Pettitt, Knowler, Bennett, Aleck, & Baird, 1987; Pi-Sunyer et al., 2007), which are implicated in the development of type 2 diabetes (Figure 1.3).

Figure 1.3 Risk factors identified for the development of type 2 diabetes. Greater age is common to type 2 diabetes and fracture risk.
* Lifestyle factors include high caloric intake, sedentary lifestyle, sleep duration, cigarette smoking. Note: greater age is common to diabetes risk and fracture risk.



Type 1 diabetes is caused by an autoimmune driven pancreatic β-cell destruction resulting in inadequate insulin production (Eisenbarth, 1986), and accounts for 5 – 10% of the diabetes cases in Canada (PHAC, 2011). In general, individuals with type 1 are diagnosed during childhood or adolescence, while the majority of type 2 diabetes diagnoses are made in adults (Evans, MacDonald, Leese, Ruta, & Morris, 2000).

Both types of diabetes cause elevated blood glucose levels and share diagnostic criteria based on the measurement of plasma glucose levels, which

are strongly associated with microvascular complications (Diabetes Control and Complications Trial, 1995; Stratton et al., 2000). A diagnosis of diabetes is given when fasting plasma glucose is equal to or greater than 7.0 mmol/L, or when random plasma glucose is equal to or greater than 11.1 mmol/L and symptoms of diabetes are present. In a 75 gram oral glucose tolerance test, 2 hour plasma glucose of 11.1 mmol/L or greater also indicates diabetes (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008). Screening individuals over age 40 every three years for type 2 diabetes is imperative because the clinical sequelae that develop as a result of chronic hyperglycemia include coronary artery disease (Lee, Cheung, Cape, & Zinman, 2000), chronic kidney disease (Reenders et al., 1993), retinopathy (Diabetes Control and Complications Trial, 1995) and neuropathy (Reenders et al., 1993).

1.3 The Interaction Between Osteoporosis and Type 2 Diabetes: Fracture Risk

Albright and Reifenstein (1948) suggested over six decades ago that poor skeletal health is another complication associated with diabetes. A meta-analysis of 8 prospective studies published before 2006 concluded that the risk of hip fracture was 38% greater in adults with type 2 diabetes (relative risk [RR] 1.38 [95% confidence interval, CI 1.25-1.53], $p < 0.05$) (Vestergaard, 2007). A lower wrist fracture risk was reported from analyses of 3 studies (RR 1.19 [95% CI, 1.01-1.41], $p < 0.05$) and there was no increased risk of vertebral fracture in adults with type 2 diabetes (RR 0.93 [95% CI, 0.63-1.37], $p > 0.05$) (Vestergaard,

2007). In another meta-analysis which included 13 epidemiologic studies, the risk of hip fracture was 80% greater for adults with type 2 diabetes (RR 1.8 [95% CI, 1.5-2.2], $p < 0.05$), while the risk was not elevated for wrist, vertebral, proximal humerus or ankle fracture (Janghorbani et al., 2007).

Duration of type 2 diabetes, propensity to fall and type 2 diabetes-related complications are involved in the observed elevated fracture risk. In adults who were diagnosed with type 2 diabetes for less than 5 years, hip fracture risk was 13% higher compared to controls without type 2 diabetes (rate ratio [RR] 1.13 [95% CI, 1.00-1.28], $p < 0.05$), whereas in adults with longer-standing type 2 diabetes (*i.e.,* more than 5 years), hip fracture risk was 40% higher compared to controls (RR 1.40 [95% CI, 1.28-1.53], $p < 0.05$) (Leslie et al., 2007). Amongst men and women with type 2 diabetes living in the community and in long-term care, falls are common (Maurer, Burcham, & Cheng, 2005; Schwartz et al., 2002; Schwartz et al., 2008). The risk of falling increases with the presence of common diabetes complications, hypoglycemic events, reduced functional ability and poor muscle strength (Gregg et al., 2002; Richardson, 2002; Schwartz et al., 2008; Strotmeyer et al., 2005; Vestergaard, Rejnmark, & Mosekilde, 2009). However, an increased risk of fracture in adults with type 2 diabetes remains even after adjustment for these extra-skeletal factors.

1.4 The Interaction Between Osteoporosis and Type 2 Diabetes: BMD

It is well-known that for most adults, there is an inverse relationship between fracture risk and BMD (Marshall et al., 1996; Papaioannou et al., 2005). However, this is not the case for adults with type 2 diabetes. Fracture risk remains elevated despite higher than normal femoral neck and lumbar spine BMD (de Liefde et al., 2005; Gerdhem, Isaksson, Akesson, & Obrant, 2005; Hanley et al., 2003; Isaia et al., 1999). A meta-analysis of 15 cross-sectional, cohort and case-control studies demonstrated that men and women with type 2 diabetes had elevated BMD compared to controls (Ma et al., 2012). In adults with type 2 diabetes, BMD was greater by a difference of +0.06 g/cm^2 (95% CI, 0.04-0.07, $p < 0.05$) at the lumbar spine, +0.04 g/cm^2 (95% CI, 0.02-0.05, $p < 0.05$) at the femoral neck, and +0.06 (95% CI, 0.04-0.08, $p < 0.05$) at the total hip (Ma et al., 2012). There was a greater magnitude of difference when the data for women only were analyzed (Ma et al., 2012). Similar findings have been reported for Canadians. In a study that was not included in the meta-analysis, women with type 2 diabetes in the Canadian Multicentre Osteoporosis Study (CaM*os*) had higher lumbar spine and femoral neck BMD compared to women without type 2 diabetes (Hanley et al., 2003). Between-group differences only existed for men at the lumbar spine (Hanley et al., 2003). There are various factors that could contribute to higher lumbar spine and femoral neck BMD. One possibility is that lumbar spine BMD is over-estimated in adults with type 2 diabetes due to vertebral disk narrowing, osteophyte formation, sclerosis, aortic calcification and

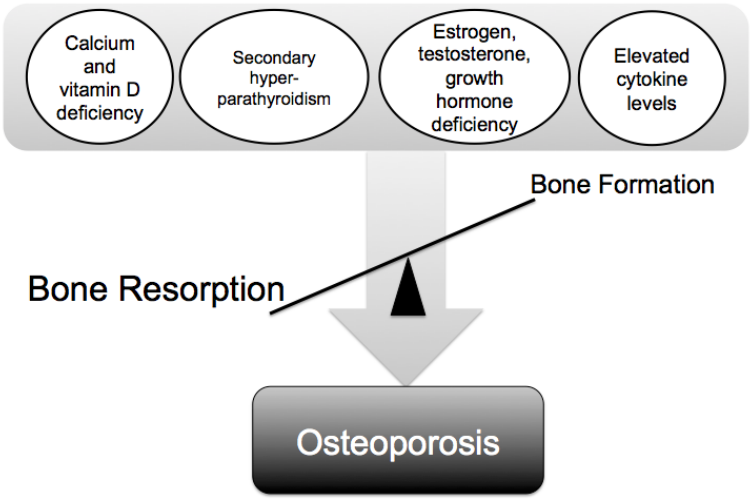
diffuse idiopathic skeletal hyperostosis (DISH or Forestier's disease) (Di Franco, Mauceri, Sili-Scavalli, Iagnocco, & Ciocci, 2000; Guglielmi et al., 2005; Orwoll, Oviatt, & Mann, 1990; Sahin et al., 2002; Yu, Gluer, Grampp, et al., 1995). Osteoarthritis can increase the size of the vertebral bodies, making the amount of bone in a projected area (areal BMD) appear greater (Carr et al., 2008). BMI and glycemic control (HbA1c) are also strong predictors of elevated BMD (Ma et al., 2012). However, studies have adjusted for BMI and HbA1c in multivariate regression analyses and found that BMD remains higher in adults with type 2 diabetes compared to controls without type 2 diabetes (de Liefde et al., 2005; Hanley et al., 2003; Lunt et al., 2001; Register et al., 2006). In order to further understand the counterintuitive relationship between fracture risk and BMD in adults with type 2 diabetes, potential mediators ought to be examined.

1.5 Pathophysiology of Osteoporosis

Bone is an active organ that changes in size, shape and mineral content throughout life. An important determinant of BMD is the activity level and number of cells in the basic multicellular unit, which is made up of osteoclasts and osteoblasts (Frost, 1969). Following activation by a stimulus, osteoclasts are recruited to resorb bone and osteoblasts follow to form bone in the resorption pit (Frost, 1969). This process of activation, resorption and formation is known as bone remodeling (Frost, 1969). Depending on an individual's life-stage, the activity and number of osteoclasts and osteoblasts varies, which consequently

impacts BMD. For example, in the second and third decade of life, bone formation dominates over resorption and peak BMD is achieved (Baxter-Jones, Faulkner, Forwood, Mirwald, & Bailey, 2011). During times of BMD loss, bone resorption dominates over bone formation. For women, femoral neck BMD is stable between age 25 and 40 years and significant losses begin between age 40 and 44 years, with accelerated losses occurring in the fifth decade of life (Berger et al., 2008). In men, a steady decline in femoral neck BMD is apparent after age 35, with accelerated losses occurring after age 65 (Berger et al., 2008). Various biological factors favour bone resorption over formation (Bischoff-Ferrari et al., 2005; Cauley et al., 2007; Chapuy et al., 1996; Greendale, Edelstein, & Barrett-Connor, 1997; Manolagas, Weinstein, Jilka, & Parfitt, 1998; Recker et al., 1996; Rosen, Donahue, & Hunter, 1994), contributing to the pathophysiology of osteoporosis (Figure 1.4).

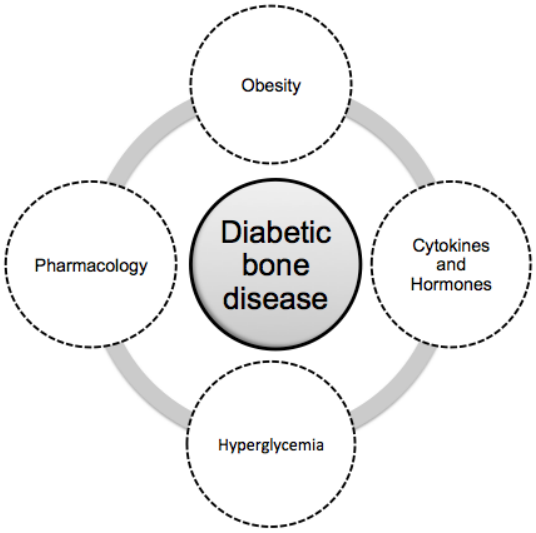
Figure 1.4 Biological factors involved in the pathophysiology of osteoporosis.



1.6 Pathophysiology of Diabetic Bone Disease

In this thesis, diabetic bone disease refers to the paradigm of elevated BMD and elevated fracture risk in adults with type 2 diabetes compared to controls without type 2 diabetes. There are various mediators potentially involved in the pathophysiology of diabetic bone disease (Figure 1.5).

Figure 1.5 Potential mediators involved in the pathophysiology of diabetic bone disease.



1.6.1 Obesity-Related Mediators

A high BMI is a strong predictor of BMD in adults with type 2 diabetes (Ma et al., 2012; Vestergaard, 2007). A greater proportion of Canadians with type 2 diabetes are in the obese category (BMI ≥ 30 kg/m^2) compared to the non-obese category, which may explain why BMD is elevated particularly at weight-bearing sites (Tjepkema, 2005). The mechanism may involve increased mechanical

loading due to weight-bearing and dynamic loading at the proximal femur and spine (Aloia, McGowan, Vaswani, Ross, & Cohn, 1991; Felson, Zhang, Hannan, & Anderson, 1993; Forwood & Turner, 1995; Kanazawa, Yamamoto, Yamauchi, Yano, & Sugimoto, 2008; Lanyon & Rubin, 1984; Ozcivici et al., 2010). Obesity is also implicated in fracture risk, where fracture risk is elevated in individuals with high BMI (Nielson et al., 2011; Pirro et al., 2010). However, reduced physical function in obese individuals may contribute to this elevated fracture risk (Nielson et al., 2011).

1.6.2 Hyperglycemia-Related Mediators

High glucose concentration inhibits the differentiation of bone marrow stromal cells into osteoblasts, osteocalcin secretion, and mineralization (Guan et al., 2009; Inaba et al., 1995; Kim, Kim, & Kim, 2009). In humans, elevated HbA1c reduces bone formation and resorption (Clowes, Allen, Prentis, Eastell, & Blumsohn, 2003; Shu et al., 2012), impairs renal calcium absorption and increases urinary calcium excretion, leading to negative calcium balance in the blood (McNair et al., 1979; Takizawa et al., 2008). Improvement of glycemic control normalizes urinary calcium excretion (Gregorio, Cristallini, Santeusanio, Filipponi, & Fumelli, 1994). There is no consensus on the effect of hyperglycemia on BMD in humans (Bridges, Moochhala, Barbour, & Kelly, 2005; Majima et al., 2005; Vestergaard, 2007), but studies suggest that hyperglycemia (Gagnon et al.,

2010) and hypoglycemia (Schwartz et al., 2008) are risk factors for falls and fractures.

A consequence of systemic hyperglycemia is the production of advanced glycation end-products (AGEs). AGEs are formed through the Maillard reaction where a reducing sugar (*i.e.,* glucose) reacts with an amino residue on a protein to form a glycated end-product (Dyer, Blackledge, Thorpe, & Baynes, 1991; Monnier & Cerami, 1983). Two chemically well-defined AGEs, pentosidine (Grandhee & Monnier, 1991) and *N*ε-carboxymethyl-lysine (Reddy, Bichler, Wells-Knecht, Thorpe, & Baynes, 1995), are elevated in adults with type 2 diabetes and are associated with diabetes complications (Boehm et al., 2004; Monnier et al., 1999; Saxena et al., 1999). *In vitro,* AGE-modification of type 1 collagen inhibits the maturation of preosteoblasts to osteoblasts and osteoblast proliferation (Katayama, Celic, Nagata, Martin, & Findlay, 1997; McCarthy, Etcheverry, & Cortizo, 2001; McCarthy, Uemura, Etcheverry, & Cortizo, 2004). AGEs also bind to receptors on osteoblasts and trigger apoptosis, causing bone loss in diabetic rats (Alikhani et al., 2007; Stolzing, Sellers, Llewelyn, & Scutt, 2010). AGEs also stimulate the production of interleukin-6, which enhances osteoclast activity (Dong, Qin, Xu, & Wang, 2011; Katayama, Akatsu, Yamamoto, Kugai, & Nagata, 1996; Miyata et al., 1997; Takagi et al., 1997). The accumulation of AGEs in bone may also be detrimental to skeletal health, as pentosidine has been identified in bone and may contribute to a reduction in bone mechanical integrity (Hernandez et al., 2005; Vashishth et al., 2001). Higher

levels of urinary pentosidine have been identified in type 2 diabetic adults with fractures (Schwartz et al., 2009), suggesting that AGEs may be involved in type 2 diabetes-related skeletal fragility.

1.6.3 Cytokine and Hormone-Related Mediators

Cytokines are proteins that influence the action of cells in an autocrine, paracrine and endocrine manner. When produced by adipocytes, cytokines are called adipokines, and one of the first adipokines to be discovered, leptin, may be involved in the pathogenesis of diabetic bone disease (Coppack, 2001). In obese adults and adults with type 2 diabetes, levels of leptin are higher than non-obese, non-diabetic adults (Kanabrocki et al., 2001). Leptin interacts with bone when in circulation, and through activation of the sympathetic nervous system. *In vitro,* leptin promotes osteoblast differentiation, proliferation and collagen synthesis and inhibits osteoclastogenesis (Gordeladze, Drevon, Syversen, & Reseland, 2002; Hamrick et al., 2005; Holloway et al., 2002). In humans, leptin is an independent determinant of BMD at weight-bearing sites (Vasilkova, Mokhort, Sharshakova, Hayashida, & Takamura, 2011) and the distal radius, after adjustment for BMI and HbA1c (Tamura et al., 2007). Complicating the perception that higher levels of leptin may be involved in increasing BMD in obese adults and adults with type 2 diabetes, leptin-deficient *ob/ob* mice have higher BMD compared to wildtype mice (Ducy et al., 2000). When leptin treatment is administered through intracerebroventricular infusion, bone loss occurs (Ducy et al., 2000), suggesting

that there are opposite skeletal effects resulting from peripheral and sympathetic nervous system treatment with leptin. In adults with type 2 diabetes, it has been speculated that central leptin resistance explains elevated BMD in the face of elevated leptin levels, but this remains controversial.

Chronic low-grade inflammation is a common feature of obesity and newly diagnosed type 2 diabetes (Fried, Bunkin, & Greenberg, 1998; Katsuki et al., 1998; Rodriguez-Moran & Guerrero-Romero, 1999; Temelkova-Kurktschiev, Henkel, Koehler, Karrei, & Hanefeld, 2002). Inflammatory cytokines such as interleukin-6, tumor necrosis factor-α, and C-reactive protein favour bone resorption (Bertolini, Nedwin, Bringman, Smith, & Mundy, 1986; Manolagas, 1998; Mukai et al., 2007). *In vitro* studies have revealed that interleukin-6 and tumor necrosis factor-α promote bone resorption by increasing osteoclastogenesis and increasing the activity of osteoclasts (Bertolini et al., 1986; Manolagas, 1998). Tumor necrosis factor-α also reduces bone formation by inhibiting osteoblast development and promoting osteoblast apoptosis (Mukai et al., 2007). In humans, elevated inflammatory factors increase the risk of fracture (Cauley et al., 2007).

Estrogen and insulin-like growth factor-1 (IGF-1) may be involved in the pathogenesis of diabetic bone disease. Estrogen promotes osteoblast development and proliferation, and inhibits bone resorption (Fujita et al., 2002; Kameda et al., 1997; Okazaki et al., 2002). Elevated whole-body adiposity and marrow adiposity increases the aromatization of androgens (Longcope, Baker, &

Johnston, 1986), leading to higher levels of circulating estrogen. Men and postmenopausal women with type 2 diabetes often have higher levels of estrogen (Ding, Song, Malik, & Liu, 2006), which may explain elevated BMD associated with obesity and type 2 diabetes (Khosla et al., 1998).

IGF-1 is an important local regulator of bone cell function. IGF-1 is secreted by osteoblasts (Nakasaki et al., 2008) and acts in a paracrine manner on the IGF-1 receptors located on osteoblasts (Middleton, Arnott, Walsh, & Beresford, 1995) and osteoclasts (Hou, Sato, Hofstetter, & Foged, 1997; Middleton et al., 1995). Through stimulating osteoblast recruitment and matrix deposition (Hock, Centrella, & Canalis, 1988; McCarthy, Centrella, & Canalis, 1989) and promoting osteoclast recruitment and activation (Hill, Reynolds, & Meikle, 1995; Mochizuki et al., 1992), IGF-1 maintains coupling between osteoblasts and osteoclasts. Lower levels of IGF-1 have been reported in adults with type 2 diabetes, and levels of IGF-1 are inversely related to the duration of type 2 diabetes and fracture (Jehle, Jehle, Mohan, & Bohm, 1998; Kanazawa, Yamaguchi, & Sugimoto, 2011a, 2011b). Levels of IGF binding protein-3 and IGF binding protein-5, which stimulate the action of IGF-1, are also lower in adults with type 2 diabetes and inversely related to diabetes duration (Jehle et al., 1998).

1.6.4 Pharmacologic-Related Mediators

Clinical practice guidelines in Canada recommend the use of metformin, an oral anti-hyperglycemic agent, as first line treatment for the pharmacologic management of type 2 diabetes (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008). *In vitro* evidence suggests that metformin is osteogenic, stimulating the proliferation and activity of osteoblasts (Cortizo, Sedlinsky, McCarthy, Blanco, & Schurman, 2006) and inhibiting osteoclast-mediated bone resorption (Lee et al., 2010). After ovariectomy, metformin prevents bone loss in rats by increasing bone formation and reducing resorption (Mai et al., 2011), resulting in an increase in BMD (Gao, Li, Xue, Jia, & Hu, 2010). In humans, metformin treatment does not affect femoral neck or lumbar spine BMD (Borges et al., 2011), nor is it associated with higher or lower fracture risk (Monami, Lamanna, Marchionni, & Mannucci, 2008).

If the glycemic target of HbA1c < 9.0% cannot be attained with metformin, an insulin-sensitizing thiazolidinedione (TZDs), such as rosiglitazone or pioglitazone, can be added to the management regime (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008). TZDs are selective ligands for the transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ), which is highly expressed in adipose tissue (Yki-Jarvinen, 2004). A meta-analysis revealed that elevated fracture risk with TZD use was more prominent in women compared to men, and lumbar spine and hip BMD loss was greatest in TZD users (Loke, Singh, & Furberg, 2009). Upon PPAR-γ

activation by TZDs, mesenchymal stem cells are preferentially differentiated into adipocytes instead of osteoblasts, which chiefly explains reduced bone formation in TZD users (Gruntmanis et al., 2010; Sottile, Seuwen, & Kneissel, 2004). Reduced osteoblast differentiation, increased osteoblast and osteocyte apoptosis and increased osteoclastogenesis may also explain the adverse skeletal effects observed in adults with type 2 diabetes using TZDs (Ali et al., 2005; Soroceanu et al., 2004; Wan, Chong, & Evans, 2007).

Insulin is recommended in the pharmacologic management of type 2 diabetes when patients with longer-standing type 2 diabetes have difficulty controlling hyperglycemia with oral anti−hyperglycemics (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008). When insulin binds to receptors on osteoblasts (Thomas, Hards, Rogers, Ng, & Best, 1996), there is an increase in osteoblast replication, collagen synthesis and mineral apposition rate (Hickman & McElduff, 1989; Kream, Smith, Canalis, & Raisz, 1985; Verhaeghe et al., 1992). When insulin binds to receptors on osteoclasts (Thomas et al., 1998), maturation of osteoclasts is reduced and the expression of the RANK receptor is suppressed, reducing osteoclast-mediated bone resorption (Huang et al.). In humans, insulin levels are positively associated with radius and spine BMD after adjustment for BMI, estrogen use, and physical activity level (Barrett-Connor & Kritz-Silverstein, 1996). Although insulin appears to have a protective effect on bone, prospective studies indicate that adults with type 2 diabetes prescribed insulin are at greater risk of fracture compared to adults with

type 2 diabetes not prescribed insulin (Ahmed, Joakimsen, Berntsen, Fonnebo, & Schirmer, 2006; Forsen L, 1999; Janghorbani, Feskanich, Willett, & Hu, 2006; Lipscombe, Jamal, Booth, & Hawker, 2007). In a large cohort study using a provincial database, Lipscombe and colleagues found that the risk of fracture associated with type 2 diabetes increased by 20-40% when the cohort was stratified by insulin use (Lipscombe et al., 2007). However, adjustment for insulin use does not entirely explain the elevated fracture risk in adults with type 2 diabetes (Bonds et al., 2006). The mechanism linking insulin therapy to increased fracture risk is not known, but it may be that insulin use serves as a surrogate for disease duration and severity (UK Prospective Diabetes Study Group [UKPDS], 1998).

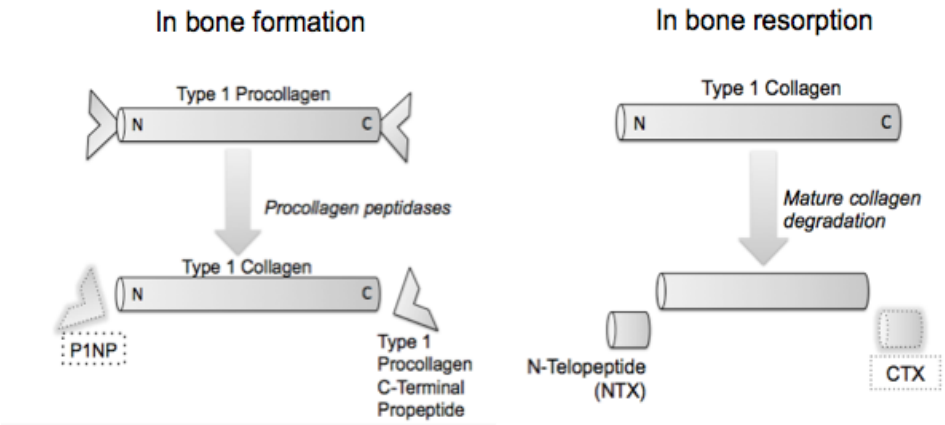
1.7 Assessment of Bone Status: Bone Turnover Markers

Bone turnover markers provide insight into the pathophysiology of osteoporosis and disease-related bone loss, as levels reflect the metabolic activity of osteoblasts and osteoclasts (Bonde, Fledelius, Qvist, & Christiansen, 1996; Garnero, Vergnaud, & Hoyle, 2008). Postmenopausal women have elevated levels of bone turnover markers compared to men (Ebeling et al., 1996; Garnero, Sornay-Rendu, Chapuy, & Delmas, 1996; Recker, Lappe, Davies, & Heaney, 2004). Higher levels of bone turnover markers predict fracture in women (Garnero, Hausherr, et al., 1996; Sornay-Rendu, Munoz, Garnero, Duboeuf, & Delmas, 2005) and men (Bauer et al., 2009). A number of national and

international organizations have recommended the use of serum procollagen type 1 N-terminal propeptide (P1NP) and C-terminal cross-linking telopeptide of type 1 collagen (CTX) as reference standards for bone formation and resorption, respectively (Brown et al., 2009; Vasikaran et al., 2011).

Procollagen is secreted by osteoblasts during bone formation and is cleaved in the process, sending P1NP into circulation, making it a marker of bone formation. During bone resorption, type 1 collagen cross-links are degraded, causing CTX to be released into serum and urine, marking bone resorption (Brown et al., 2009; Herrmann & Seibel, 2008) (Figure 1.6).

Figure 1.6 The generation of procollagen type 1 N propeptide (P1NP) fragment from procollagen molecule during bone formation process, and C-terminal cross-linking telopeptide of type 1 collagen (CTX) from type 1 collagen molecule during bone resorption.



Other bone turnover markers have been identified, however their value is compromised because of various biologic and external factors causing measurement variability, lack of quality reference data, and poor quality control of measurement technique (Brown et al., 2009; Vasikaran et al., 2011). While the

use of bone turnover markers is not recommended for the diagnosis and management of osteoporosis in all provinces in Canada (Papaioannou et al., 2010), bone turnover markers can aid in understanding the pathophysiology of bone diseases (Szulc & Delmas, 2008).

1.7.1 Bone Turnover in Adults with Type 2 Diabetes

A summary of cross-sectional studies that have explored levels of P1NP and CTX in the last decade in adults with type 2 diabetes can be found in Table 1.1.

Table 1.1 A summary of studies reporting levels of bone turnover markers (P1NP and CTX) in adults with type 2 diabetes.

Year	First Author	Sample size (T2D/CON) T2D age, years Mean (SD) Or median (IQ range)	Duration of T2D, years, Mean (SD)	CTX levels in T2D versus control	P1NP levels in T2D versus control
2012	Garcia Martin	74/50 57.7 (6.5)	13.5 (7.5)	↓	NA
2012	Shu	14/14 63.4 (7.0)	8.5 (7.0)	=	↓
2011	Reyes-Garcia	78/55 55.1 (39-66)	13.3 (7.6)	↓	NA
2011	Iglesias	20/24 61.3 (12.0)	Newly diagnosed	=	=
2006	Oz	52/48 53.9 (6.0)	4.75 (5.0)	↓	NA
2006	Dobnig	583/1081 82.8 (5.9)	NA	↓	NA
2005	Gerdhem	67/961 > age 75	9.8	↓	NA

* *Abbreviation:* interquartile, IQ; type 2 diabetes, T2D; standard deviation, SD; not assessed, NA.
= indicates no difference in levels between type 2 diabetes and control group.

In most studies, men and women with type 2 diabetes had lower levels of serum CTX compared to age-matched controls without type 2 diabetes (Dobnig et al., 2006; Garcia-Martin et al., 2012; Gerdhem, Isaksson, Akesson, & Obrant, 2005; Oz et al., 2006; Reyes-Garcia et al., 2011). P1NP, which has been less thoroughly investigated in adults with type 2 diabetes than CTX, also appears to be lower in women with type 2 diabetes compared to controls (Shu et al., 2012). A smaller study reported no difference in P1NP levels in adults with type 2 diabetes compared to normoglycemic adults, but this lack of difference may be due to small sample size (Iglesias et al., 2011). When considering other bone turnover markers, bone remodeling is suppressed in both men and women with type 2 diabetes (Achemlal et al., 2005; Akin, Gol, Akturk, & Erkaya, 2003; Cakatay et al., 1998; Cloos et al., 1998; Dobnig et al., 2006; Gerdhem et al., 2005; Iglesias et al., 2011; Oz et al., 2006; Shu et al., 2012; Takizawa et al., 2008).

There is discordance between the evidence linking the proposed biochemical mediators of diabetic bone disease to skeletal health, suppression of bone turnover markers, and the epidemiological evidence for elevated BMD in adults with type 2 diabetes. A hypothesis to explain this discordance is that the importance of each mediator may vary depending on the stage of type 2 diabetes development and progression (Table 1.2).

Table 1.2 Potential biochemical mediators of diabetic bone disease in adults with type 2 diabetes

Mediator	Effect on osteoblasts	Effect on osteoclasts	Is bone formation or resorption favoured?
Early in disease… uncoupling of bone formation and resorption			
Elevated leptin	↑	↓	**Favours bone formation**
Elevated estrogen	↑	↓	**Favours bone formation**
Hyperinsulinemia	↑	↓	**Favours bone formation**
Metformin use	↑	↓	**Favours bone formation**
Elevated AGEs	↓	↑	Favours bone resorption
Elevated inflammatory cytokines	↓	↑	Favours bone resorption
Thiazolidinedione use	↓	↑	Favours bone resorption
Later in disease… suppression of bone formation and resorption			
Persistent hyperglycemia	↓	↓	**Neither**
Suppressed IGF-1, IGF binding proteins-3 and 5	↓	↓	**Neither**

Before impaired glucose tolerance arises in obese individuals, adiposity and the associated rise in estrogen and leptin may be the predominant factors increasing bone formation and BMD. As impaired glucose tolerance develops, hyperglycemia will result. An adult can live with hyperglycemia for many years prior to a diagnosis of type 2 diabetes (Tabak et al., 2009), which may promote the formation of AGEs and increases inflammatory cytokine levels, favouring bone resorption. This may be the phase where some bone destruction occurs, however BMD levels remain above normal. The negative effects of hyperglycemia may be blunted in some individuals because hyperinsulinemia, which favours bone formation, is stimulated by hyperglycemia. If insulin resistance is not improved and adults with impaired glucose tolerance develop

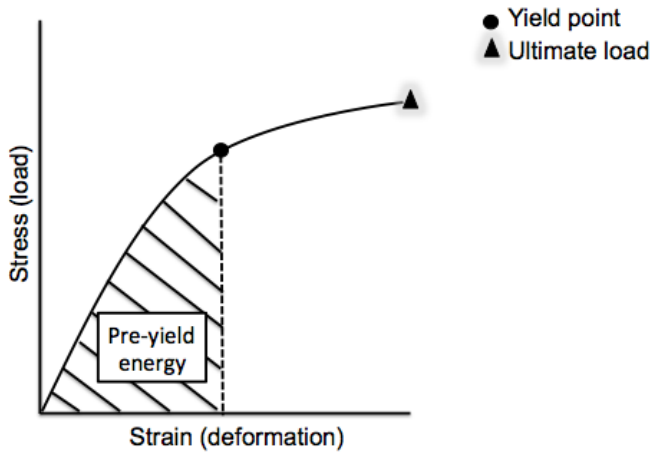
type 2 diabetes, treatment with metformin may have an osteogenic effect on bone, although *in vivo* data supporting this is lacking. If glycemic targets are not met, TZDs may be prescribed and contribute to bone destruction early in the development of type 2 diabetes. Treatment with insulin later in the disease may counteract the negative effect of TZDs, increasing BMD. Regardless of when each factor plays a role in the pathophysiology of diabetic bone disease, the overall net effect must favour bone formation because BMD is elevated in adults with type 2 diabetes.

The majority of the data on bone turnover markers in adults with type 2 diabetes is from adults with longer-standing type 2 diabetes (*i.e.,* greater than 5 years). These data suggest that bone remodeling is suppressed. The factors that could be driving suppressed bone turnover in adults with longer-standing type 2 diabetes include persistent hyperglycemia, reduced insulin sensitivity and low levels of IGF-1. Hyperglycemia is related to suppressed P1NP (Shu et al., 2012) and CTX (Achemlal et al., 2005), and poor insulin sensitivity, a marker of disease severity, is related to lower levels of bone turnover markers (Basu, Peterson, Rizza, & Khosla, 2011). Lower levels of IGF-1 and IGF-binding protein-3 are related to lower bone formation (Jehle et al., 2003) and resorption (Jehle et al., 1998).

1.8 Beyond BMD: Introduction to *Bone Quality*

There are various skeletal factors that modulate bone strength and fracture risk. In biomechanical terms, a fracture occurs at the point of ultimate load when bone is unable to absorb energy, or when the factor-of-risk, defined as the ratio of applied forces to bone strength, exceeds one (Bouxsein et al., 2006). The yield point, or elastic limit, is the point where irreversible damage occurs within the bone, and, depending on the material composition and geometric properties of the bone, the strain that can be endured for a given stress before the yield point is reached varies (Figure 1.7).

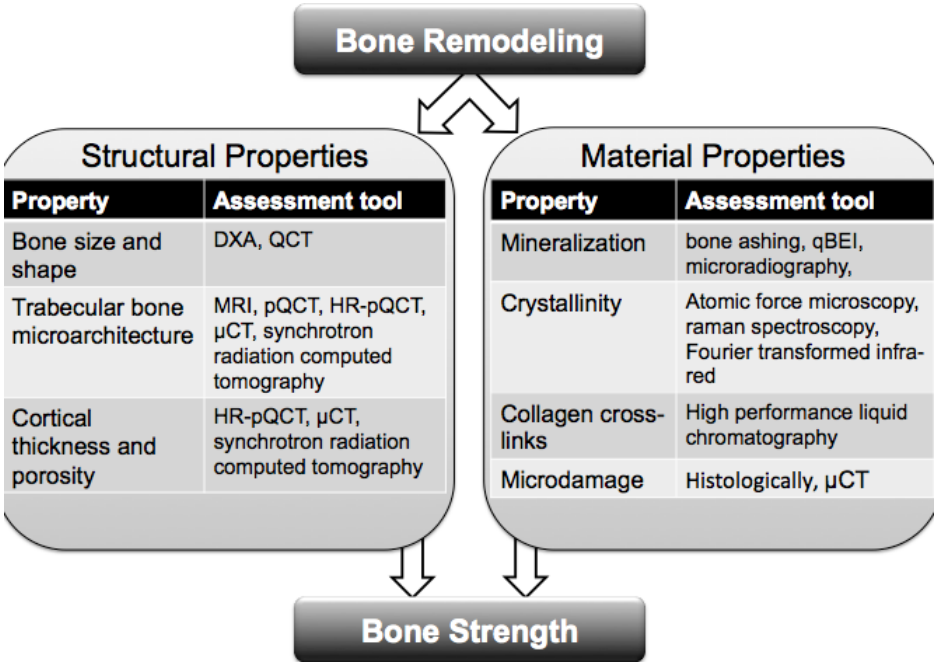
Figure 1.7 Stress-strain curve for normal bone.



Skeletal factors controlling bone strength can be classified as material or structural, both of which are influenced by the activity of osteoblasts and osteoclasts and the rate of bone remodeling. Some of the important structural characteristics are bone size and shape, trabecular bone microarchitecture and cortical bone thickness and porosity (Boonen et al., 1995; Faulkner et al., 1993; Gordon, Webber, & Nicholson, 1998; Parfitt et al., 1983; Zebaze et al., 2010).

The intrinsic material properties of bone include the accumulation or removal of microdamage, mineralization and crystallinity, collagen denaturation and covalent cross-links between collagen microfibrils (Mashiba et al., 2000; Paschalis, Betts, DiCarlo, Mendelsohn, & Boskey, 1997; Saito, Fujii, Mori, & Marumo, 2006; Zioupos, Currey, & Hamer, 1999). These skeletal factors are examples of *bone qualities* and are components of the Bone Quality Framework (Figure 1.8) (Felsenberg & Boonen, 2005).

Figure 1.8 The *Bone Quality* Paradigm and characteristics that can be classified as material or structural *bone qualities*. *Adapted from (Chappard, Basle, Legrand, & Audran, 2011; Davison et al., 2006).*



According to a consensus reached by experts at the National Institutes of Health Bone Quality Conference, *bone quality* is an umbrella term for characteristics of bone that influence bone's resistance to fracture (Fyhrie, 2005).

Others have expanded on this definition and define *bone qualities* as properties that influence the “unpredicted portion of fracture risk” in fracture risk assessment tools relying on BMD (Fyhrie, 2005).

Therefore, elevated fracture risk in adults with type 2 diabetes may be explained by impairments in other *bone qualities* that are not assessed by DXA.

1.9 Assessing a Structural *Bone Quality*: Trabecular Bone Microarchitecture

Trabecular (cancellous) bone is made up of individual trabeculae, which are approximately 100-300 micrometers (μm) wide (Whitehouse & Dyson, 1974). This porous structure resembles a honeycomb of mineralized trabeculae in the shape of rod-like structures connected by plate-like structures. Compared to the diaphysis of long bones, trabecular bone is present in greater volumes at the epiphysis and metaphysis. In relation to total bone volume, the femoral neck is approximately 50% trabecular bone, distal radius is 50-70% trabecular bone, and vertebral bodies are 70-80% trabecular bone (Dempster, 2006; Nilas, Norgaard, Podenphant, Gotfredsen, & Christiansen, 1987). The spatial arrangement of trabeculae is known as trabecular bone microarchitecture.

Trabecular bone microarchitecture is traditionally assessed by iliac crest bone biopsy. Two-dimensional histomorphometric measurements of trabecular bone can be made on sections of bone using the parallel-plate method, as described by Whitehouse in 1974 (Whitehouse, 1974). Interest was sparked in this field when trabecular bone microarchitecture at the iliac crest appeared to be

less intact in older individuals (Compston, Mellish, & Garrahan, 1987), and in women with fractures compared to women without fractures (Chappard, Alexandre, & Riffat, 1988; Parfitt et al., 1983). These early histomorphometry studies suggested that trabecular bone microarchitecture might be important in understanding skeletal fragility. Ensuing *ex vivo* biomechanical and finite element modeling studies demonstrated that trabecular bone microarchitecture indeed contributes to bone strength. Approximately 70-90% of the variability in the elastic modulus (Young's modulus) of bone can be explained by bone volume fraction (BVTV, or BV/TV), a component of trabecular bone microarchitecture (Newitt, Majumdar, et al., 2002; Ulrich, van Rietbergen, Laib, & Ruegsegger, 1999). In a study using excised bone cubes from the calcaneus, 67% of elastic modulus was explained by BVTV, and this increased to 78% when the number of trabeculae (Tb.N) was added to linear regression with BVTV (Ulrich et al., 1999). In a study using cadaveric radii, BMD accounted for 50% of bone strength, and the size of the trabecular bone holes explained an additional 25% of the variability in strength (Gordon, Webber, et al., 1998). Trabecular bone microarchitecture may contribute less to overall bone strength at cortical bone rich-sites, such as the diaphysis of the tibia, however, these studies demonstrate that trabecular bone microarchitecture is important to consider at trabecular bone rich sites, such as the distal radius.

Technological advances have facilitated the non-invasive assessment of trabecular bone microarchitecture. Micro-computed tomography (μCT)

(Feldkamp, Goldstein, Parfitt, Jesion, & Kleerekoper, 1989), synchrotron radiation computed tomography (SRCT) (Peyrin et al., 2001), peripheral quantitative computed tomography (pQCT) (Muller, Hildebrand, Hauselmann, & Ruegsegger, 1996), and magnetic resonance imaging (MRI) can be used to image and measure trabecular bone microarchitecture (Wehrli et al., 1998). Due to high levels of radiation exposure with μCT and SRCT, these methods are presently reserved for use in rodent and *ex vivo* studies only. Non-invasive *in vivo* imaging of human trabecular bone is most commonly performed with pQCT, high-resolution pQCT (HR-pQCT) and MRI. Although pQCT and MRI have various qualities in common, such as the ability to scan peripheral sites (*i.e.,* distal radius, distal tibia) with reasonable scan time (3-12 minutes), there are notable differences in these methodologies.

1.9.1 Assessment with pQCT

HR-pQCT and pQCT are suitable tools for investigating trabecular bone microarchitecture because they produce images of bone at appendicular sites with low nominal voxel size (three-dimensional element making up a digital image, 82-165μm^3) (Boutroy, Bouxsein, Munoz, & Delmas, 2005; Muller et al., 1996). Quantitative computed tomography provides volumetric density, geometric metrics and indirect measures of bone strength in addition to trabecular bone microarchitecture measurements (Table 1.3) (Boutroy et al., 2005; Gordon, Webber, et al., 1998; Kontulainen et al., 2008; Muller et al., 1996).

Table 1.3 Trabecular bone microarchitecture measurements that can be obtained from pQCT and HR-pQCT images.

Measure	pQCT	HR-pQCT
Cortical volumetric BMD (g/cm^3)	✓	✓
Trabecular volumetric BMD (g/cm^3)	✓	✓
Bone volume fraction (BVTV)	✓	✓
Trabecular Thickness (Tb.Th)	✓	✓
Trabecular Number (Tb.N)	✓	✓
Trabecular Separation (Tb.Sp)	✓	✓
Trabecular bone hole characteristics (size, #)	✓	NA
Cortical thickness (site-dependent)	✓ ×	✓
Cortical porosity	×	✓
Cross-sectional area	✓	✓
Stress-strain/stability index (SSI) or other surrogates of bone strength	✓	✓

* *Abbreviation:* trabecular bone volume fraction, BVTV; trabecular number, Tb.N; trabecular separation, Tb.Sp; trabecular thickness, Tb.Th; not available, NA (signifies no literature to support)

When compared to µCT, strong correlations exist for HR-pQCT derived BVTV, Tb.N, and trabecular separation (Tb.Sp) in cadaver radii (MacNeil & Boyd, 2007). The reproducibility of density and trabecular bone microarchitecture measures with pQCT and HR-pQCT is satisfactory, reflected by low precision errors reported in reproducibility studies (Boutroy et al., 2005; Szabo et al., 2010). There are limitations with using pQCT and HR-pQCT for clinical research. There is radiation exposure associated with the scan, although some consider this dose (3–4 µSv) to be negligible (Boutroy et al., 2005; Burghardt et al.). The scans are also susceptible to motion artifact causing measurement error (Boutroy et al., 2005). Beam hardening can produce inaccurate measures if not corrected using post-processing algorithms (Fajardo et al., 2009). Because trabeculae are on the same order of magnitude as image resolution, partial volume effects occur which

reduces measurement accuracy (Kothari et al., 1998). Increasing image resolution comes with higher radiation exposure, operating costs, scan time and processing time for image analysis (Tjong, Kazakia, Burghardt, & Majumdar, 2012). Nonetheless, pQCT and HR-pQCT have been employed by numerous research groups around the world to explore changes in cortical and trabecular bone microarchitecture during growth and in different disease states (Brancaccio et al., 2003; Dalzell et al., 2009; Kirmani et al., 2009; Kroger et al., 1999).

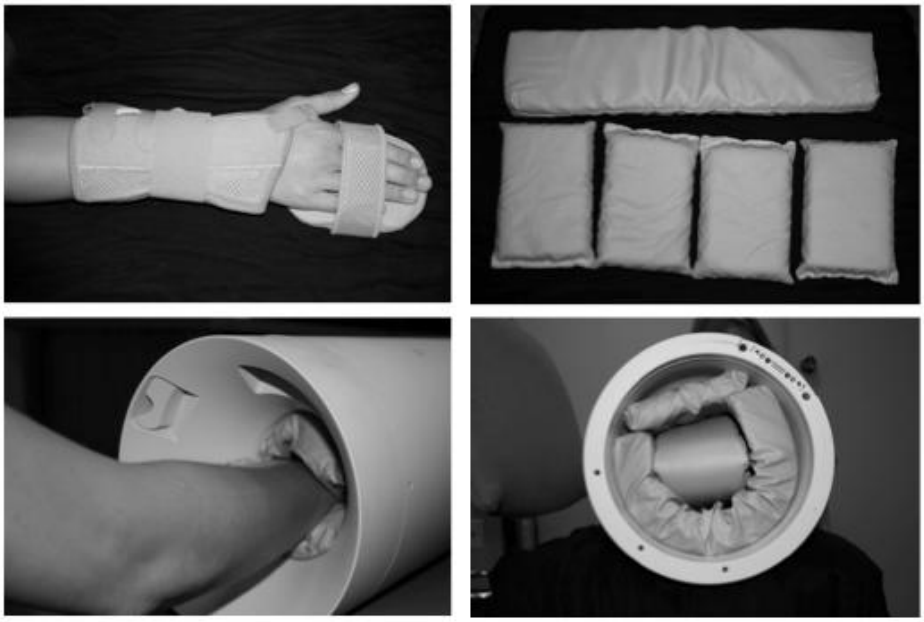
1.9.2 Assessment with MRI

MRI is an attractive tool for assessing trabecular bone microarchitecture at appendicular sites (*i.e.,* distal radius, distal tibia and calcaneus) and at the proximal femur. There is no radiation exposure and the images generated have superior signal-to-noise ratio compared to HR-pQCT and pQCT, allowing for improved differentiation between bone, bone marrow and surrounding tissues (Kazakia et al., 2008; Wehrli et al., 2002). MRI uses a strong, uniform magnetic field (measured in Tesla) to align the nuclear magnetic moments of hydrogen atoms in tissues of the region of the body being scanned. MRI systems with magnetic strengths ranging from 1.0 Tesla to 7.0 Tesla have been used for the assessment of trabecular bone microarchitecture (Iita, Handa, Tomiha, & Kose, 2007; Krug, Carballido-Gamio, Banerjee, et al., 2008). A radiofrequency pulse is used to shift the direction of the nuclear magnetic moments away from the external magnetic field, and when the radiofrequency pulse stops, the nuclei will

reorient themselves and line up once again with the external magnetic field (Brown, 1995). This reorientation, defined as magnetic resonance relaxation is classified as either T_1 or T_2 relaxation times. Relaxation times are influenced by molecular interactions between liquid and solid phases (*i.e.,* dipole-dipole interactions between bone and marrow constituents) and the composition of the tissue (Brown, 1995). Bone has low hydrogen atom density due to low water content and a short T_2 relaxation time resulting in negligible to very low signal output, making bone appear black in MR images (Wehrli et al., 2002). Conversely, fatty marrow (not hematopoietic marrow) has a higher proton density and longer T_2 relaxation time (Fernandez-Seara, Wehrli, & Wehrli, 2003). This produces high signal intensity and allows for visualization of bone tissue indirectly in MR images. Image resolution and voxel size are influenced by a number of factors, including radiofrequency coil size, magnetic field strength, scan time and pulse repetition time (TR) (Wehrli, 2007). Research groups have used different MRI systems with varying image resolutions (*i.e.,* 117 x 117 x 300 μm (Majumdar et al., 1998), 195 x 195 x 500 μm (Link et al., 1998), 195 x 195 x 800 μm (Gordon, Webber, Christoforou, & Nahmias, 1997), 234 x 234 x 1500 μm (Krug et al., 2005)) to assess trabecular bone microarchitecture. Peripheral MRI systems are ideal for imaging trabecular bone because of the ability to select different radiofrequency coils based on the anatomical site being imaged. The smaller the diameter of the coil, the better the signal-to-noise ratio, differentiation between bone and non-bone tissues and image resolution (Wehrli, 2007). The higher the

image resolution, the more important it becomes to prevent motion from causing image artifact. Rotational motion as small as 1° can cause measurement errors as great as 20%, but padding can be applied around the limb of the participant being scanned to prevent motion (Gomberg et al., 2004) (Figure 1.9).

Figure 1.9 An example of a forearm brace and padding used for to reduce motion while scanning the distal radius in a 100 mm radiofrequency MRI coil.



Following scanning, images are obtained, segmented and skeletonized to yield measures of trabecular bone microarchitecture (Table 1.4). This is often completed with in-house software developed at a research institution (Gordon et al., 1997).

Table 1.4 Trabecular bone microarchitecture measurements that can be obtained from MRI images.

Measure	MRI
Cortical volumetric BMD (g/cm^3)	×
Trabecular volumetric BMD (g/cm^3)	×
Bone volume fraction (BVTV)	✓
Trabecular Thickness (Tb.Th)	✓
Trabecular Number (Tb.N)	✓
Trabecular Separation (Tb.Sp)	✓
Trabecular bone hole characteristics (size, #)	✓
Cortical thickness (site-dependent)	×
Cortical porosity	×
Cross-sectional area	✓
Stress-strain/stability index (SSI) or other surrogates of bone strength (dependent on specialized software)	✓×

* *Abbreviation:* trabecular bone volume fraction, BVTV; trabecular number, Tb.N; trabecular separation, Tb.Sp; trabecular thickness, Tb.Th

MRI-derived measures correlate well with measures derived from high-resolution μCT and contribute to the prediction of biomechanical measures of bone strength (Krug, Carballido-Gamio, et al., 2008; Majumdar et al., 1996). The reproducibility of measuring two dimensional trabecular bone microarchitecture variables with MRI varies with imaging system, image resolution and anatomical site of assessment (Table 1.5) (Benito et al., 2005; Gomberg et al., 2004; Newitt, van Rietbergen, & Majumdar, 2002).

Table 1.5 Summary of MRI studies reporting reproducibility measurements for assessment of trabecular bone microarchitecture.

Year	First Author	MRI system	Site	Image resolution (μm)	RMSCV% or CV%
2005	Benito	1.5 Tesla	Distal Tibia	137 x 137 x 410	BVTV- 2.3% Tb.Th- 0.4% SCR- 6.7%
2004	Gomberg	1.5 Tesla	Distal Radius	137 x 137 x 350	BVTV- 4.6%
2004	Gomberg	1.5 Tesla	Distal Tibia	137 x 137 x 410	BVTV- 6.1%
2002	Newitt	1.5 Tesla	Ultra-Distal Radius	156 x 156 x 500	BVTV- 5.2% Tb.N- 4.1% Tb.Sp- 8.3% Tb.Th- 3.4%
2002	Newitt	1.5 Tesla	Distal Radius	156 x 156 x 500	BVTV- 2.2% Tb.N- 2.2% Tb.Sp- 3.2% Tb.Th- 2.2%

* *Abbreviation:* root mean squared coefficient of variation, RMSCV %; coefficient of variation, CV %; trabecular bone volume fraction, BVTV; trabecular number, Tb.N; trabecular separation, Tb.Sp; trabecular thickness, Tb.Th; surface to curve ratio, SCR

There are limitations with using MRI for the assessment of trabecular bone microarchitecture. Chiefly, trabecular and cortical volumetric BMD cannot be obtained with MRI assessment, and achievable image resolution with some MRI systems does not permit assessment of cortical thickness and porosity. There are obstacles associated with improving MR image resolution. For example, in order to increase image resolution and maintain good signal-to-noise ratio to accurately resolve small features like trabeculae, scan time must be increased and radiofrequency coil size decreased, which may not be feasible. Like pQCT and HR-pQCT, MRI is limited by partial volume effects, which overestimate trabecular

bone microarchitecture measures such as Tb.Th (Boutry et al., 2004; Majumdar et al., 1996). Adults with implanted metal devices and pacemakers cannot be scanned because of the strong magnetic field (Shellock & Kanal, 1991). Finally, the size of the gantry of transmit receive coils may only permit assessment of trabecular bone microarchitecture at the distal radius, and not the distal tibia.

Despite limitations, MRI is one of the most widely used tools for assessing trabecular bone microarchitecture. MRI has been used to explore age and gender-related differences in trabecular bone microarchitecture (Hudelmaier et al., 2005; Majumdar et al., 1997; Sode, Burghardt, Kazakia, Link, & Majumdar, 2010). A study using MRI in postmenopausal women with a history of a fracture revealed that BVTV and Tb.N are lower while Tb.Sp is higher compared to postmenopausal women without a fracture (Majumdar et al., 1997). Similarly in men, MRI-derived BVTV and Tb.Sp are strongly related to prevalent fractures (Boutry, Cortet, Dubois, Marchandise, & Cotten, 2003). MRI-derived measures of trabecular bone microarchitecture have also been used to differentiate postmenopausal women with and without vertebral fracture (Link et al., 1998) and some measures such as trabecular bone hole size are better at differentiating postmenopausal women with and without a fracture than volumetric BMD (Gordon, Webber, et al., 1998; MacIntyre, Adachi, & Webber, 2003). Considering that voxel size must be of the same order of magnitude as the feature being examined (in 2 of 3 spatial directions) (Wehrli, 2007), it may be more appropriate to use MRI for measuring features such as trabecular bone hole size (200-2000

μm in diameter) (Amstutz & Sissons, 1969), given that correlations are greater between MRI-derived and μCT-derived measures of larger features and lowest for smaller features, such as Tb.Th (Krug, Carballido-Gamio, et al., 2008).

1.9.2.1 Change in Trabecular Bone Microarchitecture with MRI

Numerous studies have explored changes in trabecular bone microarchitecture at the radius due to osteoporosis-related treatment or hormone replacement in women (Chesnut et al., 2005; Folkesson et al., 2011; Greenspan et al., 2010; Wehrli et al., 2008) and in men (Benito et al., 2005). Two studies report changes in non-traditional metrics of trabecular bone microarchitecture (*i.e.,* surface and curve density, erosion index, measures of anisotropy) and no change in traditional measures (*i.e.,* those derived using the parallel-plate model) after 12 months (Greenspan et al., 2010) and 24 months of bisphosphonate treatment (Folkesson et al., 2011). Other studies report changes in trabecular bone microarchitecture at the distal tibia and not the radius, suggesting that weight-bearing might influence changes in trabecular bone microarchitecture (Folkesson et al., 2011). In addition, in a prospective study over 24 months of hypogonadal men who received testosterone replacement therapy, the largest changes were apparent for surface to curve ratio (+11.2%) and erosion index (-7.5%), compared to BVTV (5%) or Tb.Th (1.5%) despite good reproducibility of BVTV and Tb.Th (Benito et al., 2005). These studies suggest that changes are detectable in some, but not all, trabecular bone microarchitecture measures. The

site of assessment (radius versus tibia) may also influence whether changes are apparent.

In summary, trabecular bone microarchitecture is an important structural *bone quality* to consider in bone fragility. BMD measurements may not be sensitive enough to detect changes in microarchitectural integrity that impact bone strength (Silva & Gibson, 1997). pQCT, HR-pQCT and MRI are suitable tools for the non-destructive measurement of trabecular bone microarchitecture at appendicular sites, such as the distal radius. While these tools are not yet used in clinical practice for assessing trabecular bone microarchitecture and osteoporosis, promising research exists that provides evidence of the validity, reproducibility and feasibility of use in clinical settings in the future. Each imaging modality has strengths and limitations that must be considered when a tool is employed.

1.10 Assessing a Material *Bone Quality:* Bone Mineralization

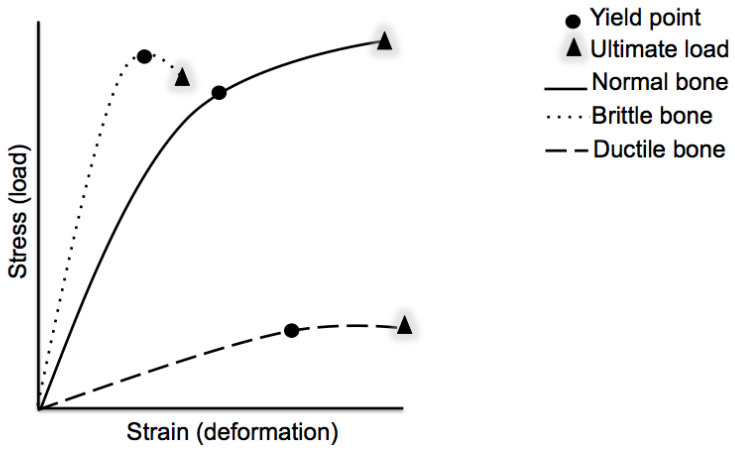
The mechanical behaviour of bone is dependent on material properties, in addition to structural properties. Bone is a composite material, which can absorb energy and provide stiffness for load-bearing strength and resistance to deformation. Bone mineral comprises approximately 50-70% of bone material and is believed to be in the form of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], which aggregates into mineral structures surrounding type 1 collagen fibrils and in the gap zones within the fibrils (McNally, Schwarcz, Botton, & Arsenault, 2012). This

mineral component contributes to bone stiffness and strength by increasing the elastic modulus. The major non-mineral constituent of bone is osteoid, which is synthesized and secreted by osteoblasts during bone formation. Osteoid, comprised of 85-90% type 1 collagen, provides ductility and toughness because of the triple helical structure of type 1 collagen and it's organization in bone (Fantner et al., 2004; Zioupos, 2001). It also acts as a scaffolding for biomineralization, perhaps by providing nucleation sites for crystal formation and subsequent expansion (Boskey, 1998).

The relationship between mineral content and bone strength was highlighted in the early 1980s by Currey (1984), who reported that, for most species, there was a near linear relationship between bending strength and mineral content. However, the tympanic bulla of a fin whale, which has over 80% mineral, had a much lower bending strength, lower work to fracture and higher elastic modulus compared to specimens that were composed of 50% mineral (Currey, 1984). In humans, there are a number of disease states that demonstrate the importance of fine balance between mineral and osteoid in bone. Individuals with osteogenesis imperfecta have a point mutation in type 1 collagen, causing bone to have reduced toughness (Jepsen et al., 1997). In osteopetrosis, a disease characterized by defective osteoclast resorption and elevated BMD (Balemans, Van Wesenbeeck, & Van Hul, 2005), pathologic fractures may be due to elevated bone mineralization. Altering the bone mineral

to matrix ratio is detrimental to optimal skeletal health (Figure 1.10), and it is important to consider bone mineralization as a factor influencing *bone quality.*

Figure 1.10 Stress-strain curve for normal bone, bone with too much mineral content (brittle bone), bone with too little mineral content (ductile bone).



1.10.1 Assessment with Ashing Technique

The amount of mineral in bone can be determined by measuring ash content, calculated by dividing the weight of bone ash (due to incineration) by the original dry weight (Mueller, Trias, & Ray, 1966). This method yields a measurement of the bulk mineral content in bone and is the gold standard for validating other methods for measuring bone mineralization, including BMD (Johanson et al., 1993; Skedros, Bloebaum, Bachus, Boyce, & Constantz, 1993; Vajda EG, 1996). However, because bone samples are incinerated, this method excludes the secondary use of samples. Furthermore, this method does not provide information about bone mineralization at the microscopic level, which may be useful for better understanding skeletal fragility.

1.10.2 Assessment with Microradiography

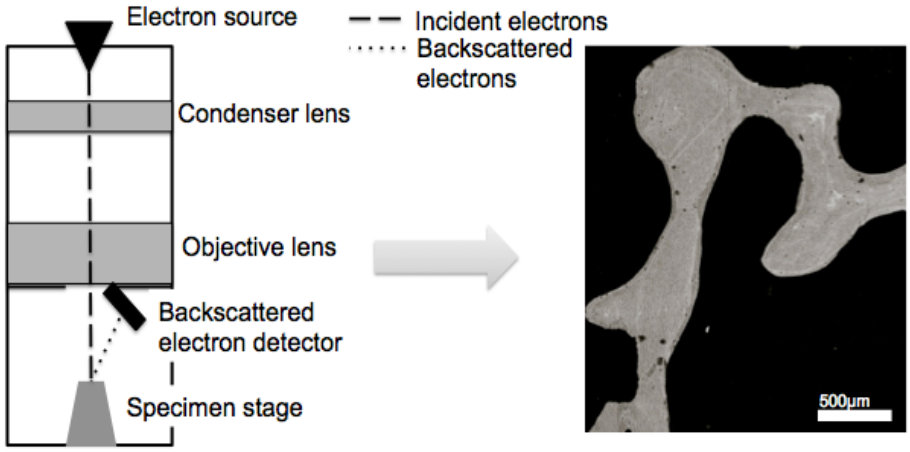
One of the first methods used to study bone mineralization at the microscopic level was microradiography (Jowsey et al., 1965). The basis of this method is that the absorption of x-rays by the bone sample is dependent on the amount of mineral present (Boivin & Meunier, 2002). This method has been improved with technological advances to assess bone mineralization using a combined contact microradiography microdensitometry computerized method (Boivin & Meunier, 2002). Using an x-ray diffraction unit equipped with a diffraction tube, copper Kα radiation is generated and directed at a 100 μm-thick sample of bone, most often from the iliac crest (Boivin & Meunier, 2002; Boivin, Chavassieux, Santora, Yates, & Meunier, 2000). Subsequently, the high-resolution film is exposed, and differences in bone mineralization microradiographs can be seen. Bone packets that are less mineralized appear dark gray, whereas bone packets that are more mineralized are brighter (Boivin & Meunier, 2002). The gray level values obtained from the microradiographs can be converted to measurements of mineral (gram of mineral/cm^3) using a calibration curve of measurements obtained with an aluminum step-wedge (Boivin & Meunier, 2002). The outcomes are the mean degree of mineralization of bone and heterogeneity index, which reflects the uniformity of bone mineralization (Boivin & Meunier, 2002). The intra-observer and inter-observer reproducibility of this method are 3% and 7%, respectively (Boivin & Meunier, 2002; Boivin et al., 2000). Measurement error can occur when bone samples do not have uniform

thickness, which is a limitation associated with the method. A constant thickness of 100 μm is most often used because a thicker section can produce confusing microradiographs with multiple layers of trabecular bone, and thinner sections make differentiating bone from non-bone more difficult (Boivin & Meunier, 2002). To overcome this, some research groups use a tool called a comparator to ensure that samples are precisely 100 μm thick (Boivin & Meunier, 2002). Another limitation is that projection errors can occur at the edges of trabeculae. This so called “edge effect” suppresses the lowest mineralization measurements in the sample, but this effect is minimized with calibration (Boivin & Meunier, 2002). Despite these limitations, microradiography is a popular method for measuring bone mineralization. It has been used for exploring the impact of osteoporosis-related treatment (Boivin et al., 2000; Farlay, Boivin, Panczer, Lalande, & Meunier, 2005) and hormone therapy (Boivin, Vedi, Purdie, Compston, & Meunier, 2005) on bone mineralization in animals and humans.

1.10.3 Assessment with qBEI

This technique is based on analysis of a polished section of bone in a scanning electron microscope (SEM) and capturing the backscattered electrons with a detector in the microscope chamber (Boyde, 2012). A digital gray scale image is produced, and the backscattered electron signal intensity and image gray level are proportional to the average atomic number of the elements present in the specimen (Ball, 1981) (Figure 1.11).

Figure 1.11 Schematic diagram of a scanning electron microscope chamber showing the specimen stage and backscattered electron detector used for quantitative backscattered electron imaging (qBEI) and a resulting gray scale image of bone.



Skedros and colleagues applied this imaging technique to simulated bone tissues with mineral contents ranging from 30-50% mineral by volume, and found that image gray level was highly related to mineral content (Skedros, Bloebaum, Bachus, & Boyce, 1993). Using chick bones, they validated the use of this tool for measuring bone mineralization, reporting strong correlations between weighted mean gray levels of bone in the images and mineral content derived by ashing (Skedros, Bloebaum, Bachus, Boyce, et al., 1993). Other groups have also demonstrated the validity of the qBEI technique for assessing bone mineralization (Bloebaum, Skedros, Vajda, Bachus, & Constantz, 1997).

There are many advantages to using qBEI. This technique is highly reproducible, with low inter-assay and intra-assay technical variance (< 3.5%) (Roschger, Fratzl, Eschberger, & Klaushofer, 1998; Vajda, Skedros, & Bloebaum,

1998). The sensitivity of this technique in detecting changes in mineralization due to disease or treatment is superior to microradiography, with inter-individual variance of 0.3% (Roschger et al., 1998) compared to approximately 10% for microradiography (Boivin & Meunier, 2003). The problem of “edge effects” that arise with microradiography is not a concern with qBEI because the image resolution for qBEI (approximately 5 μm) is better than microradiography (Roschger, Paschalis, Fratzl, & Klaushofer, 2008). However, when employing qBEI for measurement of bone mineralization, care must be taken to address the limitations with the method.

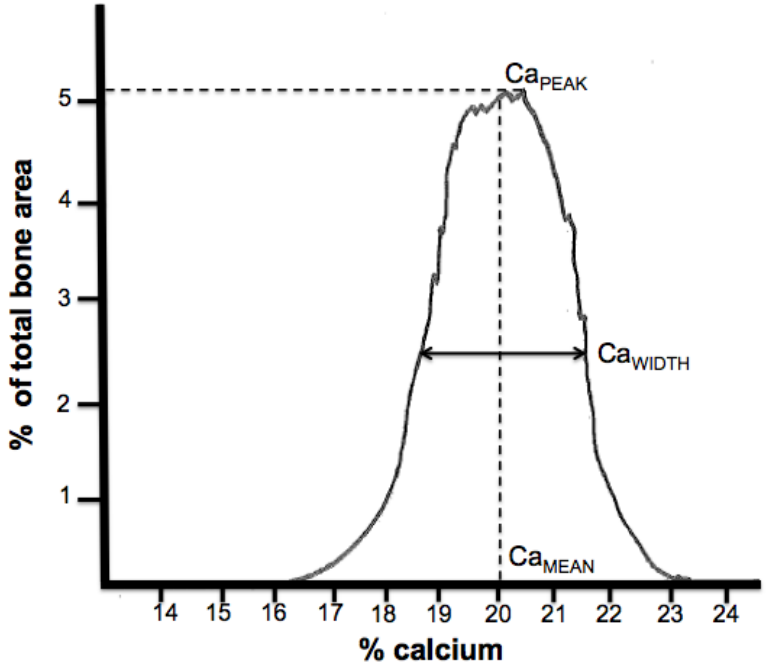
Instrument stability is a concern when using qBEI for measuring bone mineralization. Standards of known average atomic number can be scanned before and after bone samples, and at the start of each imaging session to ensure that microscope variability will not influence mineralization measurements of bone (Roschger et al., 1998). Measuring the same sample on multiple days will allow researchers to quantify the error associated with instrument variability (Roschger et al., 1998). In addition, constant electron beam intensity is important, as large fluctuations (~1pA) are associated with measurement error (Roschger et al., 1998), therefore the use of a picoampere meter is recommended. Care must also be taken to polish the surface of bone samples because surface scratches can increase measurement error (Vajda, Humphrey, Skedros, & Bloebaum, 1999). Finally, when a bone specimen is bombarded by an electron beam at high magnification, “bleaching” of the bone can occur, therefore it is recommended

that magnifications less than 200X be used for qBEI measurement of bone mineralization (Bloebaum, Holmes, & Skedros, 2005).

1.10.3.1 Bone Mineralization Density Distribution (BMDD)

Roschger and colleagues standardized the measurements obtained with qBEI in terms of weight percent calcium values (wt % Ca) to show the relationship between gray level values and actual bone mineral content (Roschger, Plenk, Klaushofer, & Eschberger, 1995). The outcomes obtained with qBEI are derived from a histogram curve of bone calcium concentrations that are present in a bone sample. This histogram has been designated the bone mineralization density distribution (BMDD) curve, and the following outcome measures are derived: 1) Ca_{MEAN}, which is the weighted mean calcium concentration of the bone area imaged (based on weighted mean gray level) (Boyce, Bloebaum, Bachus, & Skedros, 1990), 2) Ca_{PEAK}, which is the peak height of the distribution indicating the most frequently occurring calcium concentration in the area of bone imaged, 3) Ca_{WIDTH}, which is spread of calcium concentration at the full-width half maximum of the distribution, indicating the heterogeneity of mineralization of the area of bone imaged (Roschger et al., 1998; Roschger et al., 2003; Roschger et al., 2008) (Figure 1.12).

Figure 1.12 Typical bone mineralization density distribution (BMDD) curve with mineralization outcomes.

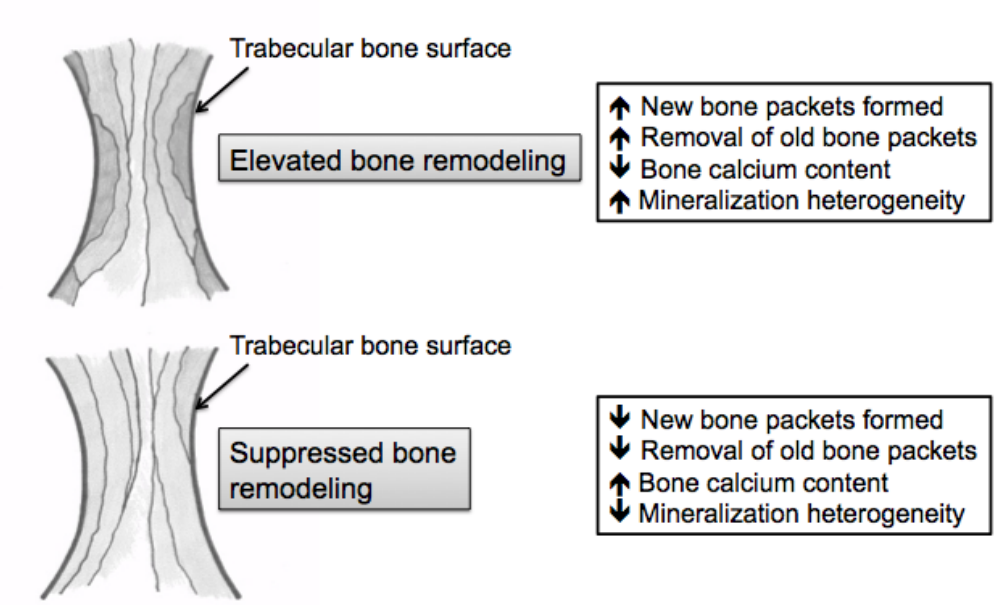


1.10.4 The Link Between Bone Remodeling and Mineralization

In the 1980's, it was suggested that bone mineralization is a reflection of the bone remodeling process (Burnell, Baylink, Chestnut, Mathews, & Teubner, 1982). Since then, histomorphometric measures of bone remodeling have been linked to bone mineralization (Misof et al., 2003; Roschger et al., 2007). These studies verify the theory that bone remodeling influences the age of trabecular bone packets (Ruffoni, Fratzl, Roschger, Klaushofer, & Weinkamer, 2007). Increased bone remodeling leads to the removal of more "old" bone and the formation of more "new" bone. Reduced bone remodeling leads to the removal of less "old" bone and the formation of less "new" bone. Interrupted secondary

mineralization is believed to decrease bone mineralization in the case of increased bone remodeling, whereas prolonged secondary mineralization increases bone mineralization in the case of decreased bone remodeling (Meunier, 1997). Bone remodeling also influences the heterogeneity of mineralization, as bone is more heterogeneous in the case of increased bone remodeling. Bone is less heterogeneous in the case of decreased bone remodeling (Boivin & Meunier, 2003b; Meunier, 1997) (Figure 1.13).

Figure 1.13 Diagram showing the link between bone remodeling and bone mineralization.



Various studies have used qBEI to explore the impact of bone remodeling on bone mineralization at the microscopic level. In patients with mild primary hyperparathyroidism and elevated bone remodeling, Ca_{MEAN} and Ca_{PEAK} are lower, while Ca_{WIDTH} is greater compared to a healthy reference population (Roschger et al., 2007). In postmenopausal women with osteoporosis,

bisphosphonate treatment induces an increase in Ca_{MEAN} and a reduction in Ca_{WIDTH}, indicating that the suppression of bone remodeling with bisphosphonates impacts bone mineralization at the microscopic level (Roschger et al., 2001). Given that bone remodeling is suppressed in adults with type 2 diabetes, measuring bone mineralization with qBEI may be useful for further understanding diabetic bone fragility.

1.11 The Knowledge Gap

Whether structural and material *bone qualities* are different in adults with type 2 diabetes is not known. Studies in rodents suggest that impaired bone structure might be a possible explanation for diabetic bone fragility. Early studies revealed that long-bone width, length, trabecular bone volume and cortical shell cross-sectional area were reduced and cortical porosity increased in diabetic rodents compared to control animals, resulting in reduced overall bone strength (Hou, Zernicke, & Barnard, 1993; Verhaeghe et al., 1994; Verhaeghe, van Herck, et al., 1990). Kawashima and colleagues reported that femur bones were more slender and trabecular bone microarchitecture was less intact (increased Tb.Sp, reduced BVTV) in a mouse model of type 2 diabetes (Kawashima et al., 2009). These studies in rodents provide evidence that structural *bone qualities* may be different in adults with type 2 diabetes. In addition, how structural *bone quality* changes over time in adults with type 2 diabetes is not known.

Studies in rodent models of type 2 diabetes have suggested that diabetic bone is more brittle than non-diabetic bone (Verhaeghe et al., 1994; Verhaeghe, Visser, Einhorn, & Bouillon, 1990). However, bone brittleness was not explained by alterations in bulk mineral content from ash density measurements (Verhaeghe et al., 1994; Verhaeghe, Visser, et al., 1990). Whether material *bone quality* is different on the microscopic level in adults with type 2 diabetes is not known.

1.12 Study Objectives and Hypotheses

The global research objective was to explore structural and material *bone qualities* in adults with type 2 diabetes. The hypothesis was that there are abnormalities in the structural and material properties of bone that are not reflected in BMD measurements and may further explain diabetic bone fragility.

In Chapter Two, structural *bone quality* in postmenopausal women with type 2 diabetes is explored. The aim of this cross-sectional study was to use MRI to determine whether there were differences in trabecular bone microarchitecture, particularly regarding trabecular bone hole size at the distal radius between a group of postmenopausal women with type 2 diabetes and a group of postmenopausal women without type 2 diabetes. The study hypothesis was that trabecular bone hole size would be greater in adults with type 2 diabetes. Other trabecular bone microarchitecture metrics were examined, such as the number of

trabecular bone holes, BV/TV, trabecular thickness, separation, and number, and measures of connectivity (branch density, nodal density).

In Chapter Three, the change in structural *bone quality* in postmenopausal women with type 2 diabetes is explored. In this study, the aim was to compare the prospective changes in trabecular bone microarchitecture in the same groups of postmenopausal women who were enrolled in the first study. The hypothesis was that there would be a greater increase in trabecular bone hole size in women with type 2 diabetes compared to women without diabetes. It was also hypothesized that women with type 2 diabetes would experience greater increases in the number of trabecular bone holes, trabecular separation and branch density, and greater losses in BVTV, trabecular thickness and number and nodal density compared to participants without diabetes.

In Chapter Four, material *bone quality* in men and women with type 2 diabetes is explored. In this study, the aim was to determine whether quantitative backscattered electron imaging derived bone mineralization density distribution measurements are different in femoral neck trabecular bone samples from adults with type 2 diabetes compared to adults without type 2 diabetes. The hypothesis was that Ca_{MEAN} and Ca_{PEAK} would be greater and Ca_{WIDTH} would be lower in adults with type 2 diabetes compared to controls without diabetes.

CHAPTER TWO

AUTHOR'S PREFACE TO CHATPER TWO

In this chapter, we employed a cross-sectional study design and used a 1 Tesla MRI system to describe the differences in trabecular bone microarchitecture in postmenopausal women with and without type 2 diabetes. This work is the first to demonstrate larger trabecular bone holes at the distal radius in postmenopausal women with type 2 diabetes compared to controls of similar age. This study provides a structural *bone quality* explanation for elevated fracture risk observed in women with type 2 diabetes.

The material presented in Chapter Two has been published in the peer-reviewed journal, *Arthritis Care & Research* for the special issue*, Muscle and Bone in the Rheumatic Diseases.* I was responsible for designing the study, recruiting study participants, acquiring the images and data, performing statistical analysis, interpreting the data and composing the manuscript. The expertise of Drs. Giangregorio, Atkinson, Beattie, Punthakee, Adachi and Papaioannou was valuable for designing the study, interpreting data and providing comments on the manuscript. Dr. Inglis assisted with MRI operation training and designed the in-house software (OsteoQ) used to obtain trabecular bone microarchitecture measurements from MRI images. Dr. Ioannidis provided valuable statistical analysis support throughout the study.

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Postmenopausal women with type 2 diabetes have larger holes in the trabecular bone at the distal radius compared to controls

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2.1 ABSTRACT

Objective: Adults with type 2 diabetes have an elevated fracture risk despite normal bone mineral density (BMD). The study objective was to compare trabecular bone microarchitecture of postmenopausal women with type 2 diabetes and women without type 2 diabetes**.**

Methods: An extremity 1-Tesla MRI system was used to acquire axial images (195 x195 x 1000 μm^3 voxel size) of the distal radius of women recruited from outpatient clinics or by community advertisement. Image segmentation yielded geometric, topologic and stereologic outcomes; number and size of trabecular bone network holes (marrow spaces), endosteal area, trabecular bone volume fraction (BVTV), nodal and branch density, apparent trabecular thickness (Tb.Th), separation (Tb.Sp), and number (Tb.N). Lumbar spine (LS), and proximal femur bone mineral density (aBMD) were measured with dual-energy x-ray absorptiometry (DXA). Microarchitectural differences were assessed using linear regression, and adjusted for percent body fat, ethnicity, timed-up-and-go test, Charlson Index, calcium and vitamin D intake. Areal BMD differences were adjusted for body mass index.

Results: Participants with type 2 diabetes [n=30, mean age 71.0(4.8) years] had larger holes (+13.3%, p=0.001) within the trabecular bone network than women without diabetes [n=30, mean age 70.7(4.9) years]. LS aBMD was greater in women with type 2 diabetes, however after adjustment for BMI, LS aBMD did not differ between groups.

Conclusion: In women with T2D, the average hole size within the trabecular bone network at the distal radius is greater compared to controls. This may explain the elevated fracture risk in this population.

2.2 INTRODUCTION

Individuals with type 2 diabetes have an elevated risk of hip, vertebral, proximal humerus, wrist, ankle and foot fractures, despite normal or elevated areal bone mineral density (aBMD) (1,2). Potential factors contributing to elevated aBMD in adults with type 2 diabetes include greater body mass index (BMI), hyperinsulinemia, higher fat mass leading to higher estrogen levels and the presence of diffuse idiopathic skeletal hyperostosis (3-6). Higher than normal aBMD in the presence of elevated fracture risk may limit fracture risk stratification based on aBMD. It also implies that bone strength in those with type 2 diabetes is altered in ways not captured by dual-energy x-ray absorptiometry (DXA) (7).

Bone strength is dependent on material and structural properties, in addition to aBMD and volumetric BMD (vBMD) (8-10). These include bone geometry, morphology, trabecular bone microarchitecture (*ie:* trabecular bone network hole size, trabecular separation, thickness and number) and cortical porosity (*ie:* number and size of cortical bone pores) (8,10,11). Microarchitecture is typically assessed invasively by bone biopsy and histomorphometry, and non-invasively by *in vivo* imaging with quantitative computed tomography (QCT) and magnetic resonance imaging (MRI) (9, 12, 13). Analyses of MRI and QCT scans can yield information about bone microarchitecture, such as trabecular bone volume fraction (BVTV), trabecular number (Tb.N), trabecular separation (Tb.Sp) and trabecular thickness (Tb.Th) (14). While bone microarchitecture measurements obtained from MRI and high-resolution peripheral QCT (HR-

pQCT) are highly related, the advantages of utilizing MRI for the image-based assessment of trabecular bone microarchitecture are superior signal-to-noise ratio between bone and bone marrow, and the lack of radiation associated with scans (15, 16). In addition, small dedicated radio frequency coils are used in MR imaging for enhanced image resolution.

A recent finite element modeling study demonstrated that trabecular bone components contribute 16% to bone strength at the radius (17). MRI-based measures of trabecular bone microarchitecture may provide insight on alterations in bone quality that occur in adults with type 2 diabetes. It has been effective in detecting differences in BVTV, Tb.N, Tb.Th and Tb.Sp in groups differing in osteoporosis diagnosis and fracture history, suggesting a relationship between 2-dimensional variables of trabecular bone microarchitecture derived from MR images and fracture (18, 19). It has been argued however, that the resolution of MR images limits the accurate assessment of such 2-dimensional variables because the images are acquired at the current limits of clinical spatial resolution and can be confounded by partial volume effects (20). However, MR-based assessment of microarchitecture has proven to be promising because these ‘apparent’ 2-dimensional measures derived using MRI correlate well with measures derived by direct histomorphometry and higher resolution imaging (9, 14, 21).

Trabecular bone network hole size may be less affected by image resolution and still provide an assessment of the structural integrity of the

trabecular bone network (22, 23). MacIntyre and colleagues demonstrated that postmenopausal women with a prior wrist fracture had a greater mean hole size at the distal radius compared to non-fracture controls, despite no difference in radial aBMD and other apparent measures of bone strength (24). Therefore, assessing trabecular bone network hole size in women with type 2 diabetes may yield important information about the trabecular bone network.

The purpose of this study was to compare hole size at the distal radius in postmenopausal women with type 2 diabetes to postmenopausal women without type 2 diabetes using MRI. We hypothesized that mean hole size would be greater in patients with type 2 diabetes, despite elevated aBMD, suggesting a more perforated trabecular network at the distal radius. We also explored the impact of type 2 diabetes on other geometric, stereologic and topologic variables, including endosteal area, number of holes within the trabecular network, branch density, nodal density, BVTV, and apparent Tb.N, Tb.Sp and Tb.Th.

2.3 PATIENTS AND METHODS

Study Design

In this cross-sectional study, two groups of postmenopausal women were recruited: a group with a diagnosis of type 2 diabetes; and control group without type 2 diabetes. Individuals with diabetes were recruited from Hamilton Health Sciences Diabetes Clinics in Hamilton, Canada in 2008. Women without diabetes were recruited through poster advertisements at local hospitals, clinics and

community centres in 2009. All study participants were ≥ 65 years and postmenopausal for > 5 years where menopause was defined as 12 months after the cessation of the menstrual cycle. To ensure that women with type 2 diabetes had long-standing disease, we included only those who had a diagnosis ≥ 5 years, by applying the Canadian Diabetes Association diagnostic criteria (25).

Study participants were excluded if they had any one of the following: 1) use of medication in the previous 24 months known to affect bone including hormone therapy (HT), calcitonin, selective estrogen receptor modulator, parathyroid hormone, bisphosphonates; 2) chronic systemic glucocorticoid exposure (≥ 3 months, dose ≥ 2.5 mg/day); 3) history of metastatic cancer in the past 5 years; 4) diagnosis of Paget's disease; 5) untreated malabsorption syndrome; 6) hyperparathyroidism or hypoparathyroidism or; 7) renal impairment (Figure 1). Participants with ferromagnetic implants or pacemakers were excluded in accordance with MRI safety standards. This study was approved by the McMaster University Faculty of Health Sciences/Hamilton Health Sciences Research Ethics Board.

Medical history and lifestyle data

Participants completed a series of interviewer-administered questionnaires to capture current health status, current medication use (including multivitamins and supplements) and major osteoporotic fracture history (hip, wrist, vertebral, proximal humerus). Participants were classified as Caucasian or non-Caucasian. The age-adjusted Charlson Index served as a co-morbidity index, reflecting the

presence of weighted comorbid conditions (26). Physical activity levels were assessed using a modified Paffenbarger Physical Activity Questionnaire, which quantifies the number of kilocalories (kcal) expended per week based on the number of stairs climbed up, miles walked and participation in recreational activities during a usual week. Each participants' average dietary intake of calcium and vitamin D was estimated using a food frequency questionnaire (FFQ), previously validated by us for use in postmenopausal community-dwelling women (27). Laboratory biochemistry (random glucose, HbA1c, creatinine) was abstracted from the medical charts of the participants with type 2 diabetes. The Cockcroft-Gault equation was used to estimate glomerular filtration rate (GFR).

Participants' height was captured to the nearest 0.1 cm using a wall-mounted stadiometer and weight was obtained from the whole body DXA scan to the nearest 0.1 kg, from which BMI was calculated. Waist and hip circumference were also measured. Grip strength was assessed using an isometric dynamometer (Takei T.K.K.5001 Grip A Dynamometer, Takei Scientific Instruments Co. Ltd. Niigata-City, Japan) and average grip strength was calculated from three assessments with the dominant hand. A timed-up-and-go (TUG) test was used to assess the participant's physical mobility. A normative cut-off point of 12.0 seconds was used for TUG test performance (28).

MR Imaging

The non-dominant distal radius was imaged with a 1-Tesla extremity MRI system (OrthOne^{TM}, GE Healthcare, United Kingdom) by the same operator.

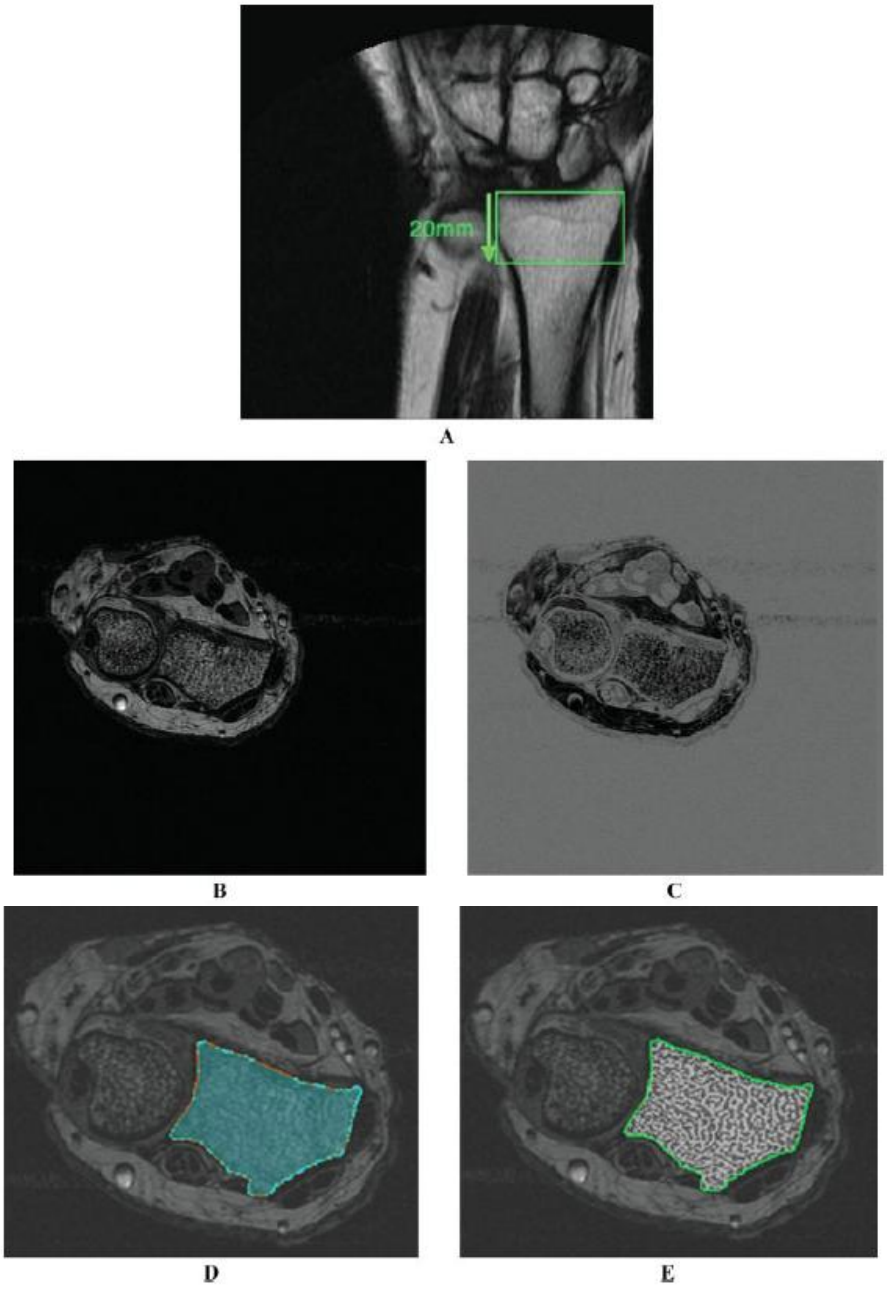
Participants were seated in a chair with their wrist in a prone position in a 100 mm diameter transmit/receive coil. Bracing and padding were applied to enhance patient comfort and the potential for motion artifact. A fast spin echo (FSE) sagittal localizer was performed followed by a FSE coronal localizer, wherein reference lines were placed at the most distal articular surface on the medial aspect of the radius, and at 20 mm proximal to this line (Figure 2). A spoiled 3D gradient-echo sequence was used to acquire 20 axial images of the wrist at 1.0 mm slice thickness with the following sequence parameters: TR 47 ms, TE 23.8 ms, 40° flip angle, 15-kHz bandwidth, 1 NEX, 100 mm x 100 mm FOV, 195 x 195 x 1000 μm^3 voxel size,12:09 minute scan time. An anthropometric phantom was scanned on a daily basis to ensure system quality assurance.

Image Analysis

Axial images were uploaded for slice-by-slice segmentation performed using in-house software developed at our institution. The software uses a graph-based technique to identify the endosteal boundary of the radius (29). The area inside the endosteal perimeter (*ie*: cortical bone excluded) served as the region of interest (ROI) for each slice. A local thresholding technique was applied to the ROI to separate the trabecular bone (no signal) and marrow (signal) phases (Figure 2.1) (30). Finally, a skeletonization algorithm was applied to the segmented trabecular bone images to enable topographic analyses (31). Similar software programs have been used by groups at our institution and others to derive variables of bone microarchitecture at peripheral sites, such as the radius

and tibia (9, 23, 24). The central six slices were selected for analysis for each participant.

Figure 2.1 Coronal (A) and axial images of the non-dominant distal radius acquired with MRI (B and C [inverted]), and images showing semiautomatic segmentation (D) and thresholding (E).



Apparent geometric, stereologic and topographic measures were derived, including mean hole size (mm^2), number of holes, endosteal area (mm^2), trabecular bone volume fraction (BVTV, %), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), trabecular number (Tb.N, mm^{-1}), nodal density (number of nodal points/ mm^2) and branch density (number of branches/ mm^2) (9, 12, 23, 32). Briefly, BVTV was calculated as the area occupied by pixels corresponding to bone divided by the total area within the endosteal boundary. A region growing technique was utilized to determine hole size within the trabecular bone network. It is defined as,

$$\text{Hole size} = \Sigma A_i \div n \qquad (1)$$

where A_i denotes the area of the ith hole, and n denotes the number of holes present in the trabecular network (33). Within the skeletonized trabecular network, branches (single pixel wide line segments) and nodes (defined as points at which three or more branches join) were identified and used to compute nodal and branch density (34). The parallel plate model was used to estimate Tb.Th, Tb.Sp and Tb.N from the perimeter lengths of network holes (35). Reproducibility of the image analysis technique was assessed by analyzing 30 sets of scans (15 from the group of women with type 2 diabetes and 15 from the group of women without type 2 diabetes) in duplicate during independent analysis sessions (Table 2.1).

Table 2.1 Reliability of the image analysis technique for trabecular bone microarchitecture variables at distal radius.

	RMSCV%	ICC
Hole size, mm^2	4.67	0.98
Number of holes	4.50	0.97
Endosteal area, mm^2	4.90	0.95
BVTV, %	1.10	0.83
Tb.Th, mm	1.66	0.94
Tb.Sp, mm	3.57	0.99
Tb.N, mm^{-1}	2.68	0.90
Nodal density	1.54	0.97
Branch density	4.11	0.87

Abbreviation: root mean square coefficient of variation, RMSCV%; intraclass correlation coefficient, ICC; bone volume fraction, BVTV; trabecular thickness, Tb.Th; trabecular separation, Tb.Sp; trabecular number, Tb.N

Areal bone mineral density

A DXA (Hologic, Discovery QDR4500A) scan was acquired to determine total aBMD (g/cm^2) at the lumbar spine (LS) (L1-L4) and proximal femur (femoral neck [FN] and total hip). Proximal femur *T*-scores were computed using NHANES III reference data. Total mass, fat free (lean) mass and fat mass were measured in a whole-body DXA scan from which percent body fat was derived. Standard DXA quality assurance protocols were followed, including use of daily spine phantoms and weekly step phantoms. The short-term *in vivo* operator precision, expressed as the root mean square coefficient of variation (RMSCV%) for the LS, FN, and total hip aBMD were 0.96%, 1.70%, and 1.47%, respectively, which was determined by performing triplicate lumbar spine and left hip scans on 13 young, healthy volunteers. DXA scans were analyzed by a certified DXA technician and radiologist who were blinded to the study purpose and participant allocation.

Statistical Analyses

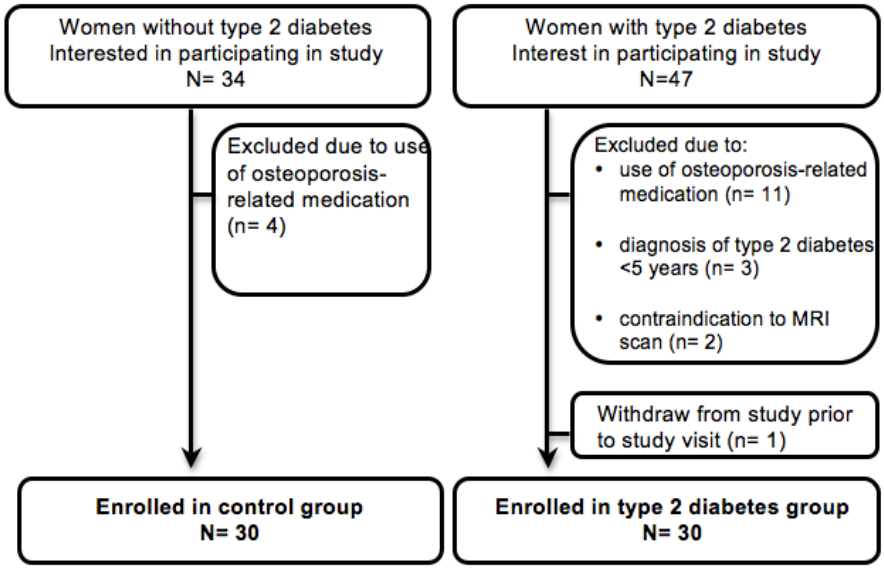
The mean ± standard deviation (SD) was determined for continuous variables, and frequency (%) for categorical variables. Differences between groups for the independent variables presented in Table 2.2, and for the dependent variables in the unadjusted analysis presented in Table 2.4 were assessed using an unpaired Student's *t*-test (for continuous variables) or Chi-square test (for categorical variables). Linear regression was used to explore the association between the dependent variable (variables of microarchitecture) and independent variables. *A priori,* percent body fat, ethnicity, age-adjusted Charlson Index, TUG test result, total calcium intake and total vitamin D intake were forced into the statistical model to examine the differences in variables of bone microarchitecture in women with and without type 2 diabetes (36-38). The other independent variables were collected for descriptive purposes. We also adjusted for BMI when comparing LS and FN aBMD, as BMI is related to LS aBMD (3). We verified that the following assumptions in regression analysis were true for our model: 1) linear relationship between dependent and independent variables; 2) normality of errors; 3) homogeneity of variance; 4) independence of errors associated with each observation. In addition, we found that the independent variables included in the regression model were not highly collinear. The Holm's procedure for multiple comparisons was performed for the comparison of secondary bone microarchitecture variables between groups (39). The reliability of the MRI image analysis technique is expressed as RMSCV% and type 2,1

Intraclass Correlation Coefficient (ICC) for 30 scans analyzed in duplicate. We based our sample size calculation on a previous study investigating bone microarchitecture in women with and without a prior wrist fracture using pQCT (in-plane voxel size: 333µm) (24). The investigators found a mean difference in trabecular bone network hole size between the two groups of 1.98mm^2 with an average standard deviation of 2.7mm^2. Using a power of 80%, an alpha level of 0.05 we aimed to enroll 30 participants/ group. All analyses were performed with SPSS v.18.0 for Windows (IBM Corporation, Somers, NY, USA). A p-value of <0.05 was considered significant for this study.

2.4 RESULTS

The study included 30 women with diabetes, and 30 women without diabetes (Figure 2.1).

Figure 2.2 Path outlining participant recruitment and enrollment in the study.



Descriptive characteristics for both study groups are summarized in Table 2.2. A greater proportion of participants with type 2 diabetes had a diagnosis of osteoarthritis of at least one joint [16/30 (53.3%) versus 5/30 (16.7%), p=0.008] (data not shown). Women with type 2 diabetes had a higher mean BMI (p<0.001), and fewer of these participants completed the TUG test in under 12 seconds, a normal cut-off point for community dwelling adults (p=0.011) (28). The serum biochemistry and anti-hyperglycemic medications used for participants with type 2 diabetes are summarized in Table 2.3.

Table 2.2 Descriptive characteristics of study participants.

	Women with type 2 diabetes n= 30	**Women without type 2 diabetes n= 30**	***P-value***
Age, years	71.0 (4.8)	70.7 (4.9)	NS
Caucasian, n (%)	23 (79.3)	30 (100.0)	NS
Height, cm	158.6 (6.8)	160.1 (5.0)	NS
Weight, kg	86.9 (18.7)	71.3 (13.7)	**<0.001***
BMI, kg/m^2	34.6 (7.6)	27.9 (5.5)	**<0.001***
Waist:hip ratio	0.89 (0.07)	0.83 (0.06)	**0.002***
Body fat percentage, %	40.3 (6.1)	37.2 (6.5)	0.057
Time since menopause, years	22 (7)	22 (8)	NS
Ambulation with an aid[1], n (%)	7 (24.1)	2 (6.7)	NS
Number of prescription medications	6.6 (3.4)	1.9 (2.2)	**<0.001***
Current smoker, n (%)	2 (6.9)	0	NS
History of an osteoporotic fracture after age 40[2], n (%)	5 (17.2)	6 (20.0)	NS
Age-adjusted Charlson Index	1.5 (2.2)	0.3 (1.4)	**0.023***
Total calcium intake, mg/day	1594 (696)	2075 (597)	**0.007***
Calcium intake from supplements, mg/day	446 (481)	678 (482)	NS
Calcium intake from food, mg/day	1148 (564)	1397 (335)	**0.046***
Total vitamin D intake, IU/day	806 (622)	1197 (922)	NS
Vitamin D intake from supplements, IU/day	626 (573)	982 (921)	NS
Vitamin D intake from food, IU/day	179 (142)	195 (130)	NS
Energy expenditure, kcal/day	1904 (2364)	2557 (2170)	NS
TUG Test, seconds ≤12 seconds >12 seconds	12.8 (4.0) 14 (56.0) 11 (44.0)	9.4 (2.7) 26 (86.7) 4 (13.3)	**<0.001*** **0.011***
Grip strength, mean (SD), kg	18.8 (4.8)	21.7 (6.3)	NS
LS BMD, g/cm^2	1.07 (0.15)	0.98 (0.18)	**0.045***
LS *T-score*	0.15 (1.40)	-0.61 (1.66)	NS
FN BMD, g/cm^2	0.73 (0.11)	0.69 (0.10)	NS
FN *T-score*	-1.11 (1.02)	-1.40 (0.89)	NS
Total hip BMD, g/cm^2	0.87 (0.21)	0.86 (0.11)	NS
Total hip *T-score*	-0.58 (0.99)	-0.70 (0.95)	NS

Values are mean (SD), unless otherwise specified. * P<0.05 is considered significant. *Abbreviations*: not significant, NS; body mass index, BMI; timed-up-and-go, TUG; bone mineral density, BMD; Lumbar spine, LS; Femoral neck, FN. [1] Ambulation aid includes single point cane, four-point cane, standard walker, rollator walker. [2] Major osteoporotic fracture defined as a hip, wrist, clinical spine or humerus fracture that occurred from a fall from standing height or less or a fall from <4 stairs.

Table 2.3 Descriptive data for participants with type 2 diabetes.

	Women with type 2 DM (n=30)
Years with type 2 DM diagnosis, mean ± SD	16.6 ± 11.1
Type of antihyperglycemic medication used	
Insulin	18 (60.0)
Biguanide	12 (40.0)
Insulin secreting sulfonylurea or nonsulfonylurea	4 (13.3)
Thiazolidinedione	1 (3.3)
Serum biochemistry	
Random glucose, mean ± SD, mmoles/L	8.3 (3.7)
Glycosylated hemoglobin, %	7.8 (1.7)
Glomerular filtration rate, mean ± SD, ml/minute	78.4 (28.4)

Values are number (percent) unless indicated otherwise.
Abbreviation: diabetes mellitus, DM

Bone microarchitecture

The reliability of the image analysis technique was good, as evidenced by high ICC and low RMSCV% values for each microarchitecture variable (Table 2.1). The comparison between bone microarchitecture variables for women with type 2 diabetes and controls is presented in Table 2.4. Six distal radius MRI scans were considered unacceptable for analysis due to motion artifact as assessed by an independent, observer blind to subject identifier group, resulting in 29 and 25 analyzable image sets for the type 2 diabetes and control groups, respectively. The participants with discarded scans did not appear to be different from the rest of the control group, with respect to the descriptive characteristics in Table 2.2 (data not shown). The unadjusted comparison of bone microarchitecture variables revealed that in women with type 2 diabetes, trabecular bone network holes were 13.4% larger in area [2.20(0.45)mm^2 versus

1.94(0.33)mm^2, p=0.011]. After adjusting for multiple comparisons, no differences were detected for number of holes, endosteal area, BVTV, nodal density, branch density, apparent Tb.Th, Tb.Sp, and Tb.N in the model (Table 2.4). After considering multiple comparisons and adjusting for percent body fat, ethnicity, age-adjusted Charlson Index, total calcium and vitamin D intake and TUG result, only trabecular bone network hole size was 13.3% greater in women with type 2 diabetes [2.22(0.47)mm^2 versus 1.96(0.26)mm^2, p=0.001] (Table 2.4).

Table 2.4 Values for trabecular bone microarchitectural variables at the distal radius in women with type 2 diabetes and controls without type 2 diabetes.

	Unadjusted analysis			Multivariate-adjusted analysis			
	Women with type 2 DM (n = 29)	Women without type 2 DM (n = 25)	*P*	Women with type 2 DM (n = 29)	Women without type 2 DM (n = 25)	*P*	Holm's adjusted *P*
Hole size, mm^2	2.20 ± 0.45	1.94 ± 0.33	0.011	2.22 ± 0.47	1.96 ± 0.26	0.001†	
No. of holes	72.5 ± 16.9	82.9 ± 15.5	0.025	71.9 ± 17.6	81.9 ± 15.8	0.022	0.154
Endosteal area, mm^2	310.24 ± 54.98	323.33 ± 56.34	0.397	308.76 ± 56.73	322.83 ± 57.87	0.319	0.957
BVTV, %	51.2 ± 2.2	51.6 ± 1.7	0.454	51.3 ± 2.1	51.5 ± 1.9	0.450	0.957
Tb.Th, mm	0.55 ± 0.04	0.55 ± 0.02	0.591	0.56 ± 0.04	0.55 ± 0.02	0.228	0.912
Tb.Sp, mm	0.92 ± 0.05	0.93 ± 0.04	0.287	0.92 ± 0.06	0.93 ± 0.04	0.085	0.425
Tb.N, mm^{-1}	0.53 ± 0.05	0.52 ± 0.04	0.250	0.53 ± 0.05	0.52 ± 0.04	0.067	0.402
Nodal density	0.69 ± 0.08	0.74 ± 0.05	0.028	0.69 ± 0.08	0.73 ± 0.05	0.014	0.112
Branch density	0.33 ± 0.03	0.33 ± 0.03	0.875	0.33 ± 0.03	0.33 ± 0.02	0.776	0.957

* Values are the mean ± SD unless indicated otherwise. DM = diabetes mellitus; BVTV = bone volume fraction; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Tb.N = trabecular number.
† Indicates statistical significance according to Holm's test for multiple comparisons. Multivariate analysis adjusted for percent body fat, ethnicity, age-adjusted Charlson Index, total daily calcium intake, total daily vitamin D intake, and timed up-and-go test result.

Bone density measurements

Lumbar spine aBMD was greater in women with type 2 diabetes [1.07(0.15) g/cm^2] compared to women in the control group [0.98(0.18) g/cm^2, p=0.045]. After adjustment for BMI, there were no differences detected between groups for LS aBMD (p=0.572), FN aBMD (p=0.663) or total hip aBMD (p=0.224) (data not shown). One LS DXA scan from a participant with type 2 diabetes was excluded from analysis due to image artifact (contrast). There were more cases of spine scoliosis (4/30 [13.3%] versus 2/29 [6.9%]) and vertebral compression (1/30 [3.3%] versus 0/29) in the control group compared to the women with type 2 diabetes (data not shown).

2.5 DISCUSSION

This is the first cross-sectional study to demonstrate that there are larger trabecular bone network holes at the distal radius in women with type 2 diabetes, after adjustment for percent body fat, ethnicity, age-adjusted Charlson Index, calcium and vitamin D intake, and timed-up-and-go result. This finding provides a possible explanation for bone fragility in this population, given the importance of hole size described in previous research. After adjusting for multiple comparisons, all other variables of trabecular bone microarchitecture were not different between groups. This study may have been underpowered to accurately assess these other microarchitecture outcomes and further investigation is needed.

Observational imaging studies in adults with a fracture history have demonstrated that reduced bone strength may be influenced by deficits in the trabecular bone network (10, 19, 24). In particular, the size of the holes in the trabecular network at appendicular skeletal sites can provide information about bone structural competence (40). Trabecular bone network hole size has been shown to be greater in women with a history of wrist fracture compared to BMI and age-matched women who had not had a prior wrist fracture (24). Other surrogate outcomes for skeletal health, including aBMD, connectivity index, and stress-strain index were not different between wrist fracture and control participants in this prior study. Furthermore, there was a highly significant correlation between hole size and prior wrist fracture (odds ratio, OR 5.4 [95% confidence interval, CI; 1.2-24.3], p=0.03), whereas no relationship was detected between aBMD and prior fracture (24). Hole size also appears to be a more powerful discriminator of vertebral fracture than Tb.Sp and Tb.Th, and can differentiate those with a fracture history with greater sensitivity and specificity than aBMD alone (33). Trabecular bone network hole size has also been shown to contribute to bone strength in an *ex vivo* study using radial bone specimens, where a strong association was demonstrated between average hole size and maximum hole area, and peak load at fracture (41).

The mechanism causing larger holes in the trabecular bone network at the radius in participants with type 2 diabetes is not fully understood. However, studies in rodent models of type 2 diabetes and in rodents fed high-fat diets have

demonstrated a reduction in osteoblast recruitment and mineral apposition rate, and an increase in osteoclastogenesis resulting in an imbalance between bone formation and resorption (42, 43). Cross-sectional findings in humans further support these data, as serum markers of bone formation, such as osteocalcin are lower, and markers of bone resorption, such as CTX, are elevated in participants with type 2 diabetes (44, 45). Increased bone resorption may be mediated by the formation of advanced glycation end-products, which stimulate osteoclast activity and may lead to an uncoupling of bone formation and resorption (46).

After the adjustment for multiple comparisons, we did not detect differences in branch density, nodal density, endosteal area, BVTV, Tb. Sp, Tb.Th or Tb.N between women with type 2 diabetes, and non-diabetic controls. This could be explained by the different methods used in deriving hole size and 2-dimensional measures, such as Tb.Sp, Tb.Th and Tb.N. Hole size analyses do not depend on any stereologic assumptions, where as the derivation of Tb.Sp, Tb.Th and Tb.N do indeed depend on sterologic assumptions (*i.e.,* parallel plate model) (35). Moreover, some suggest that comprehensive imaging studies aiming to determine histomorphometric differences between groups should have at least 50 participants per group to provide sufficient power (19). Burghardt and colleagues also found no differences in radius microarchitectural indices, including Tb.N, in a smaller pilot study in women with type 2 diabetes and age- and height-matched healthy controls using pQCT (47). The authors also reported no difference in indices of bone strength between groups, which might be

attributed to the sample size in this study. While variables of trabecular bone microarchitecture and indices of bone strength at the radius were not found to be different in this previous pQCT study, Burghardt and colleagues did report a greater number of cortical pores and greater cortical bone pore volume at the radius (47). Similar findings have been reported in men with type 2 diabetes (48). Conversely, a smaller pQCT study conducted in a cohort of postmenopausal women found no differences in trabecular bone microarchitecture or cortical porosity in women with type 2 diabetes and controls (49). In comparison to these prior studies, our study population was composed of older women who had type 2 diabetes for a greater number of years. The radius site assessed in the present study was also distal to the sites assessed in the prior studies, which may explain discrepancies in trabecular bone microarchitecture results. In addition, the in-plane image resolution of the MRI system which was employed for the present study was poorer (195 μm) compared to the image resolution of the high-resolution pQCT system (82 μm), limiting our ability to resolve the cortical bone. Others have also reported difficulties resolving the cortical bone at the distal radius with MR imaging, due in part to the low-intensity signal of neighbouring regions of connective tissue which is similar to that of bone (15). Although previous work suggests that cortical bone is more porous, our data indicate that changes to the trabecular network may also contribute to increased fracture risk in those with type 2 diabetes.

There are limitations with this study. First, partial volume effects, produced when imaging trabeculae that are smaller than the spatial resolution may confound and overestimate measures of bone microarchitecture, such as BVTV and Tb.Th (9, 20). However, studies have demonstrated that 'apparent' histomorphometric measures derived from MRI correlate well with measures derived through the use of higher-resolution modalities, such as μCT, wherein partial volume effects are mitigated (21). Second, the 195μm in-plane resolution limited our ability to resolve cortical bone, which is more porous in individuals with type 2 diabetes (47). Third, trabecular bone network hole size is a 2-dimensional measurement, which does not completely account for the anisotropic 3-dimensional nature of trabecular bone *in vivo.* Fourth, unlike pQCT, MR images do not provide information pertaining to areal or volumetric bone density and therefore we can not compare or adjust for bone density at the distal radius. Fifth, due to our small sample size and few fractures, we did not have the power to investigate the association between hole size and prevalent osteoporotic fractures and the influence of medications on hole size, such as thiazolidinediones, which are associated with elevated fracture risk (50). Finally, we did not use a clinical test to screen for peripheral neuropathy in participants with type 2 diabetes, which could influence bone quality at the distal radius.

Advances in the use of MRI have afforded insight into the impact of disease on bone microarchitecture. Our results suggest that in women with type 2 diabetes, the trabecular network at the distal radius is characterized as having

larger holes compared to non-diabetic women of similar age. Given the known contribution of bone microarchitecture to overall bone strength, these findings may explain why an elevated fracture risk has been observed in women with type 2 diabetes despite normal or elevated aBMD. Future research should clarify the independent contribution of diabetes to fracture risk, to inform risk assessment and stratification, and should focus on understanding other mechanisms behind diabetic bone fragility.

2.6 ACKNOWLEDGEMENTS

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CHAPTER THREE

AUTHOR'S PREFACE TO CHAPTER THREE

In this chapter, I describe the two year change in trabecular bone microarchitecture measurements in postmenopausal women with type 2 diabetes compared to controls without type 2 diabetes of similar age. This work is the first to demonstrate a higher percent increase in of trabecular bone holes at the distal radius in postmenopausal women with type 2 diabetes compared to controls. All other changes in trabecular bone microarchitecture measurements were not different between groups. The study provides preliminary evidence for the trabecularization of cortical bone in postmenopausal women with type 2 diabetes, but this hypothesis requires further research.

The material presented in Chapter Three was submitted for publication in October 2012 to the peer-reviewed journal, *BMC Musculoskeletal Disorders.* An acceptance or rejection response is pending. I was responsible for designing the study, following up with the study participants, acquiring the images and data, performing statistical analysis, interpreting the data and composing the manuscript. Drs. Giangregorio, Atkinson, Beattie, Gerstein, Punthakee, Adachi and Papaioannou assisted with designing the study, interpreting data and providing comments on the manuscript. Dr. Inglis designed the in-house software (OsteoQ) and Dr. Ioannidis provided valuable statistical analysis support throughout the study.

Longitudinal changes in trabecular bone microarchitecture in postmenopausal women with and without type 2 diabetes

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3.1 ABSTRACT

The aim was to compare two year changes in trabecular bone microarchitecture in women with and without diabetes. We used a 1 Tesla MRI scanner to acquire axial images (resolution 195 x 195 x 1000μm^3) of the distal radius. We report the change in the number and size of trabecular bone holes, bone volume fraction (BVTV), trabecular thickness (Tb.Th), number (Tb.N) and separation (Tb.Sp), endosteal area, nodal and branch density for each group. Using a multivariable linear regression model, we evaluated whether the percent change in the trabecular bone microarchitecture variables differed between women with and without type 2 diabetes. Of the 54 participants at baseline with valid MRI image sets, 37 participants (baseline mean±SD age, 70.8±4.4 years) returned for follow-up assessment after 25.4±1.9 months. Lumbar spine BMD was greater for women with diabetes compared to without diabetes at both baseline and follow-up. After adjustment for ethnicity, women with diabetes had a higher percent increase in number of trabecular bone holes compared to controls (10±5% versus −7±4%, p=0.010). There were no differences in the change in other trabecular bone microarchitecture variables between groups. This early evidence of accelerated trabecular bone loss in older women with diabetes warrants further investigation

3.2 INTRODUCTION

Adults with type 2 diabetes are at 30-70% greater risk of experiencing an osteoporotic fracture than those without type 2 diabetes (1-3), despite normal or higher than normal bone mineral density (BMD)(4). Various reasons for the greater fracture risk in adults with diabetes have been hypothesized and include medication use (5), accumulation of advanced glycation end-products (6), retinopathy (7), peripheral neuropathy and falls (8). Bone strength may also be compromised by changes in bone geometry or trabecular bone microarchitecture, which are not reflected in BMD measured with dual x-ray absorptiometry (DXA) (9-11).

Understanding how trabecular bone microarchitecture changes over time may provide insight into the bone fragility observed in adults with type 2 diabetes. In postmenopausal women with type 2 diabetes, we demonstrated that there are larger trabecular bone holes at the distal radius compared to women without diabetes (12), and others have reported that cortical bone is more porous in those with diabetes (13). Trabecular bone microarchitecture can be modified by osteoporosis treatments (14-16), yet whether there is skeletal response to antiresorptive medication in individuals with diabetes is controversial (17, 18).

The primary goal of this study was to explore the hypothesis that postmenopausal women with type 2 diabetes would have a greater increase in trabecular bone hole size than women without diabetes when followed over two years. Secondly, we explored whether women with diabetes would experience

greater increases in the number of trabecular bone holes, trabecular separation (Tb.Sp) and branch density, and greater losses in trabecular bone volume fraction (BVTV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and nodal density than women without diabetes, indicating a loss in bone microarchitectural integrity over time.

3.3 MATERIALS AND METHODS

Study Design and Participants

For this prospective cohort study, we recruited participants between 2008 and 2009 from Diabetes Clinics at two sites within Hamilton Health Sciences and from the community. At the time of recruitment, all participants were ≥ 65 years of age, postmenopausal for > 5 years, and those in the diabetes group had been diagnosed with type 2 diabetes for ≥ 5 years (19). Potential participants were excluded at baseline if they: 1) were taking, or had taken in the past 24 months, any medication known to affect bone, including hormone therapy, calcitonin, selective estrogen receptor modulator, parathyroid hormone, or bisphosphonate; 2) were taking oral glucocorticoids (≥ 2.5mg/day for ≥ 3 months); or 3) had a diagnosis of a disease known to affect bone (*i.e.,* metastatic cancer in past 5 years, osteogenesis imperfecta, severe renal impairment, hyperparathyroidism, hypoparathyroidism). Participants were asked to complete one study visit as a part of a cross-sectional study published previously (12), and those with valid baseline MRI image sets (absence of motion artifact) were subsequently invited

to complete a two year follow-up assessment. This study was approved by the McMaster University Faculty of Health Sciences/Hamilton Health Sciences Research Ethics Board, and all participants provided written informed consent at baseline and follow-up.

Descriptive Variables

Medical history, lifestyle and densitometry data were collected at baseline and follow-up to describe our study participants. Ethnicity (Caucasian or non-Caucasian) was used as a covariate in the multivariable linear regression model. A medical history questionnaire was used to assess number of years since menopause, number of years since a diagnosis of type 2 diabetes (if applicable), current medication use, history of major osteoporotic fractures (*i.e.,* non-traumatic fracture of the hip, wrist, vertebral, or proximal humerus) (20) and occurrence of osteoporotic fractures since baseline. The age-adjusted Charlson Index, a global comorbidity index and measure of current health status, was calculated for each participant at baseline and follow-up (21). Physical activity levels were assessed at baseline and at follow-up using a modified Paffenbarger Physical Activity Questionnaire, which quantifies the number of kilocalories (kcal) expended per week based on the number of stairs climbed up, miles walked and participation in recreational activities during a usual week (22). Each participants' average supplemental and dietary intake of calcium and vitamin D was estimated at both time-points using a food frequency questionnaire (FFQ) and self-reported supplement intake (including intake from multivitamins) (23). Anthropometric

measurements were collected at baseline and follow-up, and included height, using a wall-mounted stadiometer, weight, obtained from a whole body DXA scan, and waist and hip circumference. A test of grip strength of the dominant hand (Takei T.K.K.5001 Grip A Dynamometer, Takei Scientific Instruments Co. Ltd. Niigata-City, Japan) and a Timed-Up-and-Go (TUG) test were also completed by participants at both time-points. A normative cut-off point of 12.0 seconds was used for TUG test performance (24). DXA (Hologic, Discovery QDR4500A) scans were acquired to determine BMD at the lumbar spine (L1-L4) and proximal femur (femoral neck and total hip), for descriptive purposes. Whole body DXA scans were performed to estimate body weight and percent body fat. The DXA system's variability for BMD measurement was 0.315% from the first baseline assessment (September 2008) to the last follow-up assessment (September 2011). Short-term *in vivo* precision was less than 1.70% for BMD measurements (12). Anonymized DXA scans were analyzed by a certified DXA technician, who was blinded to group membership.

Magnetic Resonance Imaging and Image Analysis

At baseline and follow-up, each participant's non-dominant forearm was immobilized in a brace and inserted into the gantry of a 100 mm diameter coil in a 1 Tesla peripheral MRI system (OrthOne^{TM}, GE Healthcare, United Kingdom). We used a spoiled 3D gradient-echo sequence, which yielded 20 axial slices (195 x 195 x 1000 μm^3 voxel size) of the distal radius, as previously described (12). All scans were performed by the same operator at baseline and follow-up,

and a quality control phantom was scanned on a daily basis to ensure system stability.

We used image registration software (Analyze, v.10, Biomedical Imaging Resource at Mayo Clinic, USA) to match baseline and follow-up slices in the axial, sagittal and coronal planes. The first axial slice proximal to the growth plate region of the radius was selected to be the most distal slice in the volume of interest that was analyzed. Given that all participants had at least 8 contiguous slices that matched, 8 matched slices were analyzed per participant for this study. The slices were uploaded for blinded slice-by-slice semi-automatic segmentation using software, previously described (12). The segmentation of trabecular bone within the endosteal boundary of the radius generated nine apparent measures of trabecular bone microarchitecture, including number and size (mm^2) of trabecular bone holes, endosteal area (mm^2) and trabecular bone volume fraction (BVTV, %). Following skeletonization of the segmented image data, network analysis was performed to assess nodal density (number of nodal points/mm^2) and branch density (number of branches/mm^2). A model-independent method was used to estimate apparent trabecular thickness (Tb.Th, mm) and separation (Tb.Sp, mm) (25). Trabecular number (Tb.N, /mm) was derived using standard histomorphometry formulae (Tb.N= (BVTV)/Tb.Th) (26). The baseline comparison of trabecular bone microarchitecture between women with and without diabetes has been published and reflects the analysis of the central 6 MRI slices (12). The root mean square coefficient of variation

(RMSCV%) ranged from 1.10% to 4.90% and intraclass correlation coefficient ranged from 0.83 to 0.99 for the assessment of trabecular bone microarchitecture variables (12).

Statistical Analyses

The Kolmogorov-Smirnov test was used to confirm normal distribution of all variables, therefore descriptive data are presented as mean ± standard deviation (SD) for continuous variables, and frequency (%) for categorical variables. Between-group differences in descriptive variables at baseline and follow-up were determined using an unpaired Student's *t*-test or Chi-square test. For the assessment of internal validity, an unpaired Student's *t*-test was employed to compare baseline descriptive variables and trabecular bone microarchitecture variables for the participants who dropped out and returned for the follow-up visit. The absolute change in trabecular bone microarchitecture was calculated as follows: follow-up measurement−baseline measurement.

Multivariable linear regression was applied to answer the primary question of whether percent change in trabecular bone microarchitecture differed in women with diabetes compared to women without diabetes. The nine dependent variables were: percent change (absolute change/baseline measurement x 100%) in size and number of trabecular bone holes, endosteal area, BVTV, Tb.Th, Tb.Sp, Tb.N, branch density and nodal density. Inclusion of ethnicity in the model was based on previous literature suggesting that ethnicity influences BMD (27), and on the statistical principle that a covariate is significantly related to the

primary dependent variable (percent change in trabecular bone hole size) (28). Pearson correlation analysis revealed that ethnicity was related to the primary dependent variable ($r = -0.364$, $p = 0.038$). The adjusted means and SD are presented. The criterion for statistical significance was set at alpha < 0.05. All analyses were performed with SPSS version 20 (IBM Corporation, Somers, USA).

3.4 RESULTS

Study Participants

The descriptive characteristics of all study participants who completed baseline and follow-up assessments are shown in Table 3.1.

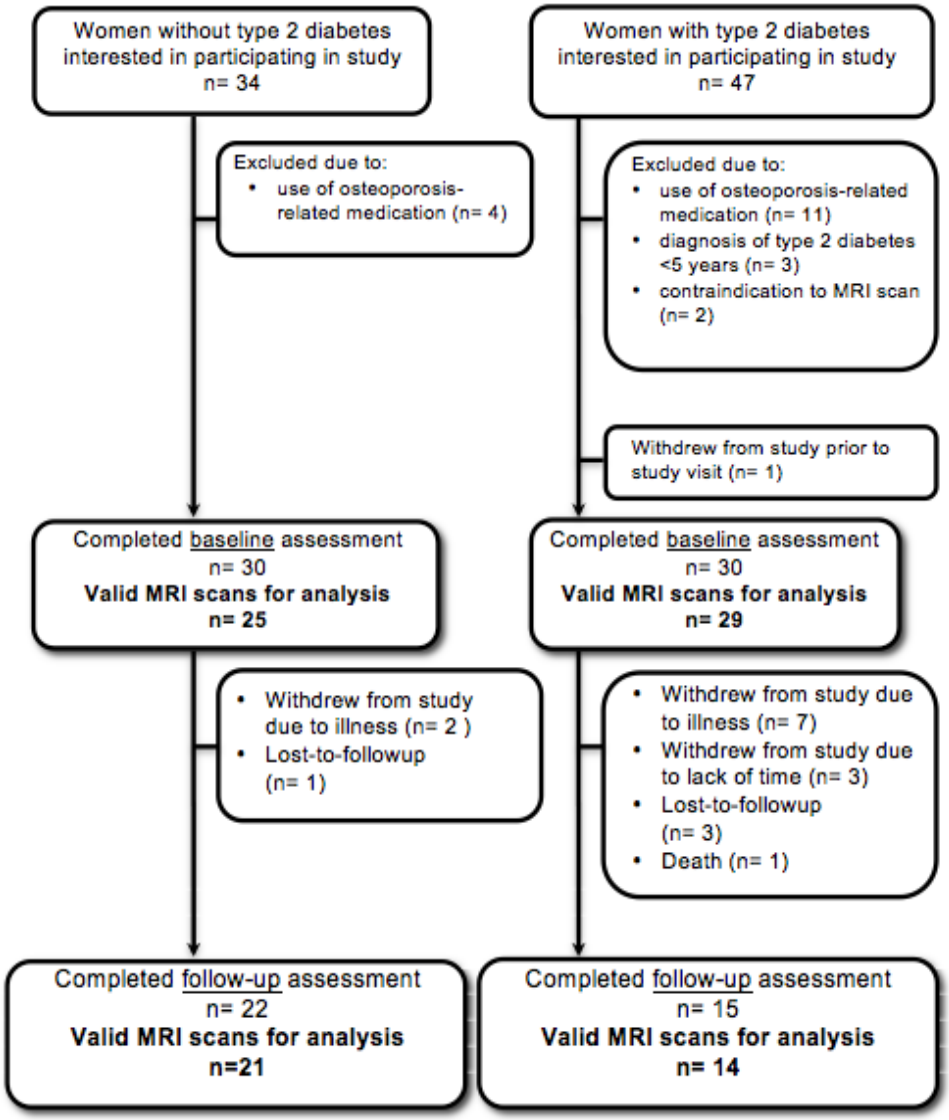
Table 3.1 Descriptive characteristics of all study participants who were enrolled at baseline and follow-up

	Baseline			Follow-up		
	Women with diabetes n= 30	Controls n= 30	Difference between groups *p-value*	Women with diabetes n= 15	Controls n= 22	Difference between groups *p-value*
Age, years	71.1 (4.8)	70.7 (4.9)	0.816	73.9 (3.6)	72.5 (4.9)	0.324
Caucasian, n (%)	23 (79.3)	30 (100.0)	0.017*	12 (80)	22 (100.0)	0.009*
History of atraumatic osteoporotic fracture[a]	5 (17.7)	6 (20.0)	0.738	-	-	-
Since age 40 years, n (%)	-	-	-	2 (13.3)	1 (4.5)	0.315
Since baseline assessment, n (%)						
BMI, kg/m^2	34.6 (7.6)	27.9 (5.5)	<0.001*	36.1 (5.7)	27.9 (4.4)	<0.001*
Waist:hip Ratio	0.89 (0.07)	0.83 (0.06)	0.002*	0.90 (0.05)	0.83 (0.06)	<0.001*
Body fat percentage, %	40.3 (6.1)	37.2 (6.5)	0.056	41.8 (9.5)	39.1 (4.2)	0.256
Time since menopause, years	22 (7)	22 (8)	0.841	24 (5)	23 (7)	0.656
Number of prescribed medications	6.6 (3.5)	1.9 (2.2)	<0.001*	8.1 (3.0)	2.4 (2.5)	<0.001*
Age-adjusted Charlson Index	4.3 (1.5)	0.1 (0.6)	<0.001*	4.5 (1.2)	0.1 (0.6)	<0.001*
Total calcium intake, mg/day	1594 (696)	2062 (590)	0.007*	1679 (890)	2019 (639)	0.697
Supplemental, mg/day	446 (481)	678 (482)	0.070	377 (480)	603 (427)	0.138
Dietary, mg/day	1148 (564)	1397 (335)	0.054	1345 (660)	1241 (473)	0.565
Total vitamin D intake, IU/day	806 (622)	1177 (912)	0.073	1316 (828)	1488 (875)	0.562
Supplemental, IU/day	626 (573)	982 (921)	0.080	993 (822)	1285 (866)	0.308
Dietary, IU/day	179 (142)	195 (130)	0.644	252 (124)	218 (155)	0.495
Weekly energy expenditure, kcal/week	1984 (2428)	2584 (2203)	0.333	959 (1129)	2255 (1443)	0.005*
TUG Test, seconds	12.8 (4.0)	9.4 (2.7)	<0.001*	14.4 (4.4)	10.0 (3.4)	<0.001*
>12 seconds, n (%)	11 (44.0)	4 (13.3)	0.011*	7 (46.6)	2 (9.1)	0.005*
Grip strength, kg	18.8 (4.8)	21.7 (6.3)	0.058	16.3 (5.0)	20.2 (6.1)	0.048*
Bone density measurements						
Lumbar spine, g/cm^2	1.07 (0.15)	0.97 (0.19)	0.025*	1.11 (0.15)	0.99 (0.15)	0.022*
Femoral neck, g/cm^2	0.73 (0.11)	0.69 (0.09)	0.254	0.73 (0.11)	0.69 (0.09)	0.254
Total hip, g/cm^2	0.87 (0.12)	0.86 (0.11)	0.639	0.88 (0.12)	0.87 (0.10)	0.759

Values are mean (SD), unless indicated. * indicates significant between-group differences at p-value <0.05. [a]Atraumatic osteoporotic fracture includes hip, wrist, spine or proximal humerus fracture. *Abbreviations*: body mass index, BMI; timed-up-and-go, TUG

At baseline, 6 MRI image sets were unacceptable for analysis due to motion artifact. Of the 54 participants with valid baseline MRI scans, 15/29 (52%) participants with type 2 diabetes and 22/25 (88%) participants without diabetes returned for the follow-up assessment (Figure 3.1).

Figure 3.1 Path outlining study participant recruitment, enrollment and follow-up from baseline to follow-up assessment.



The average time between baseline and follow-up visits was 25.4±1.9 months. At follow-up, women with type 2 diabetes had a diagnosis of diabetes for 18.8±9.7 years, and the majority of participants (12/15 [80.0%]) were taking insulin or insulin in combination with another glucose-lowering intervention. The remaining participants were either taking metformin (2/15 [13.3%]) or no medication (1/15 [6.7%]). At baseline and follow-up, the group of women with diabetes was comprised of fewer Caucasians with a greater BMI who were prescribed more medications. Lumbar spine BMD was also greater for women with diabetes at both time-points (Table 3.1).

Differences Between Study Participants and Drop-outs

The participants who dropped out of the study were not different from those who returned for the follow-up visit, regarding the majority of descriptive characteristics presented in Table 3.1. The only exception was for percent body fat in the women without diabetes, which was greater for those that returned for follow-up compared to those who dropped out (39.0±4.3% versus 31.1±9.0%, p=0.003) (remaining data not shown). Regarding baseline microarchitectural differences, trabecular bone holes were larger (2.51±0.31mm^2 versus 2.14±0.43mm^2, p=0.042), BVTV was lower (46.9±0.3% versus 47.6±0.9%, p=0.017), and branch density was greater (0.48±0.03/mm^2 versus 0.41±0.06/mm^2, p=0.003) in women with diabetes who dropped out of the study compared to those who returned for the follow-up visit. In women without diabetes, the number of trabecular bone holes was greater (92±12 holes versus

72±15 holes, p=0.031) and hole size smaller (1.75±0.17mm^2 versus 2.06±0.42mm^2, p=0.030) in those who dropped out of the study compared to those who returned for follow-up.

Between-Group Differences in Change in Trabecular Bone Microarchitecture

Two MRI scans were considered unacceptable for analysis due to motion artifact, resulting in 14 valid image sets for the type 2 diabetes group and 21 valid image sets for the control group. Unadjusted baseline, follow-up and absolute change in trabecular bone microarchitecture variables are summarized in Table 3.2 for the participants who had valid MRI image sets at baseline and follow-up.

Table 3.2 Unadjusted measures of trabecular bone microarchitecture for participants with valid MRI image sets who completed both baseline and follow-up assessments.

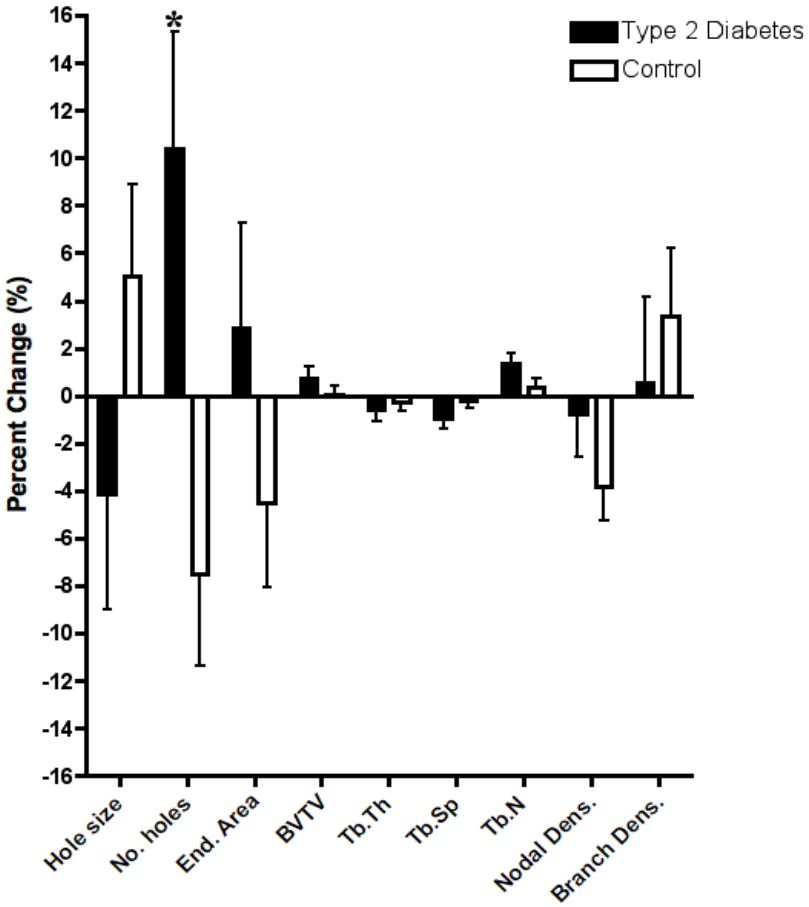
	Women with type 2 diabetes			Controls			Between group difference *p-value*
	Baseline n= 14	Follow-up n= 14	Absolute change from baseline	Baseline n= 21	Follow-up n= 21	Absolute change from baseline	
Hole size, mm^2	2.10 (0.47)	2.04 (0.37)	-0.06 (0.48)	2.06 (0.42)	2.08 (0.45)	0.03 (0.32)	0.513
Number of holes	68 (17)	69 (13)	1 (15)	72 (15)	68 (18)	-4 (12)	0.283
Endosteal area, mm^2	260.7 (51.1)	264.8 (56.9)	4.0 (39.9)	273.2 (58.4)	258.3 (48.9)	-14.9 (39.6)	0.939
BVTV, %	47.7 (1.0)	47.9 (0.8)	0.2 (0.9)	47.7 (1.2)	47.8 (1.0)	0.1 (0.7)	0.759
Tb.Th, mm	0.52 (0.01)	0.51 (0.01)	0 (0.01)	0.51 (0.01)	0.51 (0.01)	0 (0.01)	0.549
Tb.Sp, mm	0.55 (0.01)	0.54 (0.01)	0 (0.01)	0.54 (0.02)	0.54 (0.02)	0 (0.01)	0.322
Tb.N,/mm	0.92 (0.03)	0.93 (0.02)	0.01 (0.02)	0.93 (0.03)	0.93 (0.03)	0 (0.01)	0.362
Nodal density, $/mm^2$	0.16 (0.01)	0.16 (0.01)	0 (0.01)	0.16 (0.01)	0.15 (0.01)	0 (0.01)	0.574
Branch density, $/mm^2$	0.41 (0.06)	0.42 (0.05)	0.01 (0.05)	0.41 (0.05)	0.42 (0.06)	0.01 (0.05)	0.940

Values are mean (SD). *Abbreviations:* BVTV, bone volume fraction; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; Tb.N, trabecular number

Figure 3.2 shows the adjusted percent changes in trabecular bone microarchitecture variables for both groups. Women with diabetes had a

significantly higher percent increase in the number of trabecular bone holes as compared to women without diabetes (10±5% versus −7±4%, p=0.010). There were no differences between groups in the change in trabecular bone hole size (−4.15±4.88% versus 5.03±3.90%, p=0.172) or in the change in the other trabecular bone microarchitecture variables (Figure 3.2).

Figure 3.2 Adjusted percent changes over two years in trabecular bone microarchitecture variables for women with and without type 2 diabetes



3.5 DISCUSSION

Results of our study provide early evidence that women with type 2 diabetes experience a greater deterioration in trabecular bone microarchitecture than women without diabetes, as indicated by a greater increase in the number of trabecular bone holes. Other microarchitectural changes, such as the change in trabecular bone hole size, were similar for women with and without diabetes. Given that the structural integrity of bone is influenced by the number of holes within the trabecular bone network, these findings could have important implications in the context of diabetic bone fragility (29, 30). It is possible that the increase in the number of trabecular bone holes may be reflective of trabecularization of cortical bone, described as the repartitioning of cortical bone into trabecular bone (31). Trabecularization of cortical bone has been linked to reduced bone strength in patients with secondary hyperparathyroidism, and might also be a factor in reduced bone strength in patients with type 2 diabetes (32, 33). Subsequent studies using higher resolution imaging systems examining changes in trabecular bone microarchitecture relative to changes in cortical bone structure (*i.e.,* cortical thickness) are necessary to further study this hypothesis.

Skeletal change in adults with type 2 diabetes has been limited to the description of BMD change; however, whether individuals with diabetes lose bone at a faster rate than non-diabetics is unclear (34-36). Younger women with diabetes (35) and women with newly diagnosed diabetes (36) experienced greater losses in hip BMD, whereas in women with diabetes for more than twelve

years (34) and in postmenopausal women (36), no differences in the rate of BMD loss have been reported. It is possible that the greatest losses in bone occur during the years surrounding the diagnosis of type 2 diabetes when the likelihood of hyperglycemia, hypercalcuria, and generation of reactive oxygen species (ROS) is high (37-39). The negative impact of hyperglycemia and ROS on osteoblasts has been demonstrated *in vitro (40, 41),* and is a potential mechanism causing diabetic bone fragility (42). Variability exists in the concentration of these factors in adults with type 2 diabetes, depending on duration and control of diabetes (37). This may explain the discrepancy in BMD change in women with diabetes, and supports our finding of no difference in the change in the majority of trabecular bone microarchitecture variables in older postmenopausal women with long-standing type 2 diabetes given that the average length of time since diabetes diagnosis in our study was over 18 years. Furthermore, in studies with similar sample sizes to our study, non-diabetic women taking alendronate (43) and estrogen supplementation (16) experienced no change in some microarchitectural variables assessed with MRI at the radius. Similarly, nasal calcitonin does not change trabecular bone microarchitecture at the more distal sites of the radius, but does preserve microarchitecural quality at proximal radius sites (14). We speculate that losses in trabecular bone microarchitecture at more proximal sites might be apparent in women with newly diagnosed type 2 diabetes, which should be investigated in the future.

There were several study limitations. First, approximately 50% of participants with type 2 diabetes dropped out of the study, were lost to follow-up or died after the baseline assessment. To assess the internal validity of the study, we compared the baseline descriptive characteristics and trabecular bone microarchitecture variables for the participants who dropped out to those who remained in the study. In women with diabetes who dropped out, the trabecular bone microarchitecture appeared less intact, and in women without diabetes who dropped out, the trabecular bone appeared to be laden with more holes compared to those who returned. It is possible that the individuals who dropped out were more un-well in aspects that we did not assess in this study. For example, subclinical peripheral arterial disease, which we did not assess, has been linked to reduced bone mineral content (44) and to osteoporotic fractures in adults with type 2 diabetes (3). Our results may have been biased towards not detecting a difference in trabecular bone microarchitectural changes, given the baseline differences between those who dropped out and returned to complete the study. While multivariable linear regression models were used to account for the differences in ethnicity between women with diabetes and controls, the study would have been strengthened if participants were matched based on ethnicity. In addition, the resolution of the images acquired with our MRI system restricts our analyses to trabecular bone, and is not appropriate for the assessment of distal radius cortical bone. Finally, no prospective data were available at study inception on the change in the size or number of trabecular bone holes, therefore

we were unable to estimate an ideal sample size required at follow-up to capture differences in these key variables. Given our study limitations, fully powered studies with more complete follow-up and assessment of potential confounders are needed.

This study provides early evidence suggesting that women with diabetes experience an increase in the number of trabecular bone holes, while there are no differences in the change in other variables of trabecular bone microarchitecture over two years in women with and without type 2 diabetes. We speculate that an increase in the number of trabecular bone holes might reflect the trabecularization of cortical bone in women with diabetes, which warrants further investigation. Understanding whether microarchitectural adaptations with diabetes are distinctly different from age-related changes would inform future research and fracture prevention strategies in adults with type 2 diabetes.

3.6 ACKNOWLEDGEMENTS

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CHAPTER FOUR

AUTHOR'S PREFACE TO CHAPTER FOUR

In this chapter, I describe a study comparing microscopic measures of bone mineralization between two groups: one group of men and women with type 2 diabetes and a control group of men and women without type 2 diabetes. An *ex vivo* cross-sectional study design was used to explore differences in bone mineralization density distribution (BMDD) outcomes obtained with quantitative backscattered electron imaging (qBEI). This work is the first to show elevated mean bone calcium concentration and reduced mineralization heterogeneity in bone samples from men and women with type 2 diabetes compared to controls. This study provides evidence that material *bone quality* is altered in adults with type 2 diabetes, which may explain the observed elevated fracture risk in adults with type 2 diabetes.

The material presented in Chapter Four was submitted for publication in September 2012 to the peer-reviewed journal, *BONE.* An acceptance or rejection response is pending. I was responsible for designing the study, recruiting study participants, obtaining bone specimens from the surgeons, preparing the samples for imaging, performing image analysis and statistical analysis, interpreting the data and composing the manuscript. Dr. Schwarcz primarily supervised the project, and was instrumental in developing the qBEI methodology, interpreting the data and providing guidance throughout the study. Cora Tomowich assisted with qBEI methodology development, sample preparation and imaging. The expertise of Drs. Papaioannou, Giangregorio, Atkinson, Beattie and Adachi was

valuable for designing the study and providing comments on the manuscript. Orthopedic surgeons, Drs DeBeer, Winemaker and Avram, assisted with participant recruitment, conducted the surgical procedures and provided comments on the manuscript.

Bone mineralization is elevated and less heterogeneous in adults with type 2 diabetes compared to controls

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4.1 ABSTRACT

PURPOSE: The purpose of this study was to determine whether trabecular bone mineralization differed in adults with type 2 diabetes compared to adults without type 2 diabetes.

METHODS: Proximal femur specimens were obtained following a total hip replacement procedure from men and women ≥ 65 years of age with and without type 2 diabetes. A scanning electron microscope was used for quantitative backscattered electron imaging (qBEI) analysis of trabecular bone samples from the femoral neck. Gray scale images (pixel size= 5.6 μm^2) were uploaded to ImageJ software and gray level (GL) values were converted to calcium concentrations (weight [wt] % calcium [Ca]) using data obtained with energy dispersive x-ray spectrometry. The following bone mineralization density distribution (BMDD) outcomes were collected: the weighted mean bone calcium concentration (Ca_{MEAN}), the most frequently occurring bone calcium concentration (Ca_{PEAK}) and mineralization heterogeneity (Ca_{WIDTH}). Differences between groups were assessed using the Student's *t*-test for normally distributed data and Mann-Whitney *U*-test for non-normally distributed data. An alpha value of < 0.05 was considered significant.

RESULTS: Thirty-five Caucasian participants were recruited (mean ± standard deviation [SD] age, 75.5±6.5 years): 14 adults with type 2 diabetes (years since type 2 diabetes diagnosis, 13.5±7.4 years) and 21 adults without type 2 diabetes. In the adults with type 2 diabetes, bone Ca_{MEAN} was 4.9% greater (20.36±0.98 wt

% Ca versus 19.40±1.07 wt % Ca, p= 0.015) and Ca_{WIDTH} was 9.4% lower (median [interquartile range] 3.55 [2.99-4.12] wt % Ca versus 3.95±0.71 wt % Ca, p<0.001) compared to controls. There was no between-group difference in Ca_{PEAK} (21.12±0.97 wt % Ca for type 2 diabetes versus 20.44±1.30 wt % Ca for controls, p=0.121).

CONCLUSION: The combination of elevated mean calcium concentration in bone and lower mineralization heterogeneity in adults with type 2 diabetes may have deleterious effects on the biomechanical properties of bone. These microscopic alterations in bone mineralization, which may be mediated by suppressed bone remodeling, further elucidate higher fracture risk in adults with type 2 diabetes.

4.2 INTRODUCTION

Adults with type 2 diabetes have an elevated risk of hip fracture compared to those without diabetes (1, 2) despite higher bone mineral density (BMD) (3). When used in fracture risk prediction models, dual x-ray absorptiometry (DXA)-derived BMD measurements improve fracture discrimination in non-diabetic populations compared to not using BMD (4, 5). However, fracture risk prediction models incorporating BMD for adults with type 2 diabetes could be potentially misleading; there is evidence that the risk of fracture is increased in adults with type 2 diabetes even after controlling for BMD and other risk factors (6). Areal BMD can be confounded by bone size (7), degenerative changes in the spine (8), and overlying fat (9). Therefore using BMD as a measure of bone health in adults with type 2 diabetes may not be predictive of fracture in this group. Although BMD does contribute to bone strength, fracture susceptibility is also influenced by microscopic tissue qualities such as bone mineralization, which may play a greater role in bone strength in those with type 2 diabetes.

The bone remodeling process dictates bone mineralization. Suppressed bone remodeling allows the progression of secondary mineralization and mineral maturation (10, 11). Histomorphometric measures of bone remodeling are strongly correlated with the degree of bone mineralization (12, 13). In adults with type 2 diabetes, bone remodeling is suppressed compared to their non-diabetic counterparts (14-19), which could lead to delayed removal of bone packets, elevated bone mineralization and a reduction in mineralization heterogeneity.

Quantitative backscattered electron imaging (qBEI) is used for quantifying local mineralization variations in bone (20-22). The qBEI signal strength, reflected in gray level steps of an image is dependent on the elements present in a specimen (23). Therefore this method is useful for distinguishing between samples with differing average atomic number (24). When applied to bone samples, gray level is linearly related to calcium concentration (expressed as weight [wt] percent calcium [Ca], wt % Ca), where brighter regions of gray scale images of bone represent higher mineral concentration and darker regions represent lower mineral concentration (22, 25-27). This local variation in mineralization has been described as the bone mineralization density distribution (BMDD), from which the following key outcomes are derived: weighted mean calcium concentration (Ca_{MEAN}), most frequently occurring calcium concentration (Ca_{PEAK}), and the mineralization heterogeneity (Ca_{WIDTH}) (28). Variations in these BMDD variables in bone samples from populations with elevated fracture risk have been reported, suggesting the utility of this technique in quantifying meaningful variability in bone mineralization (12, 29-32). Furthermore, the use of qBEI to quantify mineral content in bone samples is both valid (25, 27) and reproducible (28).

The objective of this *ex-vivo* study was to determine whether bone mineralization assessed by qBEI is different in excised femoral neck trabecular bone samples from adults with type 2 diabetes compared to controls without diabetes. We hypothesized that Ca_{MEAN} and Ca_{PEAK} would be greater and

Ca_{WIDTH} would be lower in adults with type 2 diabetes compared to controls without diabetes.

4.3 MATERIALS AND METHODS

Study Participants

Participants were recruited from Hamilton Health Sciences Orthopedic Program at Juravinski Hospital in Hamilton, Canada to make up the convenience sample for this *ex-vivo* study. Recruitment occurred between August 2010 and December 2011, and groups were divided based on a diagnosis of type 2 diabetes (33). Participants were not matched based on age and gender as previous research suggests that these factors do not influence BMDD outcomes (34). Eligible participants were men and women who were undergoing total hip replacement due to osteoarthritis and were ≥ 65 years of age at the time of surgery. Total hip replacement procedure is indicated for patients who have severe radiographic joint degeneration and have not had adequate pain relief and improvement in function from non-pharmacologic and pharmacologic treatments (35). Potential participants were excluded from the study if they: 1) were currently taking or had taken osteoporosis-related medication (bisphosphonates, hormone therapy, selective estrogen receptor modulator, calcitonin, parathyroid hormone or denosumab) in the past 24 months; 2) had a history of metastatic cancer in the past 10 years 3); were currently taking systemic glucocorticoids for 3 months at a dose of > 2.5mg/day; or 4) had a diagnosis of severe renal disease (creatinine clearance < 30 mL/min) (36), hyperparathyroidism, hypoparathyroidism, Paget's

disease, Cushing's Syndrome, or osteogenesis imperfecta. The study protocol was approved by the McMaster University Faculty of Health Sciences/Hamilton Health Sciences Research Ethics Board.

Descriptive Data

The following information was collected for descriptive purposes: demographics, use of walking aids, history of an osteoporotic fracture (*i.e.,* non-vertebral or vertebral non-traumatic fracture (37), number of years since menopause, smoking status and presence of diseases included in the Charlson Index (38), number of years since type 2 diabetes diagnosis. Chart abstraction was performed to collect diabetes-related medication and daily calcium and vitamin D supplement intake (including multivitamins). Medication and supplement intakes were verified in the pre-operative participant interview. The Physical Activity Scale for the Elderly (PASE) was administered to estimate participation in activities over the past 7 days (39). Participants' height and weight were obtained from pre-operative consultation notes and BMI (kg/m^2) was calculated. Clinic staff ordered pre-operative random glucose and albumin, and post-operative creatinine. The Cockcroft-Gault equation was used to calculate creatinine clearance (36).

Sample Preparation

Immediately following surgical excision, each proximal femur was wrapped in saline soaked gauze for transportation. A 5 mm thick sagittal section of the femoral neck was cut at the most distal end of the sample using a handsaw at an

orientation expected to be approximately perpendicular to the Haversian canals in the cortical bone (Figure 1). Conventional methods were used for preparing the samples scanning electron microscopy (40). The anterior section was fixed in 0.2M glutaraldehyde (2% v/v) in 0.1M sodium cacodylate buffer pH 7.4 (41). Following fixation for 24 hours, samples were degreased with a series of methanol and chloroform washes and agitated for 30 minutes in an ultrasonic bath (Branson Ultrasonic Cleaner, Emerson Industrial Automation, St. Louis, MO, USA) to remove excess marrow. The samples were dehydrated in 70%, 80%, 90%, 96% and 100% ethanol washes (40) and dried at 60°C for 4 hours.

Quantitative Backscattered Electron Imaging (qBEI)

The anterior section was embedded in resin (15 parts EpoFix Resin and 2 parts Epoar EpoFix Hardener, Struers Ltd. Mississauga, ON, Canada) using evacuation (Struers Epovac, Struers Ltd. Mississauga, ON, Canada). Blocks with planoparallel surfaces were carefully prepared using 180 grit silicon carbide paper on a rotary wheel and a sequence of lapping disks (30 μm, 9 μm, 3 μm, 1 μm aluminum oxide particle size) (Allied High Tech Products Rancho Dominguez, CA, USA) with an aqueous suspension of 0.5 μm colloidal silica. Samples were rinsed with distilled water to remove residual colloidal silica and viewed under a dissecting microscope to ensure that there were no topographical artifacts (42). Samples were sputter-coated (Precision Etching Coating System, Model 682, Gatan Inc. Warrendale, PA, USA) with amorphous carbon, outlined with silver paint (High Purity Silver Paint, SPI Supplies, Structure Probe Inc. Westchester,

PA, USA) and mounted on a stub using carbon conductive tape. The thickness of each sample was measured using a digital micrometer (Digital Absolute Micrometer, Mitutoyo Canada Mississauga, ON, Canada).

A scanning electron microscope (SEM, Vega II LSU, Tescan USA Inc. Cranberry Township, PA, USA) equipped with a tungsten filament and an annular mono-crystal scintillator backscattered electron detector was used for qBEI. The electron beam accelerating voltage was 20 kV and emission current 110 pA. The emission current, which was set in a Faraday cup at the beginning of each imaging session, was checked at the end of each imaging session to ensure that major fluctuation (>1 pA) did not occur (28). A working distance of 15 mm was kept constant by adjusting the stage based on the thickness of the standards and each bone sample (mean [standard deviation, SD] thickness = 3.73 [0.41] mm). At a magnification of 50X and a scan speed of 48.6 μsec/pixel, 1024 x 1024 qBEI grayscale images were generated with a pixel size of 5.6 μm^2. The detector contrast (gain) and brightness (black) were set to 49.5% and 89.0%, respectively.

Given that qBEI intensity and gray level are proportional to average atomic number of a material, we scanned standards of known average atomic number (Z) (Carbon [C], Z = 6; magnesium oxide [MgO], Z = 10.41; aluminum [Al], Z = 13) before and after scanning each bone sample for standardization purposes. If the difference between the gray level values of the standard scanned before and after the bone sample was greater than 4 gray level steps (intra-assay technical variance of 0.27%) (28), the data from that imaging session were removed from

the analysis. The entire area of the bone sample was captured in separate frames (5.7 x 5.7 mm^2), with the number of frames captured being dependent on bone sample size (Figure 4.1). An average of 5 frames per sample were captured, to follow the methods used in other studies (12).

Figure 4.1 **A.** Schematic diagram representing a proximal femur and a typical section of femoral neck used for qBEI (anterior section). **B.** Example of the frames captured of a bone sample using qBEI and BMDD outcomes. **C.** Schematic of a BMDD histogram and outcome measurements, including the weighted mean calcium concentration (Ca_{MEAN}), the most frequently occurring calcium concentration (Ca_{PEAK}) and full width at half maximum of the histogram peak reflecting mineralization heterogeneity (Ca_{WIDTH}).

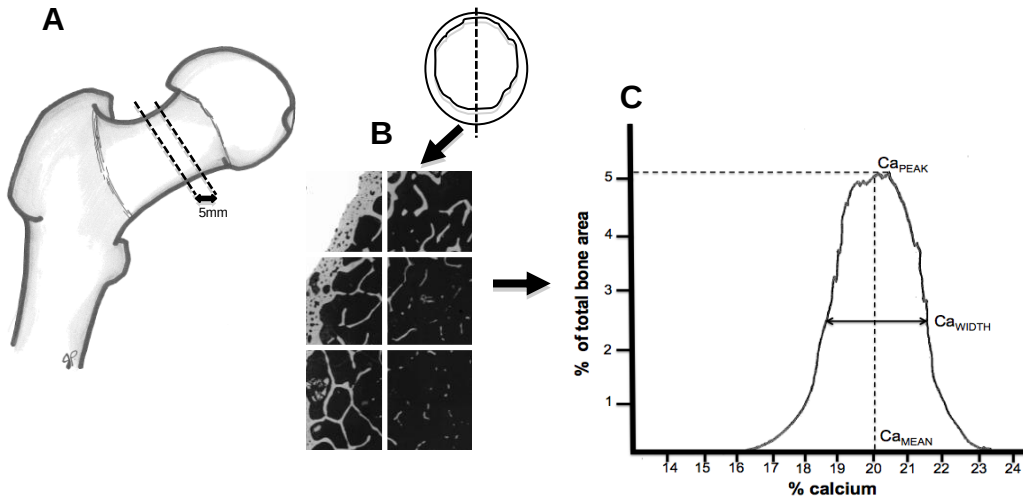


Image Analysis

Images of the standards and bone samples were uploaded to ImageJ (version 1.44o, National Institutes of Health, Bethesda, MD, USA) (24, 28). The gray scale images were composed of pixels with values between 0 and 255, where gray level = 0 corresponded to a pixel with zero backscattered electron intensity (black) and gray level = 255 corresponded to a pixel with full

backscattered electron intensity (white). Using the ‘rectangle’ selection tool, a consistent location was selected for gray level analysis for C, MgO and Al. For analysis of bone samples, a standard threshold level, GL_t, was used for image sets to differentiate between bone and marrow space, and the pixels associated with the marrow space were excluded from analysis. The maximum number of selections of trabecular bone was made in each frame using the ‘wand’ and ‘polygon’ selection tools, with the exception of areas containing artifact (*i.e.,* “bone dust” from the preparation process or organic matter). Cortical bone analysis was excluded from this study because cortical bone is composed of osteons, which undergo successive stages of Haversian remodeling (43). Because of the relatively slow rate of cortical remodeling compared to hemiosteonal remodeling of trabecular bone (44), the mineralization of cortical osteons may not entirely reflective of altered bone metabolism in adults with type 2 diabetes. That is, a significant fraction of cortical osteons could have formed and not yet been replaced prior to the onset or during the first years of diabetes. In addition, microcracks, and not bone remodeling kinetics, are believed to be the strongest stimulus for bone resorption in cortical bone (45, 46). Therefore, we focused on the more rapidly remodeling trabecular bone, which is more likely to reflect bone mineralization as a result of type 2 diabetes.

Gray level frequency histograms were exported to Microsoft Excel (Microsoft Excel for Mac 2011, version 14.1.3, Microsoft Canada Co. Mississauga, ON, Canada) where the weighted mean gray level (WMGL) was

determined according to the formula (24, 47, 48):

$$WMGL = \Sigma A_i * GL_i / A_t \qquad (1)$$

where A_i is the number of pixels with the *i*th gray level value, GL_i is the *i*th gray level > GL_t, and A_t is the total number of pixels of bone. In addition, the gray level values for the peak (mode) of the distribution and the full width at half maximum (FWHM) were determined (28) (Figure 4.1). A researcher who was blinded to group allocation completed image analyses.

Gray Level Standardization

The standards were used to graph the relationship between average atomic number and gray level, similar to the methods described by others (24, 28, 47). For each imaging session of a bone sample, a *standard trend-line* (L_i) was generated, and average y-intercept (b) and slope (m) were calculated for all sessions, yielding an *average standard trend-line* (L_μ) ($y = 0.031x+5.505$, $R^2 = 0.99$). Using a linear transformation, gray level values of bone were standardized in order for gray level values from independent imaging sessions to be compared. The following formulae was used:

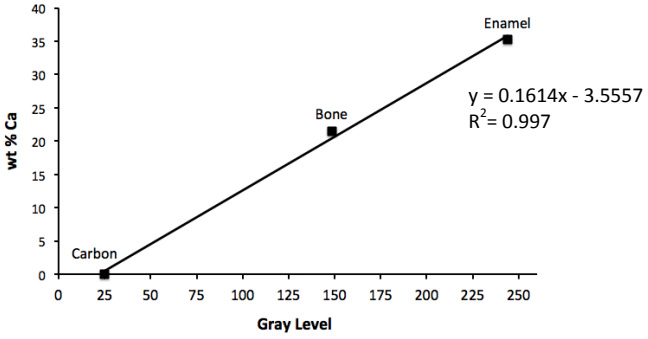
$$GL_{std} = [(m_i * GL_i) + (b_i - b_\mu)]/ m_\mu \qquad (2)$$

where GL_{std} is the standardized gray level value for the bone used in analysis; GL_i is the non-standardized gray level for the bone; m_i and b_i are the slope and y-intercept, respectively of L_i; and m_μ and b_μ are the slope and y-intercept, respectively of L_μ.

Conversion of Gray Levels to Calcium Concentration

The WMGL, peak and FWHM gray level values were converted to bone sample calcium (Ca) concentration, expressed as wt % Ca (26-28). Using the qBEI parameters and image analysis protocol described above, we determined the mean gray level of 10 points on a homogeneous area of tooth enamel (36.1 wt % Ca) (49) and carbon (0 wt % Ca). We also randomly selected a bone sample and determined the mean gray level of 10 points within a uniform area of trabecular bone. Immediately following qBEI, energy dispersive x-ray spectrometry (INCA X-max microanalysis system, Oxford Instruments, Tubney Woods, Abingdon, Oxfordshire, UK) was used to determine the wt % Ca of the points analyzed with qBEI. The probe current and working distance remained at 20 kV and 15 mm, respectively as these are the optimal settings for the x-ray detector, and magnification was set at 300X. To quantify the wt % Ca in each sample, a pure sample of calcite ($CaCO_3$) was used as a reference standard. The mean GL value and wt % Ca were plotted against each other (Figure 4.2).

Figure 4.2 Standardization of gray level values to calcium concentrations (wt % Ca) based on the linear relationship between GL and wt % Ca



Using the linear relationship ($y = 0.1614x - 3.5557$, $R^2 = 0.997$), the WMGL, mode gray level and FWHM gray level values were converted to wt % Ca. To assess the reproducibility of the qBEI and image analysis technique, the standards and one bone sample were scanned on 8 different days. The coefficient of variation (CV%) for the qBEI technique and image analysis was 1.8% for Ca_{MEAN}, 1.6% for Ca_{PEAK}, and 3.6% for Ca_{WIDTH}.

Statistical Analysis

Normality of the data was tested using the Kolmogorov-Smirnov test (50). Data are presented as mean (SD) for normally distributed data, and median (interquartile range) for non-normally distributed data. Data for Ca_{MEAN}, Ca_{PEAK}, and Ca_{WIDTH} were compared between the group of adults with type 2 diabetes and the control group without type 2 diabetes using an independent Student's *t*-test for normally distributed data, and Mann-Whitney *U*-test for non-normally distributed data. The sample size was estimated using data on bone mineralization in patients with mild primary hyperparathyroidism (12). As Ca_{MEAN} is the primary outcome for the present study, the sample size was based on the data for this variable. The difference in Ca_{MEAN} between adults with and without mild primary hyperparathyroidism was 0.57 wt % Ca with an average standard deviation of 0.53 wt % Ca (12). Using a power of 80% and an alpha level of 0.05, the estimated sample size was 14 participants per group. Analyses were completed using SPSS version 20.0 for Macintosh (IBM Corporation. Markham, ON, Canada). The criterion for statistical significance was alpha level < 0.05.

4.4 RESULTS

The study participants included 14 adults with type 2 diabetes and 21 adults without diabetes (Table 4.0).

Table 4.0 Descriptive characteristics of participants enrolled in the study.

	Participants with type 2 diabetes n= 14	**Control n= 21**	**Difference between groups *p-value***
Age, years	73.8 (6.0)	76.4 (6.8)	0.245
Female, n (%)	6 (42.9)	14 (66.7)	0.221
History of non-traumatic osteoporotic fracture[1], n (%)	3 (21.4)	3 (14.3)	0.541
BMI, kg/m^2	30.7 (7.4)	28.9 (5.4)	0.462
Time since menopause[2], years	27.6 (5.8)	31.0 (7.2)	0.348
Current smoker, n (%)	2 (14.3)	3 (14.3)	0.956
Number of prescribed medications	5.8 (2.9)	5.1 (1.6)	0.376
Age-adjusted Charlson Index	4.4 (0.8)	2.2 (2.4)	<0.001
Calcium intake from supplements[3], mg/day	463 (560)	343 (462)	0.511
Vitamin D_3 intake from supplements[3], IU/day	785 (1224)	559 (651)	0.547
PASE score	85.6 (37.8)	82.3 (82.7)	0.884
Serum biochemistry			
Random glucose, mmol/L	8.3 (2.1)	5.6 (1.1)	<0.001
Creatinine, µmol/L	75.6 (20.4)	75.4 (19.9)	0.978
Cockcroft-gault value, mL/min	96.2 (31.4)	86.2 (24.4)	0.319
Albumin, g/L	32.9 (3.5)	33.9 (4.1)	0.562

Values are mean (SD), unless indicated otherwise. *Abbreviations:* BMI, body mass index; PASE, Physical Activity Scale for the Elderly; IU, international units [1] Non-traumatic osteoporotic fracture includes hip, wrist, spine or proximal humerus fracture occurring after age 40.[2] Number of years since menopause reported from female participants only [3] Including amount from multivitamin, if applicable

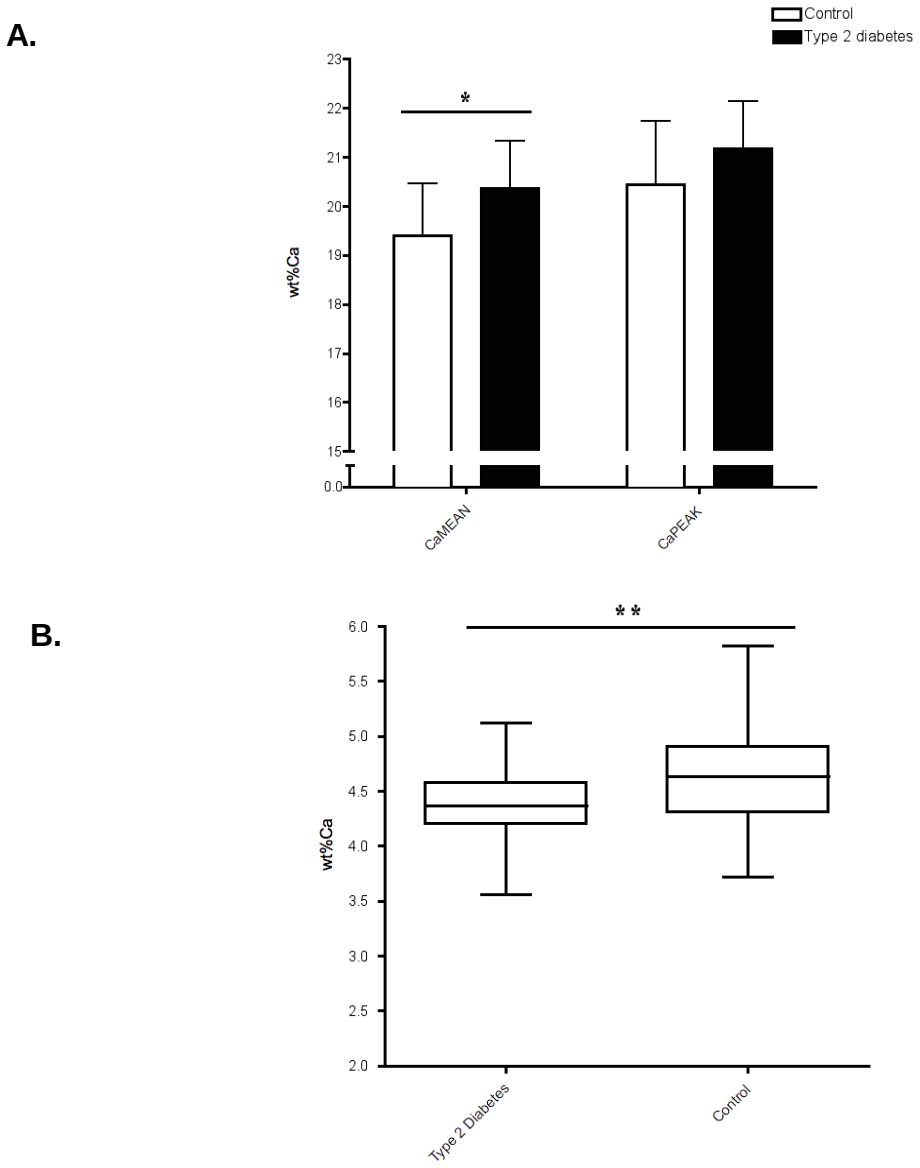
The mean (SD) age of all participants was 75.5 (6.5) years. All study participants were Caucasian, and the average time since menopause for the female participants was 30 (7) years. The majority of participants with and without

type 2 diabetes used a walking aid (9/14 [64.3%] and 13/21 [61.9%] respectively, p = 0.736). The average time since diagnosis of type 2 diabetes was 13.5 (7.4) years. In the type 2 diabetes group, three participants (3/14 [21.4%]) were diet-controlled, 7 participants (7/14 [50.0%]) were taking a biguanide or insulin secretagogue sulfonylurea, and 4 participants (4/14 [28.6%]) were on insulin therapy.

Bone Mineralization: qBEI

After removing the invalid data sets,13 samples from adults with type 2 diabetes and 19 samples from adults without diabetes were analyzed. Between-group comparison of the bone mineralization outcomes, shown in Figure 4.3, revealed that Ca_{MEAN} was 4.9% greater in the group of adults with type 2 diabetes (20.36 [0.98] wt % Ca versus 19.40 [1.07] wt % Ca, p = 0.015). Ca_{WIDTH}, which reflects the mineralization heterogeneity, was 9.4% lower in adults with type 2 diabetes compared to controls (3.55 [2.99 − 4.12] wt % Ca versus 3.95 [0.71] wt % Ca, p < 0.001). There was no difference in Ca_{PEAK} between groups (21.12 [0.97] wt % Ca for type 2 diabetes versus 20.44 [1.30] wt % Ca for controls, p = 0.121).

Figure 4.3 Comparison of BMDD outcomes (**A.** Ca_{MEAN}, Ca_{PEAK}; **B.** Ca_{WIDTH}) between adults with type 2 diabetes and adults without type 2 diabetes.



Between-group difference, * p<0.05, ** p<0.001
Abbreviations: Ca, calcium; wt%Ca, calcium concentration; weighted mean calcium concentration (Ca_{MEAN}); the most frequently occurring calcium concentration (Ca_{PEAK}); full width at half maximum of the histogram peak reflecting mineralization heterogeneity (Ca_{WIDTH}).

4.5 DISCUSSION

Our key findings were that the mean calcium concentration was higher and mineralization less heterogeneous in femoral neck trabecular bone samples from adults with type 2 diabetes compared to controls without diabetes. Given that these bone qualities can influence bone strength, our findings may provide some insight into increased bone fragility that has been observed in adults with type 2 diabetes.

Elevated bone mineralization in adults with type 2 diabetes may result in bone brittleness, lower bending strength, reduced fracture toughness and poor energy absorption (51-56). Microcracks are also more likely to form in highly mineralized cortical bone samples from the femoral neck (57) and in highly mineralized regions of trabecular bone in samples from patients with a history of an osteoporotic fracture (53). The potential mechanisms causing elevated bone calcium concentration in adults with type 2 diabetes are suppressed bone remodeling and/or accumulation of advanced glycation end-products in the bone. When bone remodeling is reduced, the secondary mineralization phase is lengthened. This causes an increase in mineral crystal size and mineral content, which may explain our finding of elevated bone calcium concentration in adults with type 2 diabetes (58, 59). Advanced glycation end-products formed by the Maillard reaction (60) are found in the urine and serum of adults with type 2 diabetes (61, 62) and can bind to the nitrogen sites of type 1 collagen in bone. The binding of advanced glycation end-products increases the number of

carboxyl groups on the surface of the collagen fibrils, which serve as nucleation sites for hydroxyapatite formation (63). Regardless of the mechanism causing elevated mineralization in bone specimens from adults with type 2 diabetes, an imbalance in mineral and collagen in bone could lead to a less ductile and more brittle material that requires less energy to fracture (55).

Suppressed bone remodeling may also explain the finding of reduced mineralization heterogeneity in adults with type 2 diabetes Suppressed bone remodeling leads to a greater amount of time between bone packet resorption and formation, resulting in a more uniform bone material comprised of bone packets of a similar calcium concentration (64). The dependence of bone calcium content and mineralization heterogeneity on the rate of bone remodeling have been shown in patients who experience an increase in bone remodeling with teriparatide (human parathyroid hormone [1-34]) (65) and in patients who experience a reduction in bone remodeling with bisphosphonates (30, 68). In postmenopausal women treated with teriparatide for one year, a 10% increase in mineralization heterogeneity is observed due to the formation of new bone packets (65). In bisphosphonate treated patients, an increase of approximately 3 − 5% in mean bone calcium concentration occurs concomitantly with a 20-30% reduction in mineralization heterogeneity, caused by the antiresorptive action of bisphosphonates (68, 69). Of note, increases in bone volume and improvements in bone microarchitecture often accompany changes in bone mineralization in bisphosphonate-treated patients, which may also contribute to the fracture

prevention efficacy of bisphosphonates (68, 70). Whether mineralization heterogeneity is important to overall bone fragility is not clear. One study reported no impact of mineralization heterogeneity on bone mechanical properties (66), while others suggest that a more heterogeneously mineralized tissue results in better defense against microcrack propagation (67). In adults with type 2 diabetes, it may be that bone calcium concentration is elevated to a point that is pathological to bone health (*i.e.,* higher than bisphosphonate-elevated bone calcium concentration). The contribution of reduced mineralization heterogeneity to bone fragility is not clear and requires further research.

Our findings in humans are in contrast to what has been observed in in a rodent model of type 2 diabetes (71). Hamann and colleagues reported no diabetes-attributable difference in mean calcium concentration in distal femur bone specimens, but did report an elevation in mineralization heterogeneity the rodent model of type 2 diabetes compared to wildtype (71). However, the skeletal phenotypes of type 2 diabetes in the rodent model and in humans are different, in that lower BMD and higher bone resorption is observed in rodents with diabetes (71), compared to higher BMD and chronic low bone remodeling in adults with longer-standing type 2 diabetes (3, 14-19).

There are several study limitations to acknowledge. This was a cross-sectional study of patients who were undergoing total hip replacement, and the amount of femoral neck on the excised specimen was dependent on the level at which the surgeon resected the femoral neck to accommodate the prosthesis.

Therefore, the 5 mm section of bone was not from a uniform location on the femoral neck, although there is no evidence suggesting that this would influence the BMDD outcomes. Bone mineralization may be elevated in patients with osteoarthritis due to osteophyte production; however our samples were obtained from the femoral neck where osteophytes were not evident. There are also no muscle insertion sites along the femoral neck ruling out the possibility that elevated mineralization was an artifact due to calcified fibrocartilaginous areas (56). Study participants with type 2 diabetes were on various types of medication for diabetes control. It was beyond the scope of the present study to investigate the influence of different medications on bone mineralization, but certain medications can modify bone remodeling (72) and potentially BMDD outcomes. Finally, we did not assess bone turnover markers, as a much larger sample size would have been required. Numerous well-powered studies have found that men and women with longer-standing type 2 diabetes have lower concentrations of bone turnover markers compared to controls without diabetes (14-19).

This study revealed that there are microscopic differences in bone mineralization in older adults with type 2 diabetes compared to controls. The mean bone calcium concentration was elevated and there was less mineralization heterogeneity, possibly explained by suppressed bone remodeling in adults with type 2 diabetes. These differences in mineralization may contribute to bone brittleness and reduced defense against microcrack propagation. The findings from our study emphasize a need for future research on osteoporosis-

related medications and bone mineralization in adults with type 2 diabetes. Suppressing bone remodeling further with bisphosphonates in adults with type 2 diabetes (Keegan et al., 2004) might further increase bone calcium concentration and bone brittleness. The impact of other interventions on bone quality and fracture prevention in adults with type 2 diabetes should be explored.

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CHAPTER FIVE

DISCUSSION

This collection of work demonstrates differences in *bone quality* in adults with type 2 diabetes compared to adults without type 2 diabetes. Differences in structural *bone qualities* were demonstrated in the cross-sectional study entitled, "*Association of larger holes in the trabecular bone at the distal radius in postmenopausal women with type 2 diabetes mellitus compared to controls*" and in the longitudinal study entitled, "*Longitudinal changes in trabecular bone microarchitecture in postmenopausal women with and without type 2 diabetes*". Differences in material *bone qualities* were demonstrated in the cross-sectional *ex vivo* study entitled, "*Bone mineralization is elevated and less heterogeneous in adults with type 2 diabetes compared to controls"*. The contribution of these studies to current literature and considerations for future research are discussed in the subsequent sections.

5.1 Structural *Bone Quality*: Contribution to the Literature

The key findings related to structural *bone quality* reported in Chapter Two and Chapter Three, respectively, are: 1) trabecular bone hole size is greater in postmenopausal women with type 2 diabetes compared to postmenopausal women without type 2 diabetes, and 2) there is greater percent increase in the number of trabecular bone holes at the distal radius over two years in postmenopausal women with type 2 diabetes compared to postmenopausal women without type 2 diabetes.

A few studies in humans have also explored bone structural differences in adults with type 2 diabetes and non-diabetic controls. Similar to the study described in Chapter Two, these studies were cross-sectional studies and used a non-invasive imaging modality (HR-pQCT) to assess structural differences attributable to diabetes. In these studies, measures of trabecular bone microarchitecture at the distal radius were not different in postmenopausal women with type 2 diabetes compared to postmenopausal women without type 2 diabetes (Burghardt et al.; Shu et al., 2011). It is possible that study power was not sufficient to detect between-group differences in multiple measures of trabecular bone microarchitecture because sample size justifications were not made in these studies. Contrary to these negative studies, we found that trabecular bone microarchitecture is different, evidenced by larger trabecular bone holes at the distal radius in postmenopausal women with type 2 diabetes. The size of trabecular bone holes was not explored in these smaller studies by Burghardt and colleagues and Shu and colleagues. Previous *ex vivo* and *in vivo* studies have shown that greater trabecular bone hole size is detrimental to bone strength (Gordon, Lang, et al., 1998; Gordon, Webber, et al., 1998; MacIntyre et al., 2003). Compared to trabecular bone connectivity and density, Gordon and coworkers demonstrated that trabecular bone hole size was more predictive of peak load at fracture for bone specimens (Gordon, Webber, et al., 1998). Women with a history of a wrist fracture had greater trabecular bone hole size at the distal radius, which was associated with greater fracture risk (MacIntyre et al., 2003).

Therefore, our results provide a structural *bone quality* explanation for increased fracture risk in postmenopausal women with type 2 diabetes.

The study described in Chapter Three is the first prospective study to investigate changes in trabecular bone microarchitecture in postmenopausal women with type 2 diabetes. Like cross-sectional studies, prospective studies are descriptive, therefore causation cannot be inferred from study results due to susceptibility to bias (Levine, 2008). An advantage to using a prospective study design over a cross-sectional design is that insight can be gained into the time-course of skeletal changes in diseases, or in response to pharmacologic therapies. This study design was employed to identify whether trabecular bone microarchitecture changes are different in postmenopausal women with type 2 diabetes compared to controls. This is important to understand because pharmacologic therapies can be employed to prevent microarchitectural loss, improve trabecular bone connectivity and reduce fracture risk (Borah et al., 2004; Dufresne, Chmielewski, Manhart, Johnson, & Borah, 2003; Jiang et al., 2003).

In Chapter Three, the study showed that the only difference in trabecular bone microarchitectural change was an increase in the number of trabecular bone holes at the distal radius in women with type 2 diabetes, which could represent the trabecularization of cortical bone. This phenomenon of trabecularization of cortical bone has been described as a process of erosion of the endocortical bone surface, producing cortical bone remnants that resemble trabecular bone, and blurring the border between trabecular and cortical

compartments (Zebaze et al., 2010). Evidence of trabecularization of cortical bone has been reported in older adults (Simmons, Pritzker, & Grynpas, 1991) and in hip fracture patients (Bell et al., 2000). Simmons and colleagues suggest that cortical and not trabecular bone resorption is responsible for age-related bone loss at the distal radius (Simmons et al., 1991). In a study using pQCT, Brancaccio and colleagues hypothesized that in patients with secondary hyperparathyroidism, the trabecularization of cortical bone lead to reduced bone strength in this patient population (Brancaccio et al., 2003). A couple of mechanisms responsible for trabecularization of cortical bone have been proposed. Elevated bone remodeling is believed to lead to the coalescence of Haversian canals in cortical bone in older adults, in hip fracture patients and in patients with secondary hyperparathyroidism (Bell et al., 2000; Brancaccio et al., 2003; Simmons et al., 1991). However, in postmenopausal women with longer-standing type 2 diabetes, elevated bone remodeling is an unlikely explanation for trabecularization of cortical bone because bone remodeling is suppressed (Dobnig et al., 2006; Garcia-Martin et al., 2012; Gerdhem et al., 2005; Oz et al., 2006; Reyes-Garcia et al., 2011). Another mechanism that could apply to adults with type 2 diabetes is disuse, as this has been shown to cause an increase in the number of pores along the endosteal border of cortical bone in turkey ulnas (Gross & Rubin, 1995). Regions of cortical bone that experience low levels of strain have more remodeling sites than sites with high levels of strain (Gross & Rubin, 1995). In adults with type 2 diabetes, sedentary living could contribute to

disuse and the trabecularization of cortical bone. This hypothesis may explain grater bone fragility in those with longer-standing type 2 diabetes, which has been documented in a population-based study (Leslie et al., 2007).

In terms of the change in the other measures of trabecular bone microarchitecture, there was no difference between postmenopausal women with type 2 diabetes and controls. Although BMD does not directly provide information about trabecular bone microarchitecture, BMD measurements are moderately related to trabecular bone microarchitecture measurements (Majumdar, 1999). Therefore, prospective studies on BMD change in women with type 2 diabetes (Hamilton et al., 2011; Kanazawa et al., 2010; Keegan et al., 2004; Khalil et al., 2011; Krakauer et al., 1995; Miazgowski et al., 2011; Schwartz et al., 2005) are informative and aid in understanding our findings. These prior prospective studies have shown that BMD loss does not appear to be different in postmenopausal women with type 2 diabetes compared to controls without diabetes 10-15 years after the menopause (Krakauer et al., 1995). BMD loss is, however, the greatest in women with and without type 2 diabetes between the perimenopausal (no menstruation in 3-11 months) and postmenopausal phases (Khalil et al., 2011). In addition, significant BMD loss occurs within the first five years of type 2 diabetes diagnosis (Levin, Boisseau, & Avioli, 1976). As type 2 diabetes progresses in an individual, β-cell function declines and treatment regimes may need to change from oral anti-hyperglycemics to insulin therapy to maintain glycemic control (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee,

2008; Turner, Cull, Frighi, & Holman, 1999). Studies in rodents suggest that insulin use might avert bone loss (Hou et al., 1993) and clinical trials are presently being conducted to determine whether sitagliptin, a dipeptidyl peptidase-4 inhibitor which increases insulin secretion, reduces bone destruction in patients with type 2 diabetes who are in the early stages of the disease (ClinicalTrials.gov Identifier: NCT00732121). A lack of change in most measures of trabecular bone microarchitecture in the study described in Chapter Three may further be explained by the fact that participants were postmenopausal for approximately two decades, had long-standing type 2 diabetes (greater than 5 years) and 80% of study participants were prescribed insulin.

5.1.1 Suggestions for Future Research

The studies presented in Chapter Two and Three provide novel preliminary data on structural *bone quality* in women with type 2 diabetes that should be explored in future research endeavours. The use of the 1 Tesla MRI system for assessing trabecular bone microarchitecture was a unique aspect in both studies. While pQCT and MRI-derived measures of trabecular bone hole size are highly related (Gordon, Webber, et al., 1998), future research should determine whether the predictability of bone strength is as strong for 1 Tesla MRI-derived measures of trabecular bone hole size as pQCT-derived measures (MacIntyre et al., 2003). A prospective study investigating the relationship between trabecular bone hole size and incident fracture in postmenopausal

women with type 2 diabetes would also be informative. Given that men with type 2 diabetes are also at high risk for fracture compared to non-diabetic controls (Ahmed et al., 2006; de Liefde et al., 2005; Forsen L, 1999; Strotmeyer et al., 2005), it would be valuable to determine whether trabecular bone hole size is also greater in men, and whether this measurement predicts fracture.

Future studies are also needed to test the hypotheses presented in Chapter Two regarding the potential mechanisms responsible for making trabecular bone holes larger in women with type 2 diabetes. If it is confirmed that AGEs and inflammatory cytokines are predominant factors causing bone destruction in obese adults and adults with impaired glucose tolerance, randomized controlled trials can focus on interventions that blunt these factors. Dietary improvements and exercise interventions (aerobic or resistance training) have been shown to reduce total body fat and circulating inflammatory markers in obese postmenopausal women (Fisher et al., 2011). The impact of exercise interventions paired with pharmacologic interventions to inhibit the production of AGEs (Thornalley, 2003) or a diet tailored to reduce the consumption of AGE-rich foods (Negrean et al., 2007) on trabecular bone holes size could be explored in future studies. Intensive glucose control with insulin reduces fasting plasma glucose compared to diet or sulfonylurea controlled type 2 diabetes (UKPDS, 1998). Therefore, intensive glucose control could inhibit the increase in trabecular bone hole size in women with type 2 diabetes by preventing the negative downstream effects of hyperglycemia over time.

The hypothesis that trabecularization of cortical bone occurs in women with type 2 diabetes ought to be tested in future research using higher resolution imaging systems such as HR-pQCT. It is important to use a modality that can sufficiently resolve cortical bone because if trabecularization of cortical bone occurs over time in adults with type 2 diabetes, a decrease in cortical thickness will be observed concomitantly with an increase in the number of trabecular bone holes. In addition, disuse should be explored as a possible mechanism involved in the trabecularization of cortical bone. The data from our study could be used for future sample size calculations for a larger prospective study involving a spectrum of premenopausal women to postmenopausal women with varying durations of type 2 diabetes. Identifying the key periods (*i.e.,* perimenopausal or postmenopausal phase) when trabecular bone microarchitecture begins to lose integrity (*i.e.,* greatest increases in trabecular bone hole size) would be helpful for designing randomized controlled trials.

For both studies, we employed a spoiled gradient-echo pulse sequence for imaging trabecular bone microarchitecture, which is widely accepted (Boutry et al., 2003; Chesnut et al., 2005). Though debate is on-going about the optimal pulse sequence for imaging trabecular bone, it would be interesting to explore the use of other pulse sequences, such as a spin-echo sequence (Ma, Wehrli, & Song, 1996) which offers less image distortion and may provide more accurate measures of trabecular bone, such as Tb.Th (Krug, Carballido-Gamio, et al., 2008). Given that the 1 Tesla MRI system is restricted to imaging appendicular

sites, it would be useful to determine how well trabecular bone hole size at the distal radius correlates with trabecular bone hole size at the spine or proximal femur measured with a higher magnetic field MRI system (1.5 or 3.0 Tesla). Other studies have found promising results and strong correlations between MRI systems of different magnetic strengths (Krug et al., 2005). Future research to expand upon the methodology employed in our studies will improve the clinical utility of the 1 Tesla MRI and make it an acceptable option for widespread non-invasive assessment of trabecular bone microarchitecture.

5.2 Material *Bone Quality*: Contribution to the Literature

The study described in Chapter Four is the first study to demonstrate: 1) elevated mean calcium concentration and, 2) lower mineralization heterogeneity in trabecular bone samples from adults with type 2 diabetes compared to controls without diabetes. Because measures of bone mineralization assessed with qBEI are similar for healthy men and women over a spectrum of ages and different races, changes due to disease or medications may be clinically important (Roschger et al., 2003).

Diabetic bone is stiffer, but cannot withstand as much load as non-diabetic bone prior to breaking (Einhorn et al., 1988; Reddy, Stehno-Bittel, Hamade, & Enwemeka, 2001; Verhaeghe et al., 1994). In rodents, neither ash measurements of mineral content or qBEI-derived measurements of calcium concentration in bone explain compromised bone strength (Hamann et al., 2011;

Verhaeghe et al., 1994; Verhaeghe, Visser, et al., 1990). Our finding of higher mean bone calcium concentration in bone samples from adults with type 2 diabetes may explain the elevated fracture risk observed in adults with type 2 diabetes because too much mineral in bone can result in increased stiffness and brittleness (Busse et al., 2009; Currey, 1979, 1984; Currey et al., 1996; McCormack et al., 2012; Vajda & Bloebaum, 1999). Microcracks are also more likely to form and propagate through highly mineralized bone (McCormack et al., 2012; Vajda & Bloebaum, 1999; Wasserman, Yerramshetty, & Akkus, 2005), and bone that has less mineralization heterogeneity. According to crack propagation theory in composite materials proposed by Kendall (1975), a microcrack will continue to grow under an applied load until it meets an interface of new material. In cortical bone, osteons of varying mineral content act as barriers to microcrack propagation (O'Brien et al., 2005). In trabecular bone, the impact of mineralization heterogeneity on crack propagation is not as well understood. Using μCT and finite element modeling, simulated trabecular bone that is made up of more heterogeneous bone packets has stiffness values that are 20% greater than more uniform bone (van der Linden, Birkenhager-Frenkel, Verhaar, & Weinans, 2001), but whether this would be advantageous or deleterious in adults with already highly mineralized bone is not known. Other studies, however, have shown that mineralization heterogeneity does not influence whole bone strength (Follet et al., 2011). Whether mineralization heterogeneity actually influences bone strength is

difficult to discern, chiefly because it is difficult to biomechanically test individual trabeculae.

5.2.1 Suggestions for Future Research

Future studies should focus on understanding the clinical implications of elevated mean calcium concentration and reduced mineralization heterogeneity in bone samples from adults with type 2 diabetes. Microcomputed tomography using synchrotron radiation could be used to obtain measurements of bone mineralization and finite element models in bone samples from adults with type 2 diabetes to estimate the impact of augmented mineralization on bone strength (Nuzzo et al., 2002). In order to relate our findings to fracture risk, a prospective study should be undertaken. It would be useful to determine whether elevated Ca_{MEAN} and reduced Ca_{WIDTH} are predictors of incident fracture. To test the hypothesis that low levels of IGF-1 and binding proteins drive the suppression of bone turnover in adults with type 2 diabetes, future studies should measure levels of IGF-1, IGF binding proteins, P1NP, CTX and qBEI-derived measures of bone mineralization. These alterations in bone mineralization in adults with type 2 diabetes raise an important clinical question. Should adults with type 2 diabetes be managed differently regarding pharmacologic therapy for fracture prevention?

In Canada, there are various pharmacologic therapies available for men and women to prevent fractures. These include antiresorptive therapies (bisphosphonates, denosumab, raloxifene) or an anabolic therapy (teriparatide)

(Papaioannou et al., 2010). Antiresorptive agents reduce the rate of bone remodeling (Eastell, Christiansen, et al., 2011; Eastell, Rogers, Ni, & Krege, 2011; Grey et al., 2010), increase BMD and reduce the risk of non-vertebral and vertebral fractures by 20-25% and 40-70%, respectively (Hopkins et al., 2011). Antiresorptive agents have also been shown to increase bone mineralization and reduce mineralization heterogeneity (Boivin et al., 2000; Roschger et al., 2001). Given that bone remodeling is suppressed in adults with type 2 diabetes and BMD is already higher than normal, it is not known whether suppressing bone remodeling further with antiresorptive agents is efficacious at preventing fractures. In one study exploring the effect of raloxifene on bone remodeling in women with type 2 diabetes, there was a treatment-associated reduction in bone resorption in the raloxifene group compared to the placebo group over 6 months (Mori et al., 2012). In another study, treatment with alendronate for 3 years lowered levels of bone resorption in women with and without type 2 diabetes, but the incidence of non-vertebral fracture was greater in women with type 2 diabetes (Iwamoto, Sato, Uzawa, Takeda, & Matsumoto, 2011). This may indicate that alendronate does not have the same anti-fracture efficacy in women with type 2 diabetes compared to individuals without type 2 diabetes (Iwamoto et al., 2011).

In terms of mean calcium concentration in bone, it may be that treatment with antiresorptive agents in adults without type 2 diabetes increases calcium concentration in bone to a “normal” level, which is effective in reducing fracture risk. Further suppressing bone remodeling and increasing calcium concentration

in bone beyond this point in adults with type 2 diabetes may make the bone more brittle, and compound the pathology related to diabetic bone disease. Future studies should verify whether mean bone calcium concentration is greater in adults with type 2 diabetes taking antiresorptive therapy compared to placebo treated adults with type 2 diabetes.

Non-antiresorptive pharmacologic therapy might be more appropriate for adults with type 2 diabetes. Teriparatide (rhPTH [1-34]), an anabolic therapy, is a synthetic segment of human parathyroid hormone. When administered intermittently, this anabolic agent increases the frequency of bone remodeling, increases bone formation (Stepan et al., 2010), increases BMD and reduces the risk of non-vertebral and vertebral fractures by 40% and 70%, respectively (Hopkins et al., 2011; Neer et al., 2001). Teriparatide also reduces mean bone calcium concentration and increases mineralization heterogeneity (Misof et al., 2003). Administration of teriparatide in diabetic rats normalizes bone turnover markers (Tsuchida et al., 2000). The impact of teriparatide on bone remodeling, bone mineralization and fracture risk reduction in adults with type 2 diabetes is not known.

Currently, a new anabolic therapy is under investigation in clinical trials. Sclerostin is a protein derived from the SOST gene, and is secreted by osteocytes to inhibit bone formation (Poole et al., 2005). In adults with type 2 diabetes, sclerostin levels are higher than in adults without type 2 diabetes, which through impaired Wnt signaling may be another cause of low bone turnover in

adults with type 2 diabetes (Gaudio et al., 2012). Pharmacologic inhibition of sclerostin using a monoclonal antibody has been shown to increase bone formation and cortical and trabecular BMD in female and male rats (Li et al., 2009; Li et al., 2010). The efficacy of sclerostin antibody for preventing fracture in adults with type 2 diabetes is not known, although a promising study of sclerostin antibody use in a rodent model of type 2 diabetes has just been published (Hamann et al., 2012). Hamann and colleagues revealed that sclerostin antibody administration improved trabecular bone microarchitecture (ie: increased BVTV, Tb.Th and Tb.N) at the distal femur and lumbar spine in a rodent model of type 2 diabetes. Improvements in femoral neck energy to failure and an increase in bone formation were also documented, which would be beneficial to skeletal health in adults with type 2 diabetes (Hamann et al., 2012). If sclerostin antibody is approved for use for osteoporosis in Canada, clinical trials should be conducted to determine whether sclerostin antibody improves trabecular bone microarchitecture and normalizes bone calcium content in adults with type 2 diabetes.

5.3 Closing Remarks

This collection of work provides preliminary evidence that structural and material *bone qualities* are different in adults with type 2 diabetes compared to non-diabetic controls. Structural and material *bone qualities* contribute to bone strength, but these qualities are not directly assessed with conventional DXA

measurement of BMD. Clinicians currently rely on BMD measurements and fracture risk assessment tools to appraise bone health in patients, but neither BMD or fracture risk assessment provides an accurate prediction of fracture risk in adults with type 2 diabetes (Giangregorio et al., 2012; Schwartz et al., 2011). Therefore, a paradigm shift may be necessary for assessing bone health and individualizing care for skeletal health in adults with type 2 diabetes. Although bone turnover markers are not yet recommended for use in assessing skeletal health in all provinces in Canada, with improved analysis techniques and reference standards, they may become valuable particularly for grading the severity of diabetic bone disease in patients. Given that bone remodeling may be different in newly diagnosed adults with type 2 diabetes compared to adults with long-standing type 2 diabetes, it may be necessary to monitor changes in P1NP and CTX as type 2 diabetes progresses. An individualized treatment approach may be needed for adults with type 2 diabetes, where antiresorptive therapy is considered when type 2 diabetes is diagnosed to prevent structural *bone quality* impairments. Anabolic therapy may be more appropriate for adults with longer-standing type 2 diabetes with suppressed bone remodeling to prevent further increases in bone calcium concentration. Diabetic bone disease is of paramount importance to continue to understand, particularly with the growth in the Canadian population age 65 years and older.

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APPENDIX

ERRATUM

In the article by Pritchard et al in the January 2012 issue of *Arthritis Care & Research* (pages 83-91) the following errors were detected. In the “Image analysis” section on page 85 (in the PDF version of the publication), the model-independent method was used to estimate Tb.Th, Tb.Sp, and Tb.N. In Table 2, daily energy expenditure should be measured in kcal/week. Reference 35 should be Hildebrand T, Ruegsegger P. A new method for the model-independent assessment of thickness in three-dimensional images. J Microsc 1997;185:67-75. We regret the errors.

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