RELATIONSHIP BETWEEN DIABETES AND BONE HEALTH STATUS IN ADULTS WITH DIABETES

BY

GHAZAL NASERI KOUZEHGANIAN

THESIS
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Adviser:

Professor Karen Marie Chapman Novakofski
ABSTRACT

Studies of bone density and diabetes have relatively strong evidence to suggest that type 1 diabetes has negative impacts on bone mass density; however, there seems to be more discrepancy in skeletal effects of type 2 diabetes. While many studies have shown normal to high BMD values in individuals with type 2 diabetes, some have found low BMD values. We studied the relationship between diabetes-related variables and bone health status, specifically osteoporosis, by examining heel ultrasound and genetic markers. The objective of this study was to compare the bone mass density (BMD) and vitamin D receptor (VDR) gene marker of adult men and women with pre-, type 1 and type 2 diabetes (n=54). Subjects were recruited from faculty, staff and students of the University of Illinois at Urbana-Champaign, as well as participants from the Senior High Rise DM Outreach groups from Champaign-Urbana Public Health Department. Anthropometric measurements including height, weight, wrist, waist and hip circumferences were taken. Participants completed a self-administered questionnaire on demographics, diabetes status, lifestyles and health behaviors, physical activity, history of falling and bone fracture, current status and/or history of medical conditions and medication use, family history of diabetes and bone health-related diseases, and female reproductive history. Heel ultrasound (Hologic Sahara) was measured for bone analysis (n=52). Saliva samples were collected for DNA genotyping (n=50) and only analyzed for the VDR gene marker of Caucasian/white ethnic group (n=45). Among the 54 subjects, mean age was 62 years (27-86 years) and median for duration of diabetes was 7 years (0.08-50 years). The majority of participants were white/Caucasian women with college/professional degree, who had type 2 diabetes, were not on insulin and had diabetes for 7 or more years. Bone mineral density was
lower in subjects with type 1 diabetes compared to those with type 2 diabetes, but this difference lacked statistical significance. However, adjusting for age resulted in lower BMD and T-score values in individuals with type 1 diabetes as well as higher T-scores in those with type 2 diabetes. Odds ratio of high BMD values were about 14.5 times greater in subjects with type 2 diabetes than those with type 1 diabetes, which increased after adjustment for BMI. Univariate analysis detected significant associations for low bone mass density with age, current status and/or history of cancer, current use and/or history of any type of bone medication intake, menopause in females, and moderate physical activity in the past 7 days. Multiple regression model showed that African-American ethnicity, calcium/vitamin D supplement use, family history of loss of height, current status and/or history of osteoporosis, and menopause in females were significant predictors of low bone mass density. No statistically significant association was found between VDR genotype and bone mass density. Only age, duration of diabetes and menopause were significant independent risk factors for low BMD, but VDR genotype or VDR polymorphism were not associated with an increased risk for low BMD. In conclusion, additional research is needed to understand the link between BMD, diabetes status and VDR genetic marker.
To my Dearest Mother, Father, and Sister
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CHAPTER 1

INTRODUCTION

Bone Function:

Bone is a highly specialized and dynamic tissue which represents about 15% to 17% of total body weight (Ott, 1998). It has a variety of different functions including mechanical, hematological and chemical properties. The mechanical function of the bone consists of providing the structural framework that supports the softer tissues and provides sites of attachment for most skeletal muscles and tendons. In addition, this framework helps with the protection of internal organs including brain, thoracic and pelvic organs from damage induced by external mechanical forces. Due to attachment of bones to skeletal muscles, this rigid structure could assist in body movements during muscle contractions (Fanghänel et al, 2006; Brandi, 2009). Bone’s hematological function consists of the ability of the red bone marrow to produce and store blood cells. Red bone marrow is a functionally active hematopoietic composite found within the marrow cavities in bones of the extremities of all newborn mammals which supports hematopoiesis. As age increases, this hematopoietic microenvironment gradually converts into mesenchymal tissue that consists mainly of adipose cells which accumulate lipids, and a few hematopoietic cells with loss of ability to support blood generation. This new composite is referred to as yellow or fatty bone marrow which can help the bone in serving as an important chemical energy reserve at older ages. Yellow bone marrow can also be converted to red bone marrow at times of severe anemia (Gurevitch et al, 2009). The chemical property of the bone tissue also involves storage of several minerals including calcium and phosphorus, which
contributes to calcium metabolism and homeostasis. Bones contain 99% of the total body calcium and 90% of the body phosphorus (Ott, 1998, Brandi, 2009).

**Bone Structure:**

The above-mentioned bone functions are possible only due to the explicit bone structure. As a connective tissue, the microstructure of the bone consists of both organic and inorganic compounds, which comprise about 35% and 65% of the bone dry weight, respectively (Chan et al, 2002). The organic component represents the flexible and tough extracellular matrix and is composed of 95% type 1 collagen fibers which account for the high elasticity of bones, and 5% proteoglycans and several noncollagenous proteins. The rigid inorganic constituent of bone imbeds within this collagen matrix and is mainly composed of calcium phosphate salts which are deposited in the form of a mineral referred to as hydroxyapatite (Brandi, 2009). The chemical formula for hydroxyapatite crystals consists of hydroxyl ions as well as 5:3 ratio of calcium to phosphate molecules (Ott, 1998).

In particular, both the collagen fibers and the calcium salts provide the bone structural strength and rigidity. The calcium salts are responsible for the great compressional strength of bones (the strength to endure squeezing forces), while collagen fibers account for the bone significant tensile strength (the strength to endure stretching forces). However, bones often lack a high level of torsional strength (the strength to endure twisting). Thus, most bone fractures occur as a result of torsional forces (Brandi, 2009).

Macroscopically, there are two main types of bones in the body: trabecular (cancellous) and cortical (compact). Trabecular or cancellous bone has a highly porous structure and is mainly
concentrated at the ends of long bones such as the femur, tibia and fibula and at the inner parts of flat bones in vertebral bodies and pelvis (Ott, 1998; Brandi, 2009). Although trabecular bone accounts for only about 20% of all bone mass, it accounts for most of the bone volume (Ott, 1998). Trabecular bone has a microstructure of struts or trabeculae which are arranged in a three-dimensional open porous configuration that form a honeycomb-like pattern. The pores consist of both yellow bone marrow and red bone marrow which are responsible for bone adiposity and production of the blood cells including erythrocytes, platelets and leukocytes, respectively (Ott, 1998). This microstructure gives directionality as well as mechanical stiffness and strength to the bone.

Cortical or compact bone comprises about 80% of the body skeleton and is primarily found in the shaft of long bones such as the humerus, ulna and radius and the outer shell of flat bones including cranial bones, sternum and scapulae (Ott, 1998; Brandi, 2009). Due to its densely packed calcified collagen fibers, cortical bone has a much more rigid structure than trabecular bone. The microscopic structure of cortical bone consists of units referred to as haversian systems which are organized into concentric layers around an extensive longitudinal network of the central haversian canals termed canaliculi. Containing the bone extracellular fluid, canaliculi are filled with blood vessels which are responsible for nourishing the inner part of the bone (Ott, 1998; Brandi, 2009).

At the outer surface of compact bones the bone is completely enclosed by a fibrous structure called the periosteum. Penetration of bone by Sharpey’s fibers from periosteum serves to anchor it to the bone. Endosteum is the inner membraneous sheath that lines haversian canals and the inner surface of the marrow cavity. Both periosteum and endosteum contain blood vessels that nourish the bone cells (Brandi, 2009).
The above-mentioned structural differences account for the diverse functions of the cortical and trabecular bones. The relative proportion of the skeletal site localization of these two types of bone varies significantly depending on the functional demand for strength and weight. Although relatively lighter in weight, trabecular bone has considerable tensile strength and weight-bearing properties than the cortical bone, since the ends of long bones are subjected to the greatest forces of compression. Additionally, the turnover rate is three to four times higher in trabecular bone due to the larger surface area which makes it more metabolically active. These properties not only make the trabecular bones more likely to receive physical stress and become more susceptible to fractures, but also more likely to show faster signs of response to therapy (Ott, 1998; Brandi, 2009).

**Bone Cells:**

There are typically four different types of bone cells: osteoprogenitor or lining cells, osteoblasts, osteocytes, and osteoclasts. Osteoprogenitor cells, osteoblasts and osteocytes derive from mesenchymal cell origin, whereas osteoclasts originate from a monocyte-macrophage lineage of hematopoietic cells (Manolagas, 2000).

Osteoprogenitor or lining cells are precursors of osteoblasts. These are undifferentiated cells which have the capacity for mitosis, resemble fibroblasts in structure, and line the entire bone surfaces. They can be proliferated into osteoblasts to participate in the initiation of bone remodeling (Manolagas, 2000; Pignolo et al, 2010).

Osteoblasts, the bone-forming cells, are responsible for the formation of bone extracellular matrix and its subsequent mineralization through calcium and phosphorus deposition and
production of mineralization regulators (Jensen et al, 2010). These bone-forming cells have well developed Golgi complex, numerous mitochondria, and prominent rough Endoplasmic Reticulum (RER), reflecting their high metabolic activity and secretion (Pignolo et al, 2010).

Osteocytes are terminally differentiated osteoblasts which are trapped in small cavities called lacunae within the bone matrix (Brandi, 2009). These resting cells exist in numbers 10 times higher than that of osteoblasts with the longest lifespan among all bone cells in the adult skeleton (Qing et al, 2009). Morphologically, osteocytes have smaller cell volume, RER and Golgi complex than osteoblasts depending on age and physical activity. Osteocytes have cell processes which form gap junctions with adjacent osteocytes and osteoblasts on the bone surface through networks of canaliculi. Osteocytes serve as mechanosensors which could detect physical changes in their environment. These properties help them to become involved in intercellular communication as well as bone formation, maintenance of bone integrity specifically the extracellular matrix, and recruitment of osteoblasts and osteoclasts during bone modeling and remodeling (Brandi, 2009; Qing et al, 2009). Additionally, Osteocytes play an active role in homeostatic regulation of calcium levels through immediate release of bone calcium into the blood during hypocalcemia (Qing et al, 2009). A recent study by Feng, et al suggests that osteocytes can also participate in bone remodeling and mineralization through phosphate regulation (Feng et al, 2009).

Osteoclasts, the bone-resorbing cells, are derived from hematopoietic stem cells in bone marrow. Osteoclasts are motile, giant multi-nucleated cells, located on bone surfaces and in tight conjunction with the extracellular matrix (Vaaninen et al, 1995). It has been shown that osteoblasts have an important regulatory effect in formation of osteoclasts from the fusion of their mononuclear osteoclast precursors (OCPs) (Boyce et al, 2009). Osteoclasts break down and
absorb both the collagen matrix and the deposited mineral of bones by secreting acid and hydrolytic enzymes (Brandi, 2009). Recent studies have identified other functions for osteoclasts in and around bone including regulation of differentiation of lining cells into osteoblasts, transition of hematopoietic cells from the bone marrow into the blood, and participation in immune responses through secretion of cytokines in inflammatory processes affecting bone (Boyce et al, 2009).

**Bone Modeling and Remodeling:**

The process through which the ability of bone to adapt its mechanical features including shape and size in response to mechanical loads is generated is referred to as “bone modeling” (Frost, 1990-a). The process involving removal of a bone part followed by the formation of new bone is called “bone remodeling” (Frost, 1990-b).

The modeling process occurs throughout the whole life cycle, from growth to adulthood (Brandi, 2009). Any mechanical load could induce bone mechanical adaptation of its shape and size through continuous bone resorption and bone formation. Since these two processes are not balanced and often occur at different locations, the microstructure of the bone can change. Due to the independent action of osteoblasts and osteoclasts, bone modeling occurs less frequently than the remodeling process (Brandi, 2009; Frost, 1990-a).

The remodeling process is a continuous phenomenon which occurs constantly throughout life. Bone remodeling is carried out at the level of the *Basic Multicellular Unit*, or BMU, which is composed of osteoclast and osteoblast cells collaborating closely on the surface of the bone (Weinstein et al, 2000). There are four distinct stages: quiescence/activation, resorption, reversal,
and formation. Bone remodeling process is initiated at a quiescent bone surface by contraction of the lining cells and recruitment of osteoclast precursors which, in turn, fuse to form active multinuclear osteoclasts on the surface of the bone. Osteoclasts completely seal off the bone-osteoclast interface area by adhering to the adjacent bone tissue matrix and create an isolated microenvironment (Roodman, 1999).

During the resorption stage, the osteoclasts acidify and dissolve the mineral compartment of bone matrix by releasing hydrogen ions through membraneous proton pumps into the resorbing compartment. Additionally, secretion of lysosomal proteolytic enzymes helps with digestion of the organic component of the bone matrix. The result is the formation of saucer-shaped resorption cavities on the surface of the bone called Howship’s lacunae. At the end of this stage, osteoclasts undergo apoptosis (Reddy, 2004).

Reversal phase, which is the transition state between bone resorption and bone formation, is characterized by transition of mononuclear pre-osteoblast cells into the resorption lacunae followed by their differentiation into osteoblasts (Ott, 1998; Brandi, 2009).

The last phase of bone remodeling is a two-step process: ossification and calcification, both of which are carried out by osteoblasts. Ossification consists of formation and organization of bone extracellular matrix through synthesis of collagen and noncollagenous proteins that form the unmineralized organic matrix referred to as osteoid. Calcification includes subsequent mineralization of the matrix through which osteoblasts regulate calcium and phosphorus deposition (Brandi, 2009; Jensen et al, 2010). During the process, some osteoblasts become encapsulated within the osteoid and differentiate into osteocytes, while others continue to synthesize bone until they convert into quiescent lining cells that completely cover the new bone surface (Brandi, 2009).
The final product of bone remodeling is a new bone structural unit referred to as osteon. Each remodeling cycle takes about 6 months, most of which includes the formation stage (Brandi, 2009). The frequency of bone remodeling depends on the type of the bone tissue. In cortical bone the BMU forms a cylindrical tunnel, whereas in trabecular bone the BMU is mainly on the bone surface. Due to the much larger surface area of trabecular bones, remodeling is processed more actively than compact bones (Ott, 1998). It has been estimated that in average about 25% of the trabecular bone and 3% of the compact bone undergo remodeling each year (Ott, 1998).

Unlike bone modeling, the processes of bone resorption and formation are tightly coupled during bone remodeling. The “coupling” mechanism includes appearance and adherence of osteoblasts only to areas where osteoclasts have already been. In a homeostatic equilibrium, the balanced coupling mechanism can ensure continuous replacement of old bone with new tissue as well as maintenance of bone mechanical integrity and microstructure. However, if bone resorption rate exceeds the formation rate, the process is no longer coupled and a net loss of bone tissue occurs, which, in turn, could result in the development of osteoporosis in the long run (Ott, 1998; Brandi, 2009).

**Osteoporosis:**

Osteoporosis is a chronic skeletal disease characterized by reduced bone mass associated with microarchitectural deterioration of bone tissue and compromised bone strength resulting in a consequent increase in bone fragility and susceptibility to fracture (Prentice, 2004; NIH, 2001). Osteoporosis is a major public health disease which according to the National Osteoporosis Foundation, affects 55% of Americans aged 50 and older. Currently in the US, 10 million people
are diagnosed with osteoporosis, 80% of which are women, and almost 34 million more have low bone mass, placing them at an increased risk for osteoporosis and bone fracture (National Osteoporosis Foundation, 2010-a). Of women aged 50 and older, 20% of non-Hispanic Caucasians and Asians, 5% of non-Hispanic African-Americans, and 10% of Hispanic women are affected by osteoporosis. The rate of low bone mass in these ethnic/racial groups is 52%, 35%, and 49%, respectively. On the other hand, 7% of non-Hispanic white and Asian men, 3% of non-Hispanic African-American men, and 4% of Hispanic men are currently diagnosed with osteoporosis. The prevalence of low bone mass density among these ethnic/racial groups is 35%, 19% and 23%, respectively. It is estimated that the risk of osteoporosis has the highest increasing rate among Hispanic women than all other ethnic/racial groups (National Osteoporosis Foundation, 2010-a).

One of the major complications of osteoporosis is bone fracture. According to National Osteoporosis Foundation, one in two women and one in four men aged 50 and over will have an osteoporosis-related fracture in their lifetime (National Osteoporosis Foundation, 2010-a). The most common sites of fragility fractures are the wrist, spinal vertebrae and hip, although these fractures can occur anywhere in the body (Prentice, 2004). It has been shown that a main cause of morbidity and disability in the elderly is osteoporotic fractures, of which the hip fracture has the highest impact on quality of life. Hip fracture, mostly resulting from simple falls is associated with increased premature mortality (Chrischilles et al, 1994). As reported by Schurch et al, up to 20% of patients who suffer from hip fracture die within six and twelve months of the event (Schurch et al, 1996). In another study by Sernbo et al, it was found that more than half of the hip fracture patients who survive the event would need long-term care (Sernbo et al, 1993). In addition, Osteoporotic fractures are a major burden to the global costs of healthcare, costing
many billions of dollars each year (Johnell, 1997). In 2005, a cost of $19 billion was spent for osteoporosis-related fractures. Experts predict that by 2025 these costs will increase to approximately $25.3 billion (National Osteoporosis Foundation, 2010-a).

Osteoporosis can be classified as primary and secondary. Primary osteoporosis is the most common form of osteoporosis which is not associated with any other chronic disorders. This type of osteoporosis is mainly a disease of the elderly as a result of cumulative bone mass loss and impaired bone microstructure (Seeman, 2003; US Department of Health and Human Services, 2004). Bone loss resulting from specific chronic diseases or use of certain medications is referred to as secondary osteoporosis (Soen, 2007). A wide variety of diseases can contribute to the development of secondary osteoporosis including endocrine or metabolic diseases such as hyperparathyroidism, diabetes mellitus, and Cushing’s syndrome; genetic disorders including cystic fibrosis and glycogen storage diseases; gastrointestinal diseases such as inflammatory bowel disease and celiac; hematologic disorders including hemophilia, thalassemia and sickle cell disease; malignancies such as leukemia; and many other chronic disorders (Harper et al, 1998; Stein et al, 2003). Some of the most important medications associated with secondary osteoporosis include: glucocorticoids, anticonvulsants, anticoagulants, chemotherapeutic drugs, and thyroxin. Long-term glucocorticoid therapy is by far the most common cause of secondary osteoporosis (Soen, 2007; Stein et al, 2003). Secondary osteoporosis may occur at any age and affects both genders equally (US Department of Health and Human Services, 2004).

Primary osteoporosis could be classified into Type I and Type II osteoporosis. The most common form of primary osteoporosis which occurs mainly in women within 15-20 years after menopause is referred to as primary type I or postmenopausal osteoporosis. The main complication of this type of osteoporosis is fractures of trabecular bones, of which distal radius
fractures (Colles fracture) caused by falls or minor accidents and lumbar vertebrae “crush” fractures due to compressional forces on the spine are the most common (Khosla et al, 1995). Also referred to as high turnover osteoporosis, postmenopausal osteoporosis is characterized by increased bone resorption and osteoclastic activity as a result of increased resorptive surfaces and higher numbers of osteoclasts (Boskey, et al, 2005). This is generally caused by the sudden estrogen deficiency and rapid bone calcium depletion in postmenopausal women resulting in bone mass loss of 2-5% per year after menopause (US Department of Health and Human Services, 2004; Arnaud et al, 1990).

Primary type II osteoporosis or senile osteoporosis occurs after age 75 in both females and males and is frequently associated with fractures of hip and vertebrae (Khosla et al, 1995). Also referred to as age-related osteoporosis, type II osteoporosis results from normal aging and is associated with bone mass loss of 1-2% per year starting at age 35-40 years (US Department of Health and Human Services, 2004). This low turnover type of osteoporosis is characterized by reduced bone formation which is a result of decreased osteoblastic activity, and lower than normal numbers of osteoclasts and resorptive surfaces (Boskey, et al, 2005).

Another form of primary osteoporosis is primary idiopathic osteoporosis which occurs in younger individuals including children, adolescents and young adults of both genders. In many cases of this rare form of disease, the exact cause of the disorder is unknown (Kulkarni et al, 2004).

Osteopenia, a precursor to osteoporosis is a much more common condition with an incidence rate of about three times higher than that of osteoporosis. Osteopenia is defined as low bone density, but not low enough to be osteoporosis, and will not necessarily develop into osteoporosis (Wehren, 2003).
Bone Strength and Bone Quality:

Since osteoporosis is the systemic disease of compromised bone strength, great interest has recently developed in the factors that affect bone strength. Bone strength is defined as the ability of the bone to resist an applied force. Bone Mineral Density (BMD) accounts for approximately 70-75% of the bone strength variance (Njeh et al, 1997), whereas the remaining variance is associated with bone quality which consists of a number of important factors including the bone turnover (rate of remodeling), mineralization, microarchitecture, and bone size and geometry (Seeman et al, 2006). The underlying concept of bone quality is that although the predicted fracture risk is similar among all osteoporotic patients, not all actually suffer from osteoporotic fractures (Ellison, 2005). A fracture is defined as a break in the continuity of bone when the external force applied to the bone exceeds its strength. The ability of the bone to resist a fracture depends on the amount of bone mass and size, bone macro- and microstructure, rate of bone remodeling, and other intrinsic properties of bone (Bouxsein, 2008).

Bone Mineral Density and Bone Densitometry:

Bone Mineral Density (BMD) measurement has four major applications in clinical practice: screening for osteoporosis, diagnosis of osteoporosis, prediction of fracture risk, and assessment of treatment efficacy and response to therapy (Baran et al, 1997). Bone mineral density or bone mineral content (BMC) refers to the bone mass per unit volume of bone. According to the World Health Organization (WHO), BMD is the only accepted quantitative measurement for accurately diagnosing osteoporosis and predicting fracture risk (World Health Organization, 1994). The WHO has defined the diagnostic criteria for osteoporosis based on the number of standard deviations that the BMC or BMD measured by bone densitometry techniques differs from the
young adult reference mean value for the population, referred to as a T-score. According to the WHO guidelines, the classification of Bone Mineral Density based on a T-score is as follows (World Health Organization, 1994):

1. **Normal** – BMD (or BMC) not more than 1 standard deviation below the young adult reference mean value (T-score ≥ -1)

2. **Osteopenia** - BMD (or BMC) between 1 and 2.5 standard deviations below the young adult reference mean value (-2.5 < T-score < -1)

3. **Osteoporosis** - BMD (or BMC) more than 2.5 standard deviations below the young adult reference mean value (T-score ≤ -2.5)

4. **Severe (established) Osteoporosis** - BMD (or BMC) more than 2.5 standard deviations below the young adult reference mean value (T-score ≤ -2.5) and the presence of one or more fragility fractures.

A Z-score can also be calculated from an absolute BMD value. Z-scores are not as appropriate as T-scores for diagnostic purposes in clinical practice because they use age-adjusted controls as their reference. As a result, Z-scores become smaller in value as the age increases (Lenchik et al, 2004). According to recommendations by the International Society for Clinical Densitometry (ISCD), for evaluating patients under 50 years of age including pediatric patients, Z-scores of equal to or less than -2.0 are associated with “low bone mineral content or bone mineral density for chronologic age” (ISCD, 2007).

There are several bone densitometry techniques available for bone mineral density measurement. Traditional methods including conventional radiographs, single- and dual-photon absorptiometry (SPA and DPA), and single-energy X-ray absorptiometry (SXA) are no longer in use in clinical settings. Plain conventional radiographs applied on the hand bone do not have enough sensitivity
for osteoporosis diagnosis in bone mass losses of lower than 50% (South-Paul, 2001). SPA, DPA and SXA methods are restricted to areas with the lowest amount of surrounding soft tissue and therefore, are only applicable to the radius and calcaneus. All three techniques provide poor resolution and low precision with an error range of 3% to 5% (Ott, 1998). The only advantage of SXA over the other two methods is lower operating costs due to less radiation exposure (South-Paul, 2001).

The development of new noninvasive techniques through molecular and cellular research has provided the opportunity to better estimate the bone strength and fracture risk by quantitative assessment of bone macro- and microstructure. Quantitative computed tomography (QCT) measurements obtained through a specialized computerized tomography (CT) scanner can be used to measure the mineral content of the bone as a true volumetric density mainly on the lumbar spine (Ott, 1998). QCT has a precision error of 5% to 15% which is affected by the marrow fat in the vertebrae. Due to the inverse relationship between marrow fat and age, QCT has the potential of underestimation of bone density in older populations (Baran et al, 1997). However, QCT is the most sensitive method among all bone densitometry techniques (South-Paul, 2001). This is due to the fact that QCT measurements are obtained only from trabecular bones which due to their higher surface area show the signs of bone loss much more rapidly than compact bones. This would allow for differentiated analysis of trabecular and cortical bones through the use of QCT method (Brandi, 2009).

Peripheral quantitative ultrasound (QUS), mainly of the calcaneus, is the most widely used screening tool for osteoporosis in primary care and field studies. The advantages include lower cost, ease of application, possibility of using a portable device, and lack of radiation exposure (American College of Radiology, 2010). QUS measures two variables: speed of sound (SOS)
and broadband ultrasound attenuation (BUA). The two variables are then combined to obtain a third variable called quantitative ultrasound index or stiffness index (QUI). Speed of sound refers to the ratio of the bone width to the transit time of ultrasound signals. Attenuation is the loss of energy during the transit of sound waves through the bone (Njeh et al, 1997). All three variables are parameters of a different quality of bone strength than BMD that could provide additional information on fracture risk, all of which are more likely to have higher values in younger/healthier individuals than older/osteoporotic subjects (American College of Radiology, 2010). The best approach with this technique is to identify individuals at higher risk for fractures and low BMD and to refer them for dual energy X-ray absorptiometry (DXA) for confirmation and diagnosis (American College of Radiology, 2010).

Dual energy X-ray absorptiometry (DXA) is the gold standard for bone densitometry and osteoporosis diagnosis in clinical practice specifically on the hip and spine, the two major sites of bone fractures (American College of Radiology, 2010). DXA scans can also be used to measure the bone density of the wrist, heel and total body (Ott, 1998). DXA measurements of the bone mineral content are expressed as a surface density rather than the true volumetric density measured by QCT, and allow for separate analysis of bone and soft tissue unlike SPA, DPA and SXA methods (Ott, 1998). DXA has the highest precision among all bone densitometry techniques with a precision error rate of 0.5% to 1% (Ott, 1998; South-Paul, 2001). Other advantages of this technique include: short scan times, low radiation exposure and lower operating costs (Ott, 1998; South-Paul, 2001).

Due to the low correlation among bone densitometry techniques, the use of different devices as well as the choice of the site of measurement could result in inconclusive diagnoses (Varney et al, 1999). A study by Faulkner et al reported the following osteoporosis diagnosis rates in
women over 60 years: 50% with QCT of the spine, 6% with DXA of the total hip, 14% with DXA of the lumbar spine, and 3% with the heel QUS (Faulkner et al, 1999).

As any other diagnostic tool, BMD has its own limitations for use. BMD has a recognized inverse and exponential relationship with future fracture risk, but there is no defined value of bone mineral density for which the fracture risk could be considered zero (Marshall et al, 1996). Additionally, individuals with normal or osteopenic T-score values could still suffer from osteoporotic fractures. A study in 2004 by Siris et al reported a high occurrence of bone fracture of about 82% with T-score values of above -2.5 calculated by peripheral bone densitometry (Siris et al, 2004). In another study in 2004, Schuit et al reported that relatively high incidences of bone fractures of about 56% and 79% in females and males, respectively, were associated with T-score values of greater than -2.5 measured for hip BMD by DXA (Schuit et al, 2004).

Although used as the basis of osteoporosis diagnosis by the WHO, bone mineral density is not useful in comparing different ethnic/racial populations. Studies on bone mineral density and fracture risks in different ethnic/racial populations suggest that East Asians who are genetically shorter and of smaller body sizes, tend to have lower bone mineral density compared to Western populations with taller statures. However, the osteoporotic fracture rates do not differ among the two populations (Aspray et al, 1996; Prentice et al, 1994; Russel-Aulet et al, 1993; Yan et al, 1997).

Despite all the above-mentioned limitations, bone densitometry is the only technology available for accurate measurement of bone mass density and prediction of bone fracture risk, to the extent that its efficacy in prediction has been shown to be as equal or even higher than that of blood cholesterol measurement for prediction of cardiovascular diseases and blood pressure testing for stroke (American College of Radiology, 2010). It is recommended that osteoporosis should be
diagnosed on the basis of BMD even before the occurrence of fractures. The use of the WHO criteria for diagnosis of osteoporosis should only be made based on DXA scans of the hip and spine (American College of Radiology, 2010).

**Bone Turnover:**

Bone remodeling can be assessed by the measurement of biochemical markers of bone turnover in the blood or urine. These markers consist of bone tissue enzymes released from bone cells and non-enzymatic peptides derived from collagen and non-collagenous proteins during bone turnover (Seibel, 2005). Markers of bone formation include serum levels of total alkaline phosphatase, bone-specific alkaline phosphatase, osteocalcin, and procollagen type I C-terminal (PICP) and N-terminal (PINP) extension peptides. Markers of bone resorption include urinary levels of pyridinoline and deoxypyridinoline, serum levels of tartrate-resistant acid phosphatase and bone sialoprotein, and serum and urinary levels of C-terminal and N-terminal cross-linking type I collagen telopeptides (Seibel, 2005). Prior to detection of any bone mineral density changes, the level of these bone turnover markers reflect changes in bone remodeling within a short period of time, ideally only the osteoblastic and osteoclastic activity.

According to the NIH consensus conference, “these biochemical markers of bone turnover do not predict bone mass or fracture risk and are only weakly associated with changes in bone mass. Therefore, they are of limited utility in the clinical evaluation of individual patients.” (NIH, 2000)

Based on the National Osteoporosis Foundation guidelines, although biochemical markers of bone turnover are useful in assessment of bone loss, risk of fracture, and efficacy of treatment,
these tests cannot be used in place of BMD testing for diagnosis of osteoporosis since they are not yet standardized (National Osteoporosis Foundation, 2010-b).

A 3-year study by Borah et al showed that high rates of bone turnover in postmenopausal women was associated with loss of bone mass, impaired trabecular bone microstructure and decreased bone mineralization, all of which were related to increased osteoclastic and reduced osteoblastic activity (Borah et al, 2004). In several studies on bone turnover markers, these remodeling rate indices were shown to predict fracture risk. A large cohort study by Garnero et al reported that increased bone turnover markers were predictors of hip fracture risk independent of BMD (Garnero et al, 1996).

**Osteoclast Differentiation and Bone Remodeling:**

Normal bone remodeling is a coupled event between bone resorption and formation. This mechanism requires occurrence of osteoblast-mediated bone formation in the exact areas of bone resorption through osteoclastic activity. These areas normally include structurally weak bone due to either bone disuse or mechanical stress. Under these circumstances, osteocytes serve as mechanosensors to detect physical changes in the networks of canaliculi. Upon detection of change in the flow of bone fluid, osteocytes send signals to osteoblasts, which in turn, stimulate osteoclast differentiation and activation, resulting in bone resorption (Sims et al, 2008; Teitelbaum, 2000).

Differentiation of osteoclast precursors into mature osteoclasts is mediated through two cytokines secreted by osteoblasts; macrophage colony stimulating factor (MCSF) (Teitelbaum, 2000; Matsuo et al, 2008), and receptor activator of nuclear factor-κB (RANK) ligand (RANKL) (Seeman, 2009). RANKL, a tumor necrosis factor (TNF)-related activation-induced cytokine
(TRANCE) (Yaturu, 2009), is the key regulator of osteoclastogenesis through binding to its receptor (RANK) on the plasma membrane of osteoclast progenitors and stimulating their differentiation (Seeman, 2009; Yaturu, 2009). Osteoprotegerin (OPG), a decoy receptor for RANKL, is also secreted by osteoblasts to inhibit RANK-RANKL interaction and osteoclast differentiation (Asagiri et al, 2007). Some systemic hormones that enhance osteoclastic activity through either upregulation of RANKL and MCSF or inhibition of OPG expression include: parathyroid hormone (PTH), 1,25-dihydroxy vitamin D, thyroid hormones, and prolactin (Teitelbaum, 2000; Miura et al, 2002; Seriwatanachai et al, 2008; Zaidi, 2007). Other stimulants of osteoclastogenesis include TNF-α, prostaglandin E2, and transforming growth factor (TGF)-β superfamily (Fuller et al, 2002; Li et al, 2000), specifically bone morphogenetic proteins (BMPs) which are members of the (TGF)-β family and are released from bone during bone resorption (Itoh et al, 2001).

The remodeling process is a continuous phenomenon which occurs constantly throughout life. Bone remodeling is carried out at the level of the Basic Multicellular Unit, or BMU, which is composed of osteoclast and osteoblast cells collaborating closely on the surface of the bone (Weinstein et al, 2000). There are four distinct stages: quiescence/activation, resorption, reversal, and formation. Bone remodeling process is initiated at a quiescent bone surface by contraction of the lining cells and recruitment of osteoclast precursors which, in turn, fuse to form active multinuclear osteoclasts on the surface of the bone. Osteoclasts completely seal off the bone-osteoclast interface area by adhering to the adjacent bone tissue matrix and create an isolated microenvironment, termed a resorption lacuna (Roodman, 1999).

During the resorption stage, the osteoclasts acidify the resorption lacuna and dissolve the mineral compartment of the bone matrix by releasing hydrogen ions through membranous proton pumps
into the resorbing compartment. During this phase, hydrogen ions in the form of proton are generated through the action of carbonic anhydrase II on carbon dioxide and water within the osteoclasts. The active transport of protons across the membrane is coupled with the passive transport of chloride through chloride channels, forming hydrochloric acid for dissolution of the inorganic compartment of bone matrix (Reddy, 2004; Supanchart et al, 2008). Additionally, secretion of lysosomal proteases and cathepsin K enzyme from osteoclasts helps with digestion of the organic component of the bone matrix, specifically type I collagen fibrils (Teitelbaum, 2000; Supanchart et al, 2008). The result is the formation of saucer-shaped resorption cavities on the surface of the bone called Howship’s lacunae. At the end of this stage, osteoclasts undergo apoptosis (Reddy, 2004).

Reversal phase, which is the transition state between bone resorption and bone formation, is characterized by transition of mononuclear pre-osteoblast cells into the resorption lacunae followed by their differentiation into osteoblasts (Brandi, 2009).

The last phase of bone remodeling is a two-step process: ossification and calcification, both of which are carried out by osteoblasts. Ossification consists of formation and organization of bone extracellular matrix through synthesis of type I collagen fibrils and noncollagenous proteins that form the unmineralized organic matrix referred to as osteoid. Calcification includes subsequent mineralization of the matrix through which osteoblasts regulate calcium and phosphorus deposition in the presence of alkaline phosphatase and two of the newly identified bone polypeptides: osteocalcin and osteopontin (Brandi, 2009; Jensen et al, 2010). Finally, mature hydroxyapatite crystals \[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\] are formed by gradual addition of hydroxide ions to calcium and phosphorus (Sims et al, 2008). During the process, some osteoblasts become encapsulated within the osteoid and differentiate into osteocytes, while others continue to
synthesize bone until they convert into quiescent lining cells that completely cover the new bone surface (Brandi, 2009). Upregulators of osteoblasts and bone formation include insulin-like growth factor (IGF)-1, insulin, and OPG (Zaidi, 2007; Seeman, 2009). The family members of (TGF)-β including BMPs seem to stimulate both resorption and formation (Seeman, 2009).

New Links Between Bone, Adipose Tissue and Glucose Metabolism:

In the last decade, emerging evidence suggests important and active metabolic roles for both skeletal and adipose tissue. Although adipose tissue was initially regarded as an inert and exclusive site of storage for fat and energy, recent findings have revealed that adipose tissue has both paracrine and endocrine roles by producing peptides and cytokines which can modulate metabolic homeostasis, both locally and systemically (Scherer, 2006). Similarly, skeleton has long been considered as a passive tissue only capable of bone remodeling and regulation of calcium homeostasis within its own structural context. However, recent evidence supports the hypothesis that bone is an endocrine organ and a regulator of global energy metabolism through control of glucose homeostasis (Fukumoto et al, 2009).

Recent findings suggest that complex relationships exist between adipose tissue and skeleton to integrate bone remodeling and glucose and energy metabolism. These interactions are well identified in rodents, but are less clear in humans. Osteoblasts and adipocytes are both present in the bone marrow and share a common mesenchymal stem cell (MSC) origin (Yaturu, 2009).

The first evidence came with a landmark study by Ducy et al in 2000 where they found that leptin, an adipocyte-secreted hormone with an already well established role as a regulator of energy balance and body weight and a stimulant of thermogenesis via hypothalamic receptors (Jéquier, 2002), can indirectly affect bone remodeling through enhanced activity of sympathetic
nervous system (Ducy et al, 2000). Increased sympathetic output by leptin activates the β2-
adrenergic receptors in osteoblasts and subsequently decreases bone formation, while increasing
bone resorption through enhanced expression of RANKL, the osteoclast differentiation factor in
osteoblast cells (Ducy et al, 2000). The influence of leptin on bone remodeling has been shown
to be independent of its role in energy balance regulation (Shi et al, 2008).

Leptin can also affect bone through mechanisms independent of the neural pathway. One
mechanism is improvement of insulin sensitivity by possibly inducing insulin-like growth factor-
binding protein 2 (IGFBP2), causing positive effects on the bone (Hedbacker et al, 2010).
Another possible effect of leptin on bone is through its receptors on osteoblasts which help in
direct action of leptin on stimulation of osteoblast formation and proliferation as well as
inhibition of osteoclast differentiation. This could result in opposite effects than those observed
with leptin acting via central nervous system (Cornish et al, 2002; Holloway et al, 2002).

Another important emerging evidence of the active regulatory effect of bone in energy
metabolism is the newly identified role of osteoblasts and their specific molecules and genes in
glucose homeostasis control. Osteocalcin, a bone-specific protein product is secreted by
osteoblasts into the bone extracellular matrix and has a regulatory role in systemic energy
metabolism. Osteocalcin undergoes post-translational vitamin K-dependent gamma-
carboxylation of three of its glutamic residues which helps the molecule to acquire higher
affinity for hydroxyapatite and to incorporate calcium ions into these crystals in the
mineralization process of the bone extracellular matrix (Clemens et al, 2011). On the contrary,
the under- or undecarboxylated form of osteocalcin has a low affinity for hydroxyapatite and is
more likely to be released into the systemic circulation for its endocrine functions. It has been
shown that the undercarboxylated osteocalcin acts as a hormone on both the pancreatic β-cell and
adipocyte proliferation and gene expression and subsequently stimulates insulin production, improves insulin sensitivity, enhances adiponectin secretion and decreases lipid accumulation. The simultaneous increase in insulin sensitivity and decrease in fat mass could result in the enhancement of energy expenditure in the body (Lee et al, 2007; Ferron et al, 2008). A recent study by Vestri et al has reported an increase in both basal and insulin-stimulated glucose transport as a result of the undercarboxylated osteocalcin activity (Vestri et al, 2008).

Adiponectin, an adipocyte-secreted adipokine in both subcutaneous and visceral adipose tissue is produced in response to osteoblastic osteocalcin secretion. Adiponectin has been shown to increase both hepatic and adipocytic insulin sensitivity (Kershaw et al, 2004; Kadowaki et al, 2005) and its low levels have been associated with insulin resistance in diabetic patients (Magni et al, 2010). This insulin-sensitizing factor could also have a negative impact on bone mineral density and seems to be an independent predictor of low bone mass (Magni et al, 2010).

A recent study by Lee et al in rodents showed that osteoblasts express the Esp gene which encodes an intracellular receptor-like protein tyrosine phosphatase, termed osteotesticular protein tyrosine phosphatase (OST-PTP). It was also found that Esp-deficient mice (ESP<sup>−/−</sup>) showed characteristics of hyperinsulinemia due to increased pancreatic β-cell proliferation, increased insulin secretion and improved insulin sensitivity, as well as increased adiponectin production and decreased visceral fat tissue. On the contrary, overexpression of Esp gene resulted in a metabolic phenotype similar to type 2 diabetes with increased insulin resistance and impaired glucose tolerance. Therefore, OST-PTP was found to be a preventative factor in glucose homeostasis by inhibiting endocrine functions of osteocalcin (Lee et al, 2008). Additionally, the exact metabolic phenotype of Esp-deficient mice has been observed in more recent studies with
overexpression of undercarboxylated osteocalcin gene, where hypoglycemia, hyperinsulinemia and increased insulin sensitivity were the typical characteristics (Lee et al, 2008).

In summary, the inactivation of Esp gene in osteoblasts results in an increase in the release of undercarboxylated osteocalcin and subsequently improves insulin sensitivity and glucose metabolism as well as decreasing adiposity. As a result, energy metabolism is positively regulated through increased energy expenditure due to enhanced glucose and lipid metabolism (Kim et al, 2010).

Osteocalcin and Glucose/Fat Metabolism in Humans:

It should be noted that Esp gene is a pseudogene in humans and a human homolog has not yet been identified. However, protein-tyrosine phosphatase 1B (PTP-1B) expressed in human osteoblasts is a close functional homolog of Esp and could possibly have the same functions (Cousin et al, 2004). Several recent studies have shown associations between serum total osteocalcin with parameters of glucose metabolism and insulin sensitivity as well as visceral fat mass in humans. Fernandez-Real et al found a strong positive impact of circulating osteocalcin concentrations on improved insulin sensitivity and lowered fasting plasma glucose and showed an increase in serum osteocalcin as a result of weight loss, supporting the hypothesis of an inverse relationship between circulating osteocalcin and fat mass in humans (Fernandez-Real et al, 2009). The same associations between osteocalcin and glucose and fat metabolism have been found in studies by Kindblom et al in older Swedish men (Kindblom et al, 2009), Yeap et al in older Australian men (Yeap et al, 2010), Saleem et al in African-Americans and non-Hispanics (Saleem et al, 2010), Im et al in postmenopausal Korean women (Im et al, 2008), Winhofer et al
in gestational diabetes patients (Winhofer et al, 2010), Pittas et al in elderly males and females (Pittas et al, 2009), and Zhou et al in Chinese population (Zhou et al, 2009).

Researches on undercarboxylated osteocalcin in humans are much fewer and more inconsistent than those carried out on total osteocalcin. In two studies by Kanazawa et al it was found that undercarboxylated osteocalcin was inversely related with fasting plasma glucose and fat mass in men, but not in postmenopausal women (Kanazawa et al, 2009; Kanazawa et al, 2010). Unlike rodent models, increased carboxylated osteocalcin level, and not the undercarboxylated form, was shown to be associated with improved insulin sensitivity in humans (Yoshida et al, 2008). Additionally, in a recent study by Shea et al only elevated circulating total and carboxylated forms of osteocalcin were associated with lower insulin resistance, and undercarboxylated osteocalcin had no such effect, despite the strong evidence in mice (Shea et al, 2009).

The inconsistent observations in human studies derive from the fact that the results are all based on measurements of serum levels of osteocalcin - unlike the use of recombinant uncarboxylated osteocalcin in therapeutic or pharmacologic doses in rodent genetic models- and that there are several limitations in the ability of accurately measuring these circulating levels in humans (Motyl et al, 2010). In addition, osteocalcin status in humans is heavily dependent on several factors including age, gender, physical activity, smoking, and nutritional status (Nimptsch et al, 2007). On the other hand, since all the aforementioned human studies are cross-sectional, they can only prove relationships but cannot establish causality between osteocalcin and glucose metabolism, or confirm whether the association between serum osteocalcin and visceral fat mass in humans is dependent or independent of insulin action. As a result, more clinical studies are needed to investigate these issues and to determine whether uncarboxylated, carboxylated or
total osteocalcin is responsible for the regulation of glucose metabolism in humans (Motyl et al., 2010).

**Insulin Regulation on Bone Function:**

The mechanism through which OST-PTP inhibits the release of undercarboxylated osteocalcin in osteoblasts and acts as a preventative factor in glucose homeostasis was unknown until recently. The finding was surprising by itself since this intracellular protein tyrosin phosphatase could not be directly involved in the carboxylation of an extracellular protein (Clemens et al., 2011). Based on the observed influence of osteocalcin on insulin, it was hypothesized that there is a reciprocal action of insulin on osteocalcin expression or activity.

Insulin receptors are present in osteoblasts in large numbers. The insulin receptor is a receptor tyrosine kinase which can be upregulated by phosphorylation through exposure to physiological levels of insulin and downregulated by dephosphorylation through the action of protein tyrosine phosphatases (Schlessinger, 2000). Protein tyrosin phosphatase OST-PTP has been reported to regulate osteocalcin carboxylation through dephosphorylation of insulin receptor in osteoblasts (Clemens et al., 2011). It has been shown that the insulin receptor is a substrate of both OST-PTP and PTP-1B in osteoblasts of rodents and humans, respectively; that is, the insulin signaling is negatively regulated by these two protein tyrosin phosphatases (Delibegovic et al., 2009). The finding of the insulin receptor as a substrate for OST-PTP or PTP-1B could be of importance for two novel hypotheses: (1) insulin signaling in bone osteoblasts is required for whole-body glucose homeostasis, supporting the emerging evidence for the regulatory role of bone in global
energy metabolism, and (2) insulin signaling could regulate osteocalcin expression or activity (Clemens et al, 2011).

A recent study by Ferron et al in 2010 showed that mice deficient in osteoblastic insulin receptor (InsR\textsubscript{ob}\textsuperscript{−/−}) have a glucose intolerance-like phenotype with hyperglycemia and hypoinsulinemia, along with decreased uncarboxylated osteocalcin levels (Ferron et al, 2010). Another experiment by the same group on double-mutant heterozygous mice lacking one allele of osteocalcin and one allele of the insulin receptor in osteoblast (Ocn\textsuperscript{−/+}; InsR\textsubscript{ob}\textsuperscript{−/+}) reported the same impaired glucose tolerance phenotype as observed in either the insulin receptor mutant mice (InsR\textsubscript{ob}\textsuperscript{−/−}) or osteocalcin-deficient mice (Ocn\textsuperscript{−/−}) (Ferron et al, 2010).

The results of Ferron et al study confirmed the hypothesis that insulin signaling in osteoblasts could regulate the activity of osteocalcin by promoting its decarboxylation. This mechanism of action of insulin signaling was further analyzed in a study by Fulzele et al. They studied the skeletal phenotype of mice deficient in insulin receptors in osteoblasts and found impaired bone acquisition and low bone density in their trabecular bones as a result of reduced osteoblastic proliferation and bone formation. Surprisingly, osteoclastic activity was also decreased in these mutant mice (Fulzele et al, 2010). These findings led the researchers to believe that insulin signaling not only impacts bone formation, but also regulates bone resorption.

The positive impact of insulin signaling on bone formation is coupled to transcriptional control of osteoblast proliferation and differentiation through suppression of Runx2 inhibitors, Twist2 and FoxO1 (Fulzele et al, 2010). Runx2 is an osteoblast transcription factor which stimulates differentiation of mesenchymal stem cells into osteoblasts (Komori, 2010; Confavreux, 2011). The transcription factor Twist2 is a downstream suppressor of osteoblast differentiation through Runx2 inhibition (Bialek et al, 2004). FoxO1, a member of the forkhead family of transcriptional
factors which promotes gluconeogenesis in hepatocytes, is a negative regulator of insulin receptors in osteoblasts, adipocytes, myocytes, hepatocytes, and pancreatic β-cells (Nakae et al, 2002; Gross et al, 2008;). FoxO1 deficiency in osteoblasts has been shown to be associated with hyperinsulinemia and enhanced insulin sensitivity related to decreased Esp gene expression and increased osteocalcin secretion, suggesting the role of osteoblasts in FoxO1 regulation of glucose metabolism (Rached et al, 2010).

The mechanism of action of insulin signaling on the upregulation of bone resorption by osteoclasts could be explained by the effect of insulin on suppression of osteoprotegrin (OPG) expression in osteoblasts (Ferron et al, 2010; Fulzele et al, 2010). As noted earlier, OPG is secreted by osteoblasts to block osteoclastogenesis by acting as the decoy receptor for RANKL and inhibiting RANK-RANKL interaction on the plasma membrane of osteoclast progenitors (Asagiri et al, 2007). Suppression of OPG expression by insulin signaling results in a decrease in the OPG/RANKL ratio and thus, causes an overexpression of Tcrg1 and cathepsin K in osteoclasts (Ferron et al, 2010; Fulzele et al, 2010). Cathepsin K is a lysosomal proteolytic enzyme which digests the organic component of the bone matrix, specifically type I collagen fibrils (Teitelbaum, 2000; Supanchart et al, 2008). Tcrg1, another osteoclast-specific gene, encodes a proton pump subunit required for acidification of the bone extracellular matrix in the resorption lacunae. This lowers the pH to an approximate acidity of 4.5, sufficient for decarboxylation and activation of osteocalcin (Ferron et al, 2010; Fulzele et al, 2010). Thus, it is the role of the osteoclasts to ultimately determine the form and function of osteocalcin produced by osteoblasts (Ferron et al, 2010). The undercarboxylated osteocalcin then acts as a circulating hormone to promote insulin secretion and sensitivity as well as adiponectin production, resulting in increased energy expenditure.
These results indicate the existence of a feed-forward bone-pancreas endocrine loop through which insulin signaling in the osteoblasts stimulates undercarboxylated osteocalcin secretion, which in turn promotes insulin secretion and sensitivity for regulation of glucose homeostasis and energy metabolism. This feed-forward mechanism adds further credence to the emerging evidence that insulin signaling in osteoblasts is required for the whole-body glucose metabolism and that bone is an important regulator of energy homeostasis (Ferron et al, 2010; Fulzele et al, 2010; Confavreux, 2011).

**Diabetes-induced Osteoporosis:**

There is relatively strong evidence to suggest that type 1 diabetes has negative impacts on bone health, specifically bone mineral density. On the other hand, there seems to be more discrepancy in skeletal effects of type 2 diabetes. While many studies have shown normal to high BMD values in individuals with type 2 diabetes, some have found low BMD values.

In a research study by Lumachi et al in 2009, a small group of adult men and women with type 1 diabetes were recruited and matched with healthy controls by age, gender and BMI in order to study the association between osteopenia and bone health variables including bone mass density. The results of this study showed that the BMD value at the lumbar spine and femoral neck in type 1 diabetic patients was about 60% lower than the controls (Lumachi et al, 2009). A similar study by Saha et al in 2009 on adolescent men and women with type 1 diabetes reported low BMD values in cortical bones and proximal femur, as well as reduced bone size compared to healthy peers. This diminish in BMD values seemed to affect male adolescents more than females (Saha et al, 2009).
Unlike type 1 diabetes, the impact of type 2 diabetes on bone health has been shown to be conflicting. Some studies have reported protective effects, whereas some others have shown negative impact. Yamaguchi et al in a research study in 2009 found higher BMD values at the femoral neck in men with type 2 diabetes, however they reported a lower prevalence of vertebral fractures in the same population (Yamaguchi et al, 2009). However, Yaturu and colleagues found significantly lower BMD values, as well as increased incidences of osteoporosis at hip bone in veteran men with type 2 diabetes compared to age-matched controls (Yaturu et al, 2009).

**Possible Mechanisms of Diabetes-induced Impaired Bone Strength and Osteoporosis:**

Hyperglycemia is a typical clinical feature of both type 1 and type 2 diabetes. It is hypothesized that hyperglycemia is the major factor which is responsible for the detrimental effects of diabetes on bone metabolism and bone quality, both directly and indirectly. Hyperglycemia increases osteoclast function through mechanisms discussed earlier, including increase in number of osteoclasts, since glucose is the main energy source for osteoclasts (Williams et al, 1997), as well as induction of TNF-α, MCSF and RANKL production from osteoblasts to activate osteoclast proliferation and differentiation. Hyperglycemia could also impair osteoblast function through decreasing osteocalcin production and in turn, osteoblast proliferation. The end-result of these mechanisms would be accelerated bone resorption and suppressed bone formation, leading to osteopenia and osteoporosis (Wongdee et al, 2011).

Hyperglycemia can also reduce the number of endothelial progenitor cells, lining the blood vessels. This could result in decreased angiogenesis, which could in turn, retard bone formation and repair at fracture sites and further aggravate bone loss (Wongdee et al, 2011). Additionally,
high blood glucose can promote an adipocyte-like phenotype by overexpression of adipocyte
differentiation markers including PPARγ. This adipogenic differentiation of mesenchymal cells
causes an osteoblast-to-adipocyte shift, and thus suppresses osteoblast differentiation and bone
formation (McCabe, 2007; Wongdee et al, 2011).

A major mechanism of hyperglycemia in impairing mechanical characteristics and bone strength
is by formation of Advanced Glycation Endproducts (AGEs) or non-enzymatic cross-links in
type 1 collagen fibers. These permanent glucose-derived cross-links can contribute to decreased
bone rigidity and bone quality and increases bone fragility in type 1 and type 2 diabetes (Saito et
al, 2010).

**Specific Mechanisms of Type 1 Diabetes:**

It is clearly known now that type 1 diabetes primarily decreases bone formation, rather than
increasing bone resorption, as its major skeletal effect. This would in turn, induce the osteoblast-
to-adipocyte shift and promote bone marrow adiposity (McCabe, 2007). Along with
hyperglycemia, hypoinsulinemia is another major contributor to bone fragility in type 1 diabetes.
Insulin is an anabolic hormone and its deficiency can negatively impact bone turnover, majorly
by reducing osteoblast proliferation and function (McCabe, 2007). Additionally,
hypoinsulinemia leads to a deficiency in insulin-like growth factor (IGF-1), which in turn, results
in low BMD values in type 1 diabetes (Botolin et al, 2007). On the other hand, GH/IGF axis is a
major mechanism contributing to decreased bone formation, since IGF-1R is required for
anabolic effects of growth hormone (GH) in osteoblasts in vivo (DiGirolamo et al, 2007).
An indirect contributor to type 1 diabetes-induced osteoporosis is impaired adipokines secretion and function including leptin (Elefteriou, 2008). The mechanism is not yet clear, but it has been shown that leptin is an activator of bone resorption (Elefteriou et al, 2005; Shi et al, 2008).

**Specific Mechanisms of Type 2 Diabetes:**

Unlike type 1 diabetes that is characterized by low body weight, hypoinsulinemia, and low IGF-1 levels which all adversely impact bone status, type 2 diabetes is characterized by overweight and obesity, and hyperinsulinemia at early stages, all of which could have potential anabolic effects on bone that can counteract the negative effects of hyperglycemia (Cutrim et al, 2007).

However, advanced insulin resistance in later stages of type 2 diabetes along with increased levels of AGEs and diabetic complications could lead to detrimental effects on mechanical bone properties (Merlotti et al, 2010). On the other hand, although obesity can have a protective role on BMD by increasing the mechanical load on the bone, an increase in adiposity as well as insulin resistance during poor glycemic control could result in upregulation of cytokines, adipokines and leptin which in turn, promotes inflammation. Inflammation is a major risk factor for high bone turnover, bone fracture and osteoporosis (Wisse, 2004; Clowes et al, 2005; Ginaldi et al, 2005; Tilg et al, 2008).

Limited research suggests a role of low vitamin D and calcium homeostasis in the development of type 2 diabetes, specifically during poor glycemic control. A systematic review and meta-analysis on the role of vitamin D and calcium in type 2 diabetes by Pittas and colleagues in 2007 suggested an association between low vitamin D status, calcium intake, and risk of type 2 diabetes in observational studies (Pittas et al, 2007). However, intervention studies have shown
small benefits of vitamin D supplementation alone, but positive effects of combined vitamin D and calcium in preventing type 2 diabetes among populations most at-risk (Hitz et al, 2007). On the other hand, diabetic nephropathy as a complication of type 2 diabetes has been shown to increase bone fragility and decrease bone quality through impaired secretion of Parathyroid Hormone (PTH), low bone turnover, and accumulation of microdamage in bone (Inaba et al, 2005).

Overall, obesity and over-glycosylation of collagen fibers are two important factors contributing to increased bone fracture risk in type 2 diabetes, independent of bone mass density (de Paula et al, 2010).

The present study was conducted due to the conflicting results of the relationship between diabetes and bone mineral density, specifically in type 2 diabetes. This study is aimed at elucidating the effect of diabetes-related variables on bone health status and bone mineral density, especially in osteoporosis, in both adult men and women.
Objectives:

In an effort to examine the bone mass density (BMD) of adult men and women with pre-, type 1 and type 2 diabetes, we aimed to:

1. compare BMD by diabetes status.

2. determine frequency of low BMD (T-score < -1) by type of diabetes.

3. determine if demographics, lifestyles and health behaviors, history of falling or bone fracture, current status or history of conditions and medication use, and female reproductive history explain any of the variance in BMD in this population.

4. determine if family history of diabetes and any of the bone diseases contribute to the variance in BMD.
Hypotheses:

1. BMD levels are significantly lower in individuals with type 1 diabetes and are significantly higher in individuals with type 2 diabetes.

2. Frequency of low BMD (T-score < -1) is significantly higher in subjects with type 1 diabetes than those with type 2 diabetes.

3. Older age and being a female are significant contributors to the variance in BMD.

4. Those with vigorous or moderate physical activity have significantly higher BMD values than those who do not have any exercise.

5. BMD is significantly lower in individuals with a history of falling or bone fracture.

6. Women who have undergone menopause have significantly lower BMD values than those who have not yet experienced it.

7. Family history of diabetes and/or bone diseases contributes significantly to the variance in BMD.
CHAPTER 2

METHODOLOGY

Population:

Individuals with type 1 or 2 diabetes were recruited to participate in this study.

Subject Recruitment:

Subject inclusion criteria were to have diabetes and be 18 years of age or older. Adults over age 65 were not specifically included nor excluded.

Various methods of recruitment were utilized. The first round of recruitment was through E-week, the weekly University of Illinois faculty/staff electronic mail newsletter with brief summaries of campus announcements. The recruitment announcement was made twice during Fall 2009 and once during Spring 2010 semesters. The announcement invited those with diabetes to participate in the bone health screening study by having their bone mass density checked through a heel ultrasound screening test, filling out a short questionnaire, having their anthropometric measurements taken and contributing their saliva to determine the relationship between diabetes and osteoporosis in terms of genetic markers (See Appendix A for the recruitment announcement).

The second round of recruitment consisted of posting flyers all around campus on public bulletin boards and placing advertisements in the Daily Illini (DI) newspaper. DI is the independent student newspaper of the University of Illinois. The flyers and advertisements contained the same information as the E-week announcements. Permission was sought from the Institutional
Review Board (IRB) at the University of Illinois at Urbana-Champaign prior to hanging up flyers and posting ads to the newspaper (See Appendix B for the recruitment flyer and Appendix C for DI ad).

Since enough samples were not recruited through previous methods, we expanded the recruiting efforts to local diabetes support groups, including Senior High Rise Diabetes Mellitus Outreach Groups at Champaign-Urbana Public Health Department (CUPHD). The recruitment consisted of the student researcher attending one of the group’s monthly meetings and explaining about the research study. This was done at two of their sites: Washington Square in Urbana, and Round Barn in Champaign. The contact information of the volunteers was collected at the end of the session and an appointment was made through the group’s coordinator.

**Data Collection:**

Subjects who were recruited through E-week, flyers, and DI ads were faculty, staff and students associated with the University of Illinois at Urbana-Champaign. These individuals were examined in Bevier Hall room 199. Participants from the Senior High Rise DM Outreach groups were visited at the same sites of CUPHD as recruitment. All the testing was conducted between September 2009 and May 2010.

Subjects were provided the email address of the student researcher in all recruitment methods. An appointment for participation in the study was scheduled upon their contact with the student researcher. An email with the exam instructions on the bone mass density test, anthropometric measurements, the health questionnaire and DNA testing as well as the reimbursements were then sent to the potential participant. Those who were unable to currently participate due to their
busy schedules or those who were unable to attend their appointment were asked if and when they could be contacted again for rescheduling an appointment. A reminder email was also sent to the participant one day before their exam appointment with detailed information about direction to the study location, parking and instructions about refraining from eating food, drinking beverages (including water), smoking, brushing teeth, and chewing gum for an hour before the appointment, provided they were interested in participating in the DNA testing.

Study approval was obtained from the Institutional Review Board at the University of Illinois at Urbana-Champaign. Participants were provided two written informed consent forms approved by the IRB at the time of enrollment. The first consent form included information on bone screening in those with diabetes and informed the participants of the research protocol, including the heel ultrasound device and bone mass density measurement, as well as the anthropometric measurements and the brief health questionnaire. The second consent form provided information about DNA testing and saliva collection. It was explained to the subject that participation in this second part of the study was completely optional. Both consent forms informed the participants of their rights as human subjects, including their right to withdraw from the study at any time, and confidentiality and anonymity of the information. Subjects were asked to sign the consent forms prior to participation (See Appendix D for consent forms).

At the beginning of the study, the participant was given a screening test consisted of three questions. The recruiter determined whether the participant was eligible to participate based on their answers to these questions. Eligibility was defined as having type 1 or 2 diabetes or pre-diabetes, not having any pins or metals in the feet/ankles and not having any swelling in the feet/ankles. Diabetes was self-reported by the subjects. No examination was conducted to diagnose diabetes in the subjects and no proof of the disease was obtained. In case a subject had
either pins/metals or swelling in the foot/ankle, the other foot was used for the bone screening test (See Appendix E for the screening test).

The main part of the exam consisted of subjects rotating through four testing areas; anthropometric measurements, self-administered health questionnaire, heel ultrasound testing, and saliva collection. Participants had anthropometric measurements taken including weight, height, wrist, waist and hip circumferences. A calibrated medical electronic scale and a standard stadiometer with a sliding head piece were used for weight and height measurements, respectively. Subjects were wearing light clothing and no shoes at the time of measurements. Wrist, waist and hip circumferences were taken with a plastic tape measure. Wrist circumference was measured around the styloid process of ulna and radius, also known as the “wrist bone”; waist circumference was taken at the umbilical level, the midpoint between the lower ridge of the ribs and the upper ridge of the pelvis; and hip circumference was measured at the widest area around the buttocks. Each measurement was taken three times for precision and was converted into metric units (kilograms for weight and centimeters for height and circumferences). Body Mass Index (BMI), frame size and Waist-to-hip ratio (WHR) were calculated and recorded based on the above-mentioned measurements. The average of the three BMI and WHR measurements were used for further analysis.

The self-administered health questionnaire was designed specifically to gather information about diabetes and bone health (See Appendix F). The first section of the questionnaire collected demographic information, including gender, date of birth, ethnicity, and education level. Age was calculated as the difference between the date of birth and the date of the exam expressed in years. The second section assessed the diabetes status, including type of diabetes, duration of diabetes, oral medications and insulin use, history of previous diabetes medications, and
Hemoglobin A1C (HbA1c) testing, as well as health behaviors and lifestyles, including smoking, alcohol use, caffeine intake, calcium/vitamin D supplement use and vegetarianism.

The third section of the questionnaire was designed to assess the physical activity of the participants, including vigorous and moderate physical activity as well as walking and sitting habits, all of which were specifically described and explained with examples. Vigorous physical activity was defined as taking hard physical effort and making breathing much harder than normal, including heavy lifting, digging, aerobics, or fast bicycling. Moderate physical activity was described as those activities that take moderate physical effort which make breathing somewhat harder than normal, and may include carrying light loads, bicycling at a regular pace, or doubles tennis. Walking included at work and at home, walking to travel from place to place, and any other walking that was done solely for recreation, sport, exercise, or leisure. Sitting was described as any time spent sitting at work, at home, while doing course work, and during leisure time, and included time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Participants were asked to report how many days and how much time in a day they had usually spent in each specific category in the last 7 days for at least 10 minutes at a time.

A history of weight gain and/or weight loss, fall, bone fracture and bone mineral density scan was also obtained. A major part of the questionnaire assessed the current status and/or history of several medical conditions including thyroid disease; intake of bone medications specifically prescribed for bone health; and other medications including antidepressants and glucocorticoids that could have an impact on bone density. Information on the family history of participants, including diabetes, osteoporosis, bone fracture, loss of height as grown older, high blood pressure, and dyslipidemia was also obtained.
The last section of the questionnaire was specifically designed to gather information on the female reproductive history, including menopause status, age at menopause, Hormone Replacement Therapy use, and history of oophorectomy, pregnancy and breastfeeding.

**Quantitative Ultrasound (QUS):**

Bone mineral density (g/cm\(^2\)) in heels was measured on all participants by QUS using Hologic Sahara Clinical Bone Sonometer (Hologic Inc., Waltham, MA, 1998). There are no known adverse effects from ultrasound testing. Heel BMD was measured with the participant seated in a stationary, straight-back chair with their shoes and socks off. The non-dominant foot was used for measurement, unless there were pins, metals or swelling in that foot. The participant’s bare heel was then covered with a small coating of ultrasound conduction gel and was placed into the ultrasound machine in between the already gelled transducer pads with the middle of the heel snug against the center of the positioning contour and the positioning line aligned with the gap between the patient’s second and third toe. The foot was then held still with the positioning aid strapped around the leg. The measurement was taken three times and the average was used for further analysis.

Participants were provided with a copy of their ultrasound results at the end of the testing (See Appendix G). Those with T-scores of more than one standard deviation below the mean were encouraged to visit their primary physician for further osteoporosis evaluation.

Quality control was performed using QC phantom each day at the beginning of the testing session to monitor system performance. Additionally, a pilot study was conducted to measure the precision (percent coefficient of variation) of repeated heel BMD measurements in humans. This
was done to ensure that the estimated coefficient of variation specified in the QUS manual was correctly obtained in the study. The precision was determined using three measurements of heel BMD from three staff volunteers as well as the student researcher and was calculated using SAS 9.2 software (SAS Inc., 2010). The coefficient of variation was 3%, and was in agreement with the reported percentage in the manual.

At the end of the rotations, willing participants were asked to spit into a vial for their saliva sample. Saliva samples were collected according to the manufacture’s protocol and stored immediately at -80 °C freezer until the DNA extraction protocol was performed (See Appendix H for the genetic study).

As compensation for participation, subjects received one $5 gift card for participation in the bone screening test and another $5 gift card for DNA testing, a recipe book on bone health or diabetes, and the opportunity to attend a class or seminar on diabetes and bone health. Participants were also provided with a copy of their heel ultrasound results. Subjects who were recruited from the University of Illinois were given gift cards to Bevier or Bevier II café, while participants at CUPHD were granted either a County Market or Jerry’s IGA gift card, depending on the store accessibility at the site of recruitment.

**Statistical Analysis:**

The statistical analysis was performed using SAS 9.2 software (SAS Inc., 2010). Power analysis and sample size calculation were conducted with SAS power and sample size 3.1 (SAS Inc., 2010). All the collected data were manually entered into Excel and were double checked by the Primary Investigator.
Of the continuous variables, all bone health variables including BMD, Tscore, BUA, QUI, and SOS were normally distributed. Age, in years, also had a normal distribution. BMI, WHR, duration of diabetes in years, length of medication intake, and HbA1c value were not normally distributed. For showing the frequency of normal variables, mean ± standard deviation was used, whereas for non-normal variables frequency was shown by using median, lower quartile (25th percentile), and upper quartile (75th percentile). Two-sample independent t-test and ANOVA test were used for the analysis of normal variables, including the association of Type of diabetes with age and all the bone health variables. Kruskal-Wallis test was used to compare non-normally distributed variables, including the association of Type of diabetes with BMI, WHR, duration of diabetes in years, length of medication intake, and HbA1c value. ANOVA with Fisher Least Squared Differences (LSD) was used to determine post-hoc mean separation for variables identified as statistically significant. Chi-square test was used to look at the associations between categorical variables. Pearson correlations were used to assess the correlation between continuous normally distributed variables, whereas Spearman correlations were used to assess the correlation between continuous non-normally distributed variables. To explain the variance in BMD as the dependent variable, stepwise multiple regression analysis was performed. Dummy variables were created for the independent categorical variables in the model. P-values were set at alpha = 0.05 for statistical significance.
CHAPTER 3
RESULTS

Overall, 54 subjects with pre-, type 1 and type 2 diabetes participated in the study. Table 1 shows some of the demographic and anthropometric characteristics of the participants. Age ranged from 27 to 86 years with a mean of 62 years, and diabetes duration ranged from 1 month to 50 years with a median of 7 years. Individuals with type 1 diabetes had higher duration of diabetes and lower waist-to-hip ratio. There were no statistical differences in age, medication duration, hemoglobin A1C, or BMI across the three diabetic groups.

Demographic characteristics are presented in Table 2 and diabetes status is shown in Table 3. The majority of participants were white/Caucasian women with college/professional degree, who had type 2 diabetes, were not on insulin and had diabetes for 7 or more years. Insulin use was higher among individuals with type 1 diabetes and those with type 1 diabetes were more likely to have diabetes for a longer time.

Figure 1 shows a pie chart of the frequency of current oral antidiabetic medication use. The majority of the participants (34.5%) were not on any type of oral medication. Of those who used oral medication, approximately 32.6% were on metformin, followed by 14.3% on a combination of metformin and sulfonylurea, and 12.2% on sulfonylureas only.

Health behaviors and lifestyles are presented in Table 4. The majority of participants were non-smokers, not regular alcohol users, not vegetarians or vegans, and were on calcium/vitamin D supplements. Regular alcohol use was higher in individuals with type 1 diabetes. Calcium/vitamin D supplement use was higher among those with type 2 diabetes.
Table 5 shows history of weight, falling and bone fracture in participants. Most of the subjects did not have a history of weight gain or weight loss of more than 15 lbs in the last year and had not experienced falling in the last 6 months. The majority of participants did not have a bone fracture during their adult life (21 years or older) and had not experienced a bone mineral density scan of whole body or hip/spine. There were no statistical differences in either of the variables across the three groups of type of diabetes.

The QUS bone sonometer was used to measure bone mineral density of the non-dominant heel, and results are presented in Table 6. Of the total 54 subjects, bone health variables of 52 participants were recorded. Four invalid bone mass values were measured for one of the subjects due to swollen ankles on both feet, and invalid measurements were recorded 8 times (4 times on each foot) from another participant due to the presence of rod in the non-dominant calf. All bone health variables were lower in subjects with type 1 diabetes compared to those with type 2 diabetes, but no statistical differences were found across the three diabetic groups in terms of BMD, T-score, quantitative ultrasound index (QUI), broadband ultrasound attenuation (BUA), or speed of sound (SOS). Power analysis showed that for a two-sample pooled t test of a normal mean difference with a two-sided significance level of 0.05, assuming a common standard deviation of 0.1, group sample sizes of 8 (type 1 diabetes) and 38 (type 2 diabetes) did not have enough power to detect a difference between BMD means (power=71%).

T-score values ranged from -4.00 to 2.23 with a mean of -0.76. Approximately 44.2% of the participants had T-scores smaller than one standard deviation below the young adult reference mean (T-score ≤ -1). Of those with T-scores less than one standard deviation below the reference mean, approximately 83% were female, 18% had type 1 diabetes, 32% were on insulin, and 62.5% had diabetes for 7 or more years (median value) (Table 7). Additionally, of those with T-
scores less than -2.5 standard deviation threshold for osteoporosis (4 individuals, 7.7% of total participants), approximately 75% were female, 25% had type 1 diabetes, and none were on insulin (Table 7). On the other hand, 23 postmenopausal white women participated in this study, among which approximately 61% had T-scores less than one standard deviation below the reference mean, and 13% had T-scores less than -2.5 standard deviation threshold for osteoporosis (data not shown).

Data for the bone mineral density values in various groups, including those for demographics, diabetes status, bone health, medical conditions, and female reproductive history are presented in Tables 8 and 9. No statistical differences were found for BMD values within gender, insulin use, duration of diabetes, medication type, history of falling and bone fracture. Power analysis showed that for a normal mean difference with a two-sided significance level of 0.05, assuming a common standard deviation of 0.1, group sample sizes of 18 (duration <7 years) and 18 (duration ≥ 7 years) have a power of 82% to detect a difference between BMD means, and group sample sizes of 37 (Non-insulin users) and 14 (Insulin users) have a power of 88% to detect a difference between BMD means. The power for detecting significant differences between BMD means for gender, history of falling and bone fracture were not strong enough (power < 80%).

However, of all the conditions and diseases, BMD values were only significantly lower among subjects with a current status and/or history of cancer compared to those who never experienced cancer (p=0.037). Of all the medications, significant lower BMD values were only found in those who currently used or had a history of using any of the bone medications compared to individuals who were never on any of the bone meds (p=0.003). Least Significant Difference to determine post hoc significance showed that in females, those who had undergone menopause had significantly lower BMD values than those who had not experienced menopause yet
(p=0.0033). Power analysis for a normal mean difference with a two-sided significance level of 0.05, assuming a common standard deviation of 0.1 for cancer, bone medication, and menopause were all above 80% to detect a statistical significant difference between BMD means.

Of those variables for medications and diseases which did not have a statistical significant relationship with BMD values, power analysis for a normal mean difference with a two-sided significance level of 0.05, assuming a common standard deviation of 0.1 for hypertension, thyroid disease and kidney problems were 92%, 86%, and 86%, respectively, to detect a difference between BMD means. Of all the medications for which no significant relationship was reported, only power analysis at significance level of 0.05 for thyroid medication and Actonel as a bone medication were strong enough to detect a difference between BMD means (86% and 90%, respectively). Additionally, only power analysis with a two-sided significance level of 0.05 for family histories of diabetes, hypertension and bone fracture were above the 80% threshold of detecting significant differences between BMD means (85%, 83%, and 81%, respectively).

Physical activity is shown in Table 10. Approximately 83% of the participants had some kind of physical activity, either vigorous, moderate, or walking during the last 7 days of their data collection, and 7.55% spent absolutely no time on physical activity. Statistical significant differences were found between BMD values of those with and without moderate physical activity and those not sure about their moderate physical activity (p=0.048). Least Significant Difference to determine post hoc significance showed that individuals who had moderate physical activity in the past 7 days had significantly higher BMD values than those who did not have any moderate physical activity or those who were not sure about their moderate activity. Power analysis for a normal mean difference with a two-sided significance level of 0.05, assuming a common standard deviation of 0.1 for moderate physical activity yielded a power of
80% for detecting significant differences between BMD means. For all other physical activity levels that were not significantly related to BMD values, the power analysis for detecting significant differences between BMD means did not yield strong enough powers (power < 80%).

Table 11 illustrates the odds ratio for BMD and T-scores among subjects with type 2 and pre-diabetes against individuals with type 1 diabetes (used as the reference group). BMD values were higher among participants with type 2 diabetes than those with type 1 diabetes and pre-diabetes, but the difference was not statistically significant. The odds ratio for high BMD values in the group with type 2 diabetes and pre-diabetes compared to type 1 diabetes was 14.54 and 2.31, respectively. That is, for a one standard deviation increase in BMD value, the odds of having type 2 diabetes and pre-diabetes increased by 1354% [95% CI: 4.916-43.027] and 131% [95% CI: 0.513-10.381], respectively. In other words, the odds of high BMD values for type 2 diabetes was about 1354% of the odds for type 1 diabetes, and the odds of high BMD values for pre-diabetes was about 131% of the odds for type 1 diabetes. On the contrary, the odds of high BMD values for type 1 diabetes was only about 0.069 times of the odds for type 2 diabetes (95% CI: 0.023-0.203; data not shown).

After adjustment for BMI, the odds of high BMD values for type 2 diabetes increased from 14.54 to 28.96 times greater than type 1 diabetes, but the odds ratio for pre-diabetes compared to type 1 diabetes decreased from 2.31 to 1.17 times (Table 11). Additionally, when adjusted for BMI, the odds ratio for high BMD values in subjects with type 1 diabetes significantly decreased from 0.069 to 0.035 times of the odds in individuals with type 2 diabetes (95% CI: 0.011-0.107; data not shown). Adjustment for age or gender did not yield statistically different results and the odds ratio and confidence intervals across all three diabetic groups were non-estimable (data not shown).
In addition, low BMD was categorized as those with T-scores below one standard deviation of
the reference mean (T-score < -1) and was compared against the control group with normal
BMD who had T-scores greater than one standard deviation below the reference mean (T-score >
-1). We found that type of diabetes was not associated with low T-scores. The odds ratio for low
BMD in type 2 diabetes and pre-diabetes compared to type 1 diabetes was 0.818 and 1.000,
respectively. In other words, the odds of low BMD values for type 2 diabetes was about 82% of
the odds for type 1 diabetes, and the odds of low BMD values for pre-diabetes was exactly the
same as the odds for type 1 diabetes. On the other hand, the odds of low BMD values was 1.22
times greater in individuals with type 1 diabetes than those with type 2 diabetes (95% CI: 0.267-
5.585; data not shown). Adjustment for age did not change the results, and adjustment for BMI
yielded non-estimable odds ratio and confidence intervals across all groups (data not shown).

The correlations of demographic, anthropometric and diabetes factors are presented in Table 12.
Only age had a moderate negative association with BMD (r=-0.574; p<0.0001). This association
was significantly stronger among type 1 and type 2 diabetes (r=-0.742,-0.673, respectively;
p<0.0001), but not in pre-diabetes (r=-0.400; p=0.60; data not shown). All other variables had
weak associations with BMD and lacked statistical significance. These associations were not
statistically different by diabetes status either (data not shown).

The unadjusted and adjusted BMD and T-score values across all three diabetic groups are shown
in Table 13. Participants with type 1 diabetes had lower BMD and T-score values than those with
type 2 diabetes. With adjustment for age, BMD values became lower in individuals with type 1
diabetes (mean=0.40), however, they were not statistically different in subjects with type 2 and
pre-diabetes. After adjustment, T-score levels became lower in type 1 diabetes and higher in type
2 diabetes (mean=-1.09, -0.59, respectively). T-score values did not show any statistical change
in pre-diabetes. Adjustment for BMI did not yield any statistical differences in either BMD or T-scores (data not shown).

Multiple regression equations were calculated for bone mineral density (BMD) as the dependent variable against independent variables in several categories: demographics, diabetes status, diabetes care and management, lifestyle and health behaviors, bone health status, oral medications, current status and/or history of medical conditions, family history, and female reproductive history. The equations are presented in Table 14. In terms of demographics, gender and African-American/black ethnicity contributed to the variance in BMD. Among variables for diabetes status, age, type 1 diabetes and oral metformin use were contributors to BMD variance. Duration of diabetes and the most current HbA1c value contributed to BMD variance in the category of diabetes care and management. In terms of lifestyle and health behaviors, consumption of calcium/vitamin D supplements as well as moderate physical activity during the last 7 days including those activities that take moderate physical effort which make breathing somewhat harder than normal contributed to the variance in BMD. Among variables describing the bone health status, only a history of BMD scan had an effect in the variance of BMD. Intake of any bone medication specifically prescribed for bone health and current status and/or history of osteoporosis separately contributed to the variance in BMD. In terms of family history, family history of osteoporosis and loss of height with aging contributed to BMD variance. Among variables for female reproductive history, menopause and breastfeeding were significant contributors to variance in bone mineral density.

An overall regression model was calculated for variables which had statistical significance in their multiple regression equations. Bone mineral density was used as the dependent variable. This model shows that African-American ethnicity, calcium/vitamin D supplement use, family
history of loss of height, current status and/or history of osteoporosis, and menopause in female significantly contribute to the variance in BMD. The R-square for this model was 0.7714, meaning that approximately 77% of the variability of BMD was accounted for by the variables in the model (data not shown). On the other hand, the adjusted R-square (0.6953) indicated that about 69% of the variability of BMD was accounted for by the model, even after taking into account the number of predictor variables in the model (data not shown).

The coefficients for each of the variables indicated the amount of change in BMD given a one-unit change in the value of that variable, given that all other variables in the model were held constant. Based on the overall model, we would expect a decrease of 0.222 in the BMD value for every one-unit increase in African-American ethnicity, a decrease of 0.082 for every one-unit increase in calcium/vitamin D supplement intake, a 0.111 decrease for every one-unit increase in family history of loss of height, a 0.181 decrease for every one-unit increase in current status and/or history of osteoporosis and a decrease of 0.101 for every one-unit increase in menopause, assuming that in each case all other variables in the model are held constant.

In order to compare the strength of the coefficients of these variables, we obtained standardized values, also called beta coefficients. These beta coefficients can be used to compare the relative strength of the various predictors within the model. Because these standardized coefficients are all in the same standardized units, i.e. standard deviation, they can be compared to assess the relative strength of each of the predictors. In our overall model, African-American ethnicity had the largest beta coefficient, -0.44, and intake of calcium/vitamin D supplement had the smallest beta, 0.278. Thus, a one standard deviation increase in African-American ethnicity led to a 0.44 standard deviation decrease in predicted BMD, with the other variables held constant.
Additionally, a one standard deviation decrease in calcium/vitamin D supplement use resulted in a 0.278 standard deviation increase in BMD with all other variables in the model held constant.
CHAPTER 4
DISCUSSION

Bone mineral density was low in our sample of individuals with type 1 diabetes compared to those with type 2 diabetes, but this difference lacked statistical significance. Calculating odds ratio for BMD showed odds of high BMD values of about 14.5 times greater in subjects with type 2 diabetes than those with type 1 diabetes. After adjustment for BMI, the odds of high BMD values associated with type 2 diabetes increased to 28.96. On the other hand, adjusting for age resulted in lower BMD and T-score values in individuals with type 1 diabetes as well as higher T-scores in those with type 2 diabetes. These findings are in agreement with numerous earlier studies that have investigated BMD by diabetes status. There is relatively strong evidence to suggest that type 1 diabetes has negative impacts on bone health, specifically bone mineral density, and that it results in higher incidence of bone fractures. On the other hand, there seems to be more discrepancy in skeletal effects of type 2 diabetes. While many studies have shown normal to high BMD values in individuals with type 2 diabetes, some have found low BMD values.

Of the most recent studies, Mastrandrea et al in 2008 measured bone mineral density using dual-energy X-ray absorptiometry on 63 young female subjects with type 1 diabetes aged 13-35 years. The results of the study showed a significant reduction in BMD at hip, femoral neck and the whole body in women aged 20 years and older. Thus, it was suggested that low BMD as well as failure to accumulate bone density after age 20 may contribute to the increased incidence of osteoporotic hip fractures in postmenopausal women with type 1 diabetes. (Mastrandrea et al, 2008). Another study by Hamilton and colleagues in 2009 on both males and females with type 1
diabetes aged 20-71 years reported lower BMD values at hip, femoral neck and spine in diabetic adult male participants. However, no significant differences were found in BMD values of diabetic female subjects compared to their age-matched controls. These results suggested that reduced bone density in male could be a result of type 1 diabetes (Hamilton et al, 2009).

The study by Soto and colleagues in 2011 on young adolescents and adult women with Type 1 diabetes was conducted with the objective of examining the relationship between bone mass density and sex steroids. Diminished bone mass was observed at spine, femoral neck and whole body of both the adolescent and adult diabetic women, but there was no association with sex steroid levels. It was suggested that the observed decrease in bone mass could be related to differences in body composition and age (Soto et al, 2011).

Unlike type 1 diabetes, the impact of type 2 diabetes on bone health has been shown to be conflicting. Some studies have reported protective effects, whereas some others have shown negative impact. The exact reason for these conflicting results is unknown, but the inconsistency most probably arises from confounding factors such as gender, diabetes complications, body mass index (BMI), ethnicity, and pharmacological agents. For instance, in a recent study by Petit et al in 2010, higher BMD values were reported in older men aged 65+ years with type 2 diabetes compared to age-matched healthy controls (Petit et al, 2010). On the contrary to this study, Yaturu and colleagues found significantly lower BMD values, as well as increased incidences of osteoporosis at hip bone in veteran men with type 2 diabetes compared to age-matched controls (Yaturu et al, 2009).

In the present study, history of bone fracture during adult life (21 years or older) and history of falling in the last 6 months were not statistically different across the diabetic groups in the present study. These non-significant observations could possibly be due to the lack of sample
size in each category which resulted in a low power unable to detect significant differences of BMD values for falling and bone fracture history (power < 80%). These findings are on the contrary to the results of previous studies which have reported higher bone fracture risk among individuals with type 1 and type 2 diabetes.

Consistent with the findings on BMD, higher bone fracture risk has been reported in type 1 diabetes. Various studies have shown increased fracture risk in men and women with type 1 diabetes (Strotmeyer et al, 2006; Miao et al, 2005; Nicodemus et al, 2001). Previous research has shown that a one standard deviation decrease in BMD at the heel, hip or spine of postmenopausal women can result in a 1.4-2.6 increased relative risk of bone fracture at any site (Cummings et al, 2002). In a study by Hofbauer et al in 2007 increases in bone fracture risk at various sites were found for type 1 diabetes. These increases ranged from 1-2 folds at the spine to 1.5-2.5 folds at the hip bone (Hofbauer et al, 2007). Another supportive evidence was a meta-analysis in 2007 which reported decreased BMD values and increased hip fracture risk in type 1 diabetes (RR=6.94; 95% CI: 3.25-14.78) (Vestergaard, 2007).

Interestingly, many studies that have shown higher to normal BMD values in type 2 diabetes have also reported increased fracture risk. In the meta-analysis by Vestergaard, BMD Z-scores were found to be increased in the spine (0.41 ± 0.01) and hip (0.27 ± 0.01) in type 2 diabetes. However, hip fracture risk was higher in those with type 2 diabetes (RR=1.38; 95% CI 1.25-1.53) compared to their age-adjusted controls (Vestergaard, 2007).

The Rotterdam study, one of the largest studies of its time, reported higher fracture risk in patients who had type 2 diabetes for a long time, but not in newly diagnosed subjects (RR=1.69 and 1.01, respectively) compared to non-diabetic controls (de Leifde et al, 2005). A more recent study in Canada confirmed these findings and showed an increase in fracture risk in subjects
with type 2 diabetes for more than 5 years compared to the newly diagnosed individuals (Lesli et al, 2007). Another supportive evidence is a study by Schwartz et al in 2005 who reported higher hip bone loss in older adults with type 2 diabetes (Schwartz et al, 2005).

All of these findings led to the conclusion that the increase in fracture risk in type 2 diabetes, despite high to normal BMD values, could be due to factors other than the bone mineral density. One hypothesis is that the stage of type 2 diabetes could be an important determinant, such that the initial phase of insulin resistance may have a protective effect on bone, whereas negative bone health effects could result from long-term type 2 diabetes (Schwartz, 2009).

High BMI is a common feature of type 2 diabetes which seems to be accounting for some of the increase in BMD values (de Laet et al, 2005). In the present study, we also found higher BMI values in individuals with type 2 diabetes compared to subjects with type 1 diabetes, but the difference was not statistically significant. Although overweight and obesity have been shown to be contributing factors to high BMD values and increased bone fracture risk, previous studies have shown the increase in BMD to be persistent even after controlling for BMI (Strotmeyer et al, 2004; Rakic et al, 2006; Vestergaard, 2007).

On the other hand, studies using quantitative computed tomography (QCT) have suggested volumetric decreases in cortical bones of individuals with established type 2 diabetes, which due to small cortical bone area results in lower compressive bone strength and higher bone fragility (Melton et al, 2008; Petit et al, 2010).

Another hypothesis for increased bone fracture risk in type 2 diabetes could be the long-term diabetes complications, including diabetic retinopathy (visual impairments) and peripheral neuropathy. Poor vision due to diabetic retinopathy and neuropathy could potentially increase the
propensity of the diabetic subject to fall and thus, increase their risk of bone fracture (Rix et al, 1999; Maurer et al, 2005). Additionally, peripheral neuropathy in type 2 diabetes could lead to Charcot osteoarthropathy, defined as bone damage and deformity around weight-bearing joints, especially in the foot. This is mostly caused by loss of sensation in the feet due to neuropathy which in turn, could result in loss of muscle support, joint instability and bone destruction (Hamilton et al, 2009).

Significant associations for low bone mass density with age and menopause in females have been observed in the present study which suggests that menopause and aging have negative effects on bone health status and thus, could contribute greatly to the potential adverse effects of type 1 diabetes on BMD and bone fragility. A study by Danielson et al on postmenopausal women reported low BMD values and high bone fracture risk associated with type 1 diabetes. They also indicated that women with type 1 diabetes experience aging and menopause-related alterations in bone turnover at a younger age which could potentially add to adverse effects of type 1 diabetes on bone health status (Danielson et al, 2009).

With regard to low BMD, defined as T-scores less than one standard deviation below the young adult reference mean, about 44% of the participants had low BMD values, who were considered to be osteopenic or osteoporotic based on the WHO guidelines (World Health Organization, 1994). A higher proportion of subjects with type 2 diabetes (73%) met the study criterion for low BMD than those with type 1 (18%) or pre-diabetes (9%), however this difference was not statistically significant. On the other hand, a smaller proportion of those with low BMD were on insulin compared to non-insulin users (33% vs. 68%), and had diabetes for less than 7 years compared to those who had diabetes for 7 or more years (37.5% vs. 62.5%), both of which were not statistically significant. Additionally, of those with T-scores less than -2.5 standard
deviations threshold for osteoporosis, approximately 75% were female, 25% had type 1 diabetes, and none were on insulin.

The statistics for low T-scores in type 1 diabetes observed in the present study is comparable to the finding of McCabe in 2007. It was estimated that 50% of those with type 1 diabetes had low bone mass density and almost 20% of patients aged 20-56 years had T-scores below -2.5 standard deviations (McCabe, 2007). Moreover, based on the Third National Health and Nutrition Examination Survey (NHANES III, 1988-1991), 34-50% of postmenopausal white women have osteopenia, and 17-20% have osteoporosis (Looker et al, 1995). This statistics is close to our finding that among the white postmenopausal women, about 61% had T-scores less than one standard deviation below the reference mean indicating osteopenia, and 13% had T-scores below -2.5 standard deviations threshold for osteoporosis.

In the present study, there was no significant relationship between gender and BMD values, and adjustment for gender did not yield significant results for the odds ratio of BMD among subjects with type 2 and pre-diabetes against individuals with type 1 diabetes (used as the reference group). However, women who had already gone under menopause at the time of the study had significantly lower BMD values than women who had not experienced menopause yet. No significant associations were found for any of the other female reproductive history variables, including hormone replacement therapy. The relationship between gender and bone mineral density as well as its influence on the association between diabetes and osteoporosis is still controversial. Some studies suggest lower BMD values and in turn, higher bone fracture and osteoporosis risk in males than females. In this regard, the use of hormone replacement therapy, oral contraceptives, and higher levels of estrogen before menopause in females could be contributing to the protective effects on the bone health status (Strotmeyer et al, 2006).
In the present study, the majority of participants who received oral antidiabetic medication, were on metformin, followed by a combination of metformin and sulfonylureas, and sulfonylurea alone. Oral antidiabetic medications also have direct negative and positive effects on bone health status, however, there was no significant relationship between intake of any of these antidiabetic medications and BMD. Metformin and sulfonylureas have been shown to increase osteoblast proliferation and differentiation by inducing alkaline phosphatase production and function and osteocalcin expression, which in turn, reduce the risk of bone fracture in patients undergoing treatment (Molinuevo et al, 2010; Ma et al, 2010; Zinman et al, 2010). Metformin acts primarily by decreasing hepatic glucose production and also has a minor increase in muscle and adipose tissue glucose uptake which may improve insulin resistance. Its major side effects are mostly gastrointestinal such as nausea, diarrhea, metallic taste, and possible lactic acidosis. Sulfonylureas acts by increasing insulin production in the pancreas, and could cause hypoglycemia, weight gain, and hyperinsulinemia as their side effects (American Diabetes Association, 2011).

On the other hand, thiazolidinediones (TZDs) such as rosiglitazone and pioglitazone act as agonists of PPARγ. TZDs effect on stimulating PPARγ would increase adipogenic differentiation of mesenchymal stem cells and thus, reduce the number of differentiated osteoblasts for bone formation (Schwartz, 2008; Adami, 2009; Debiais, 2009). TZDs act through several pathways; they can decrease insulin resistance, improve blood glucose levels through stimulation of PPARγ, induce minor decrease in hepatic glucose output, preserve pancreatic β-cell function, and decrease vascular inflammation. Their main side effect is minor weight gain of 3-6 lbs and edema (American Diabetes Association, 2011).
Univariate analysis detected significant associations for low bone mass density with current status and/or history of cancer, and moderate physical activity in the past 7 days. It has been shown that some types of cancer, including breast cancer and prostate cancer can lead to decreased bone mass density and increased bone fracture risk. This is due to the metastasis of the cancer cells to the bone, where they disrupt the normal process of bone turnover after they colonize and use osteoclasts to attack the bone tissue. This, in turn, results in a breakdown of the bone structure and a decrease in bone mineral density (Bendre et al, 2003; Clark et al, 2003). In the present study, the type of cancer was not reported.

Effect of exercise on bone health has been shown in several studies. Physical activity induces a mechanical load on the bone which is associated with increased bone mineral density as well as bone strength and quality (Singh, 2004). Various studies have shown that impact-loading exercise, defined as “exercise involving peak forces of more than three-times the body weight, including running, tennis and gymnastics” (Tanaka et al, 1999), is positively related to bone mass density (Lima et al, 2001; Nurmi-Lawton et al, 2004; Nikander et al, 2005). This type of exercise is very much like the moderate physical activity that was reported to be significantly correlated with higher BMD values in our study. We defined moderate physical activity as those physical activities that make the person breathe somewhat harder than normal, and that may include carrying light loads, bicycling at a regular pace, or doubles tennis.

This study shows the importance of sufficient sample sizes for detecting significant differences in BMD means by type of diabetes. Our power analysis shows that for a two-sample pooled t test of a normal mean difference with a two-sided significance level of 0.05, assuming a common standard deviation of 0.1, group sample sizes of 8 (type 1 diabetes) and 38 (type 2 diabetes) did not have enough power to detect a difference between BMD means (power=71%). However,
sample size calculations indicate that a sample size of 11 individuals with type 1 diabetes and 38 individuals with type 2 diabetes, would yield an 80% power to detect significant differences for BMD values by type of diabetes. Moreover, sample size analysis suggests that adding only 5 male participants to the current male to female ratio would result in the ability to observe significant differences for BMD values by gender with a power of 80%.
CHAPTER 5

CONCLUSIONS

This study examined the bone health status and vitamin D receptor gene marker of 54 male and female subjects with pre-, type 1 and type 2 diabetes, aged 27 to 86 years. All participants were faculty, staff and students from the University of Illinois at Urbana-Champaign, or individuals associated with the Senior High Rise DM Outreach groups at Champaign-Urbana Public Health Department (CUPHD). Data were collected on anthropometric measurements, bone mineral density of the heel by quantitative ultrasound testing, vitamin D receptor genetic marker using saliva samples, and information on demographics, diabetes status, lifestyle and health behaviors, physical activity, current status and/or history of medical conditions and medication use, family history of diseases, and female reproductive history through a self-administered questionnaire.

The results showed that bone mineral density was low in our sample of individuals with type 1 diabetes compared to those with type 2 diabetes, but this difference lacked statistical significance. Adjusting for age resulted in lower BMD and T-score values in individuals with type 1 diabetes as well as higher T-scores in those with type 2 diabetes. Calculating odds ratio for BMD showed odds of high BMD values of about 14.5 times greater in subjects with type 2 diabetes than those with type 1 diabetes, which increased after adjustment for BMI.

The frequency of low T-scores in type 1 diabetes observed in the present study was comparable to the statistics of the Third National Health and Nutrition Examination Survey (NHANES III, 1988-1991) with 34-50% of postmenopausal white women having osteopenia, and 17-20% having osteoporosis. Our results indicated that among the white postmenopausal women, about
61% had T-scores less than one standard deviation below the reference mean indicating osteopenia, and 13% had T-scores below -2.5 standard deviations threshold for osteoporosis.

Univariate analysis detected significant negative effects of aging, menopause in females, current status and/or history of cancer, and moderate physical activity in the past 7 days on bone health status. No significant associations were observed between history of bone fracture during adult life (21 years or older) or history of falling in the last 6 months with low BMD values.

Multiple regression model showed that African-American ethnicity, calcium/vitamin D supplement use, family history of loss of height, current status and/or history of osteoporosis, and menopause in females were significant predictors of low bone mass density.

In conclusion, additional research is needed to understand the link between BMD and diabetes status. The etiology of altered BMD associated with type of diabetes should be further studied through intervention studies.
There were several limitations to our research. First, we did not have enough sample size in many categories of the variables and this resulted in observing too many lacks of significance. Power analysis for those non-significant relationships confirmed low power for a two-sample pooled t test of a normal mean difference with a two-sided significance level of 0.05 to detect significant differences of BMD values for many variables included in this study. A further sample size calculation suggested that by adding only a couple of more subjects to each category, for example by recruiting 5 more males or 3 more individuals with type 1 diabetes, a power of 80% could be obtained, which is strong enough to detect any significant difference of BMD values for potential predictors of low BMD, including gender and type of diabetes. Thus, increasing the sample size in future studies even to the minimum numbers required for a power of 80% is suggested.

Another limitation of this study was lack of a control group. There were too many potential confounders in this study which we could have adjusted for only by recruiting a control group. Selection of an age-matched or gender-matched control group seemed to be a good option, so that other potential confounders, including BMI could be matched and adjusted for as well. Thus due to the lack of a control group, confounding of the association between diabetes status and BMD would possibly be too large. This absence of adjustment for potential confounding variables may explain some of the heterogeneity of the results of the present study compared to previous studies, as well as limited sample sizes. Therefore, it is recommended to minimize the potential effects of confounding variables through the selection of a well-matched control group.
The current study sample primarily included white/Caucasian subjects and VDR gene marker analysis was only conducted in this ethnic group. Future studies need to be done in various ethnic groups including minorities to determine if racial/ethnic differences among individuals with diabetes is associated with low BMD values and genetic markers. If significant differences do actually exist, prevention and treatment programs for bone health and diabetes status should be differentially targeted.

Another potential limitation is the observational nature of this study which limits the ability of the researchers to explain potential etiology of low BMD values associated with type 1 diabetes and odds ratio of high BMD levels in individuals with type 2 diabetes. Future studies should be designed intervention studies to be able to truly elucidate the long-term effects of bone turnover and glycemic control on BMD and fracture risk in individuals with diabetes.
TABLES AND FIGURES

Figure 1. Frequency of Current Intake of Antidiabetic Medication Type (0: No medication; 1: Metformin; 2: Sulfonylureas; 6: Metformin + Sulfonylurea; Other: TZD + Sulfonylurea; Metformin + TZD)
### Table 1. Characteristics of Participants – Total and by Type of Diabetes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total n = 54</th>
<th>Type 1 Diabetes n = 8</th>
<th>Type 2 Diabetes n = 40</th>
<th>Pre-Diabetes n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.98 ± 15.66(^1) (n=51)</td>
<td>53.25 ± 22.38 (n=8)</td>
<td>63.05 ± 13.51 (n=38)</td>
<td>63.25 ± 15.90 (n=4)</td>
</tr>
<tr>
<td>Range (years)</td>
<td>27.00 – 86.00</td>
<td>27.00 – 82.00</td>
<td>36.00 – 84.00</td>
<td>41.00 – 78.00</td>
</tr>
<tr>
<td><strong>Diabetes Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>7.00; 3.00; 12.00(^2) (n=41)</td>
<td>20.00; 9.00; 40.00 (n=7)</td>
<td>6.25; 3.00; 10.00 (n=32)</td>
<td>1.37; 0.75; 2.00 (n=2)</td>
</tr>
<tr>
<td>Range (years)</td>
<td>0.08 – 50.00</td>
<td>2.50 – 50.00</td>
<td>0.08 – 21.00</td>
<td>0.75 – 2.00</td>
</tr>
<tr>
<td>Medication duration (years)</td>
<td>7.00; 3.50; 8.00 (n=9)</td>
<td>13.00; 13.00; 13.00 (n=1)</td>
<td>6.25; 3.25; 7.50 (n=8)</td>
<td>-</td>
</tr>
<tr>
<td>Range (years)</td>
<td>0.70 – 13.00</td>
<td>-</td>
<td>0.70 – 8.00</td>
<td>-</td>
</tr>
<tr>
<td>HbA1c value (%)</td>
<td>6.80; 6.65; 7.15 (n=16)</td>
<td>7.30; 6.80; 7.80 (n=2)</td>
<td>6.80; 6.50; 7.10 (n=14)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Anthropometrics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>31.71; 27.78; 38.82 (n=53)</td>
<td>29.45; 24.83; 32.04 (n=8)</td>
<td>33.27; 28.31; 40.67 (n=39)</td>
<td>30.30; 23.68; 41.20 (n=4)</td>
</tr>
<tr>
<td>WHR</td>
<td>0.93; 0.88; 0.99 (n=50)</td>
<td>0.90; 0.80; 0.94 (n=7)</td>
<td>0.93; 0.88; 0.99 (n=37)</td>
<td>1.00; 0.89; 1.24 (n=4)</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± Standard deviation for normal variables  
\(^2\) Median; Lower quartile; Upper quartile for non-normal variables
### Table 2. Demographic Characteristics - Total and by Type of Diabetes

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Total n = 54</th>
<th>Type 1 Diabetes n = 8</th>
<th>Type 2 Diabetes n = 40</th>
<th>Pre-Diabetes n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15 (27.78)</td>
<td>2 (25.00)</td>
<td>11 (27.50)</td>
<td>1 (25.00)</td>
</tr>
<tr>
<td>Female</td>
<td>39 (72.22)</td>
<td>6 (75.00)</td>
<td>29 (72.50)</td>
<td>3 (75.00)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White/Caucasian</td>
<td>45 (84.91)</td>
<td>8 (100)</td>
<td>33 (82.50)</td>
<td>3 (75.00)</td>
</tr>
<tr>
<td>African-American</td>
<td>6 (11.32)</td>
<td>0</td>
<td>5 (12.50)</td>
<td>1 (25.00)</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>1 (1.89)</td>
<td>0</td>
<td>1 (2.50)</td>
<td>0</td>
</tr>
<tr>
<td>Latino/Hispanic</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Native American</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mexican-American</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>1 (1.89)</td>
<td>0</td>
<td>1 (2.50)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Education level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not completed High school</td>
<td>10 (19.23)</td>
<td>0</td>
<td>9 (23.08)</td>
<td>1 (25.00)</td>
</tr>
<tr>
<td>High school/GED</td>
<td>5 (9.62)</td>
<td>2 (25.00)</td>
<td>3 (7.69)</td>
<td>0</td>
</tr>
<tr>
<td>Some college</td>
<td>14 (26.92)</td>
<td>0</td>
<td>12 (30.77)</td>
<td>1 (25.00)</td>
</tr>
<tr>
<td>College/Professional degree</td>
<td>23 (44.23)</td>
<td>6 (75.00)</td>
<td>15 (38.46)</td>
<td>2 (50.00)</td>
</tr>
</tbody>
</table>

1 Frequency (Percentage)
Table 3. Diabetes Status - Total and by Type of Diabetes

<table>
<thead>
<tr>
<th>Diabetes Status</th>
<th>Total n = 54</th>
<th>Type 1 Diabetes n = 8</th>
<th>Type 2 Diabetes n = 40</th>
<th>Pre-Diabetes n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>8 (15.38)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Type 2</td>
<td>40 (76.92)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pre-diabetes</td>
<td>4 (7.69)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes duration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 7 years²</td>
<td>20 (48.78)</td>
<td>1 (14.29)</td>
<td>17 (53.13)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>≥ 7 years</td>
<td>21 (51.22)</td>
<td>6 (85.71)</td>
<td>15 (46.88)</td>
<td>0</td>
</tr>
<tr>
<td>Insulin use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>39 (73.58)</td>
<td>1 (12.50)</td>
<td>33 (82.50)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Yes</td>
<td>14 (26.42)</td>
<td>7 (87.50)</td>
<td>7 (17.50)</td>
<td>0</td>
</tr>
</tbody>
</table>

¹ Frequency (Percentage)
² Median value for duration of diabetes
### Table 4. Health Behaviors and Lifestyles - Total and by Type of Diabetes

<table>
<thead>
<tr>
<th>Health Behaviors and Lifestyles</th>
<th>Total n = 54</th>
<th>Type 1 Diabetes n = 8</th>
<th>Type 2 Diabetes n = 40</th>
<th>Pre-Diabetes n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>48 (90.57)</td>
<td>6 (75.00)</td>
<td>38 (95.00)</td>
<td>3 (75.00)</td>
</tr>
<tr>
<td>Yes</td>
<td>5 (9.43)</td>
<td>2 (25.00)</td>
<td>2 (5.00)</td>
<td>1 (25.00)</td>
</tr>
<tr>
<td>Regular alcohol use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>46 (86.79)</td>
<td>4 (50.00)</td>
<td>38 (95.00)</td>
<td>3 (75.00)</td>
</tr>
<tr>
<td>Yes</td>
<td>7 (13.21)</td>
<td>4 (50.00)</td>
<td>2 (5.00)</td>
<td>1 (25.00)</td>
</tr>
<tr>
<td>Calcium/Vitamin D supplement use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>18 (39.13)</td>
<td>1 (16.67)</td>
<td>17 (48.57)</td>
<td>0</td>
</tr>
<tr>
<td>Yes</td>
<td>27 (58.70)</td>
<td>5 (83.33)</td>
<td>17 (48.57)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Not sure/Don’t know</td>
<td>1 (2.17)</td>
<td>0</td>
<td>1 (2.86)</td>
<td>0</td>
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<tr>
<td>Vegetarian/Vegan</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>48 (94.12)</td>
<td>7 (100)</td>
<td>36 (92.31)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Yes</td>
<td>3 (5.88)</td>
<td>0</td>
<td>3 (7.69)</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Frequency (Percentage)
Table 5. History of Weight, Falling and Bone Fracture - Total and by Type of Diabetes

<table>
<thead>
<tr>
<th>Weight, Fall and Bone Fracture History</th>
<th>Total n = 54</th>
<th>Type 1 Diabetes n = 8</th>
<th>Type 2 Diabetes n = 40</th>
<th>Pre-Diabetes n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight loss &gt;15 lbs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>40 (75.47)</td>
<td>8 (100)</td>
<td>28 (70.00)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Yes</td>
<td>13 (24.53)</td>
<td>0</td>
<td>12 (30.00)</td>
<td>0</td>
</tr>
<tr>
<td>Weight gain &gt;15 lbs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43 (87.76)</td>
<td>8 (100)</td>
<td>32 (86.49)</td>
<td>3 (75.00)</td>
</tr>
<tr>
<td>Yes</td>
<td>6 (12.24)</td>
<td>0</td>
<td>5 (13.51)</td>
<td>1 (25.00)</td>
</tr>
<tr>
<td>History of falling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>40 (76.92)</td>
<td>7 (87.50)</td>
<td>29 (74.36)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Yes</td>
<td>12 (23.08)</td>
<td>1 (12.50)</td>
<td>10 (25.64)</td>
<td>0</td>
</tr>
<tr>
<td>History of bone Fracture</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32 (61.54)</td>
<td>5 (62.50)</td>
<td>23 (57.50)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>Yes</td>
<td>20 (38.46)</td>
<td>3 (37.50)</td>
<td>17 (42.50)</td>
<td>0</td>
</tr>
<tr>
<td>History of BMD scan</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>34 (65.38)</td>
<td>5 (62.50)</td>
<td>25 (62.50)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>Yes</td>
<td>18 (34.62)</td>
<td>3 (37.50)</td>
<td>15 (37.50)</td>
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</tr>
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</table>

\(^1\) Frequency (Percentage)
Table 6. Bone Health Variables - Total and by Type of Diabetes

<table>
<thead>
<tr>
<th>Bone Health Variables</th>
<th>Total n = 52</th>
<th>Type 1 Diabetes n = 8</th>
<th>Type 2 Diabetes n = 38</th>
<th>Pre-Diabetes n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>0.48 ± 0.15</td>
<td>0.43 ± 0.12</td>
<td>0.50 ± 0.17</td>
<td>0.44 ± 0.08</td>
</tr>
<tr>
<td>T-score</td>
<td>-0.76 ± 1.43</td>
<td>-0.94 ± 1.43</td>
<td>-0.67 ± 1.53</td>
<td>-1.24 ± 0.73</td>
</tr>
<tr>
<td>BUA</td>
<td>89.09 ± 24.60</td>
<td>81.25 ± 19.29</td>
<td>91.54 ± 26.81</td>
<td>82.25 ± 12.94</td>
</tr>
<tr>
<td>QUI</td>
<td>70.32 ± 23.42</td>
<td>61.79 ± 18.13</td>
<td>72.45 ± 25.60</td>
<td>65.50 ± 9.90</td>
</tr>
<tr>
<td>SOS</td>
<td>1537.77 ± 38.66</td>
<td>1529.08 ± 30.43</td>
<td>1540.88 ± 42.34</td>
<td>1527.81 ± 22.91</td>
</tr>
</tbody>
</table>

1 Mean ± Standard deviation
<table>
<thead>
<tr>
<th>Parameters</th>
<th>T-score ≤ -1</th>
<th>T-score ≤ -2.5</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4 (17.39)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Female</td>
<td>19 (82.61)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Type of diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>4 (18.18)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Type 2</td>
<td>16 (72.73)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Pre-diabetes</td>
<td>2 (9.09)</td>
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</tr>
<tr>
<td>Insulin</td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>15 (68.18)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Yes</td>
<td>7 (31.82)</td>
<td>0</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td></td>
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</tr>
<tr>
<td>&lt;7 years</td>
<td>6 (37.5)</td>
<td>2 (66.67)</td>
</tr>
<tr>
<td>≥ 7 years</td>
<td>10 (62.5)</td>
<td>1 (33.33)</td>
</tr>
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</table>

1 Frequency (Percentage)
2 Median value for duration of diabetes
Table 8. Bone Mineral Density (BMD) by Demographics, Diabetes Status, and Bone Health

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BMD</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.5448 ± 0.1457&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Female</td>
<td>0.4653 ± 0.1551</td>
</tr>
<tr>
<td>Medication type</td>
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</tr>
<tr>
<td>Metformin</td>
<td>0.5549 (15)&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>0.5515 (6)</td>
</tr>
<tr>
<td>Metformin + Sulfonylurea</td>
<td>0.4268 (6)</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.5103 ± 0.1080</td>
</tr>
<tr>
<td>Yes</td>
<td>0.4521 ± 0.1490</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td></td>
</tr>
<tr>
<td>&lt;7 years&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.5325 ± 0.1559</td>
</tr>
<tr>
<td>≥ 7 years</td>
<td>0.4719 ± 0.1379</td>
</tr>
<tr>
<td>History of falling</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.5075 ± 0.1388</td>
</tr>
<tr>
<td>Yes</td>
<td>0.4343 ± 0.2051</td>
</tr>
<tr>
<td>History of bone Fracture</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.5155 ± 0.1426</td>
</tr>
<tr>
<td>Yes</td>
<td>0.4383 ± 0.1757</td>
</tr>
</tbody>
</table>

<sup>1</sup> Mean ± Standard deviation
<sup>2</sup> Mean (Frequency)
<sup>3</sup> Median value for duration of diabetes
Table 9. Bone Mineral Density (BMD) by Medical Conditions and Female Reproductive History\(^1\)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BMD</th>
<th>P-value(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.5140 ± 0.1577</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.3993 ± 0.1191</td>
<td></td>
</tr>
<tr>
<td>Bone medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.5243 ± 0.1590</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.3507 ± 0.0842</td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.5884 ± 0.1317</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.4214 ± 0.1362</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Mean ± Standard deviation
\(^2\) Independent two-sample t-test; α=0.05
\(^*\) p<0.05
Table 10. Bone Mineral Density (BMD) by Physical Activity\(^1\)

<table>
<thead>
<tr>
<th>Physical Activity</th>
<th>Total n = 54</th>
<th>BMD n = 52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vigorous Physical Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>22 (44.00)</td>
<td>0.53 ± 0.15</td>
</tr>
<tr>
<td>Yes</td>
<td>19 (38.00)</td>
<td>0.50 ± 0.14</td>
</tr>
<tr>
<td>Not sure/Don’t know</td>
<td>9 (18.00)</td>
<td>0.40 ± 0.15</td>
</tr>
<tr>
<td>Moderate Physical Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>12 (24.49)</td>
<td>0.48 ± 0.14</td>
</tr>
<tr>
<td>Yes</td>
<td>28 (57.14)</td>
<td>0.53 ± 0.15</td>
</tr>
<tr>
<td>Not sure/Don’t know</td>
<td>9 (18.37)</td>
<td>0.38 ± 0.14</td>
</tr>
<tr>
<td>Walking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>7 (13.46)</td>
<td>0.55 ± 0.16</td>
</tr>
<tr>
<td>Yes</td>
<td>38 (73.08)</td>
<td>0.48 ± 0.14</td>
</tr>
<tr>
<td>Not sure/Don’t know</td>
<td>7 (13.46)</td>
<td>0.42 ± 0.21</td>
</tr>
<tr>
<td>Total Physical Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>4 (7.55)</td>
<td>0.53 ± 0.21</td>
</tr>
<tr>
<td>Yes</td>
<td>44 (83.02)</td>
<td>0.50 ± 0.14</td>
</tr>
<tr>
<td>Not sure/Don’t know</td>
<td>5 (9.43)</td>
<td>0.36 ± 0.15</td>
</tr>
<tr>
<td>Vigorous and Moderate Physical Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>10 (19.61)</td>
<td>0.48 ± 0.16</td>
</tr>
<tr>
<td>Yes</td>
<td>33 (64.71)</td>
<td>0.52 ± 0.14</td>
</tr>
<tr>
<td>Not sure/Don’t know</td>
<td>8 (15.69)</td>
<td>0.38 ± 0.15</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± Standard deviation
Table 11. Unadjusted and adjusted odds ratios for Bone Mineral Density (BMD) and T-score

<table>
<thead>
<tr>
<th>Bone Health Variable</th>
<th>Type 1 Diabetes n = 8</th>
<th>Type 2 Diabetes n = 38</th>
<th>Pre-Diabetes n = 4</th>
<th>Unadjusted Odds Ratio&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Adjusted for BMI&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Unadjusted Odds Ratio&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Adjusted for BMI&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>0.43 ± 0.12&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.50 ± 0.17</td>
<td>0.44 ± 0.08</td>
<td>14.543</td>
<td>28.959</td>
<td>2.308</td>
<td>1.167</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(4.916, 43.027)</td>
<td>(9.321, 89.973)</td>
<td>(0.513, 10.381)</td>
<td>(0.214, 6.366)</td>
</tr>
<tr>
<td>T-score</td>
<td>-0.94 ± 1.43</td>
<td>-0.67 ± 1.53</td>
<td>-1.24 ± 0.73</td>
<td>0.818</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.179, 3.739)</td>
<td></td>
<td>(0.091, 11.028)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Mean ± Standard deviation
<sup>2</sup> Odds ratio (95% confidence interval) for type 2 diabetes vs. type 1 diabetes
<sup>3</sup> Odds ratio (95% confidence interval) for pre-diabetes vs. type 1 diabetes
Table 12. Spearman Correlations of Demographic, Anthropometric and Diabetes Status Variables with Bone Mineral Density (BMD)

<table>
<thead>
<tr>
<th>Demographic, Anthropometric and Diabetes Factors</th>
<th>Correlation Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.574</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Body Mass Index (BMI)</td>
<td>0.078</td>
<td>0.5857</td>
</tr>
<tr>
<td>Waist-to-Hip Ratio (WHR)</td>
<td>-0.001</td>
<td>0.9944</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>-0.174</td>
<td>0.2901</td>
</tr>
<tr>
<td>Duration of medication intake (years)</td>
<td>0.146</td>
<td>0.4175</td>
</tr>
<tr>
<td>HbA1c value (%)</td>
<td>0.196</td>
<td>0.2028</td>
</tr>
</tbody>
</table>

* p<0.05
### Table 13. Unadjusted and Adjusted Bone Mineral Density (BMD) and T-score values between Type 1, Type 2 and Pre-diabetes

<table>
<thead>
<tr>
<th>Bone Health Variables</th>
<th>Unadjusted</th>
<th>Adjusted for Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type 1 Diabetes</td>
<td>Type 2 Diabetes</td>
</tr>
<tr>
<td>BMD</td>
<td>0.43 ± 0.12</td>
<td>0.50 ± 0.17</td>
</tr>
<tr>
<td>T-score</td>
<td>-0.94 ± 1.43</td>
<td>-0.67 ± 1.53</td>
</tr>
</tbody>
</table>

1 Mean ± Standard deviation
2 Adjusted for Age (as a continuous variable)
### Table 14. Stepwise Multiple Regression Equations for bone mineral density (BMD) as the Dependent Variable

<table>
<thead>
<tr>
<th>Category</th>
<th>Equation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td>0.654 – 0.088 [Gender] – 0.145 [African-American]¹</td>
<td>0.0334</td>
</tr>
<tr>
<td>Diabetes Status</td>
<td>0.821 – 0.005 [Age] – 0.097 [DMType1]² + 0.087 [Metformin]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes Care and Management</td>
<td>0.497 – 0.006 [DMDuration]³ – 0.015 [HbA1cvalue]</td>
<td>0.0086</td>
</tr>
<tr>
<td>Lifestyles and Health Behaviors</td>
<td>0.485 – 0.081 [Calcium/VitD] + 0.093 [PAmoderate]⁴</td>
<td>0.0429</td>
</tr>
<tr>
<td>Bone Health Status</td>
<td>0.531 – 0.100 [BMDscan]</td>
<td>0.0447</td>
</tr>
<tr>
<td>Medications³</td>
<td>0.524 – 0.174 [Bonemed]⁶</td>
<td>0.0030</td>
</tr>
<tr>
<td>Medical Conditions⁷</td>
<td>0.521 – 0.250 [Osteoporosis]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family History</td>
<td>0.954 – 0.007 [Age] – 0.209 [FHOOSENTOROSIS]⁸ – 0.091 [FHHTLOSS]⁹</td>
<td>0.0014</td>
</tr>
<tr>
<td>Female Reproductive History</td>
<td>0.548 – 0.143 [Menopause] + 0.093 [Breastfeeding]</td>
<td>0.0032</td>
</tr>
<tr>
<td>Overall Model</td>
<td>0.673 – 0.222 [African-American] – 0.082 [Calcium/VitD] – 0.111 [FHHTLOSS] – 0.181 [Osteoporosis] – 0.101 [Menopause]</td>
<td>0.0002</td>
</tr>
<tr>
<td>Overall model with standardized values</td>
<td>0 – 0.440 [African-American] – 0.278 [Calcium/VitD] – 0.376 [FHHTLOSS] – 0.359 [Osteoporosis] – 0.339 [Menopause]</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

¹ African-American/Black Ethnicity
² DMType1 = Type 1 diabetes
³ DMDuration = Duration of diabetes
⁴ PAmoderate = Moderate physical activity during the last 7 days
⁵ Any medications other than diabetic medications
⁶ Bonemed = History or current use of any type of bone medication
⁷ Current status and/or history of medical conditions
⁸ FHOOSENTOROSIS = Family history of osteoporosis
⁹ FHHTLOSS = Family history of loss of height as grown older
APPENDIX A

U OF I E-WEEK RECRUITMENT

Diabetes and bone-health screening study seeks subjects

If you have diabetes, join a heel ultrasound screening study. Short survey, height/weight taken. Receive cookbook, $5 gift card; can attend bone/diabetes class TBA, if desired. Option for contributing saliva samples for study of overlap between diabetes and bone health means additional $5. Contact Ghazal Naseri at naserik1@illinois.edu to schedule.

URL: http://fshn.illinois.edu/
Karen Marie Chapman-Novakofski - kmc@illinois.edu
Food Science & Human Nutrition
Appendix B

U of I Recruitment Flyer

Have Diabetes?

- Join a heel ultrasound screening study.
- Short survey with height & weight taken.
- As compensation, receive cookbook and $5 Bevier gift card.
- If desired, you can attend a bone/diabetes class TBA.
- Option for contributing saliva samples for study of overlap between diabetes & bone health, get an additional $5 Bevier gift card.
APPENDIX C

DAILY ILLINI AD

Have diabetes? Join a heel ultrasound screening study. Receive cookbook, $5 gift card, attend bone/diabetes class. Option for contributing saliva samples for study of overlap between diabetes and bone health, additional $5. Contact Ghazal Naseri at naserik1@illinois.edu
APPENDIX D
CONSENT FORMS

Consent Form 1

Informed Consent for Participation in the Research Project:
Bone Screening in those with Diabetes

You are being asked to participate in a research project conducted by Dr. Karen Chapman-Novakofski, Department of Food Science at the University of Illinois. The research project is looking at bone screening in those who have diabetes.

The bone screening itself takes only a few minutes. It is completed with a machine called a sonometer. The sonometer takes ultrasound measurements of the heel. There is no radiation and no known health risks. For the bone screening, the person is seated, and their foot is positioned in the sonometer. A pair of soft elastomer pads are brought into contact with opposite sides of the person’s heel. Inaudible high frequency sound waves produced by the sound transducers are transmitted through the heel and received by the opposite transducer. The sonometer estimates heel bone mineral density from the measured ultrasound parameters. There are no known potential adverse effects of the sonometer on health. The purpose of this study is to determine if people attending with diabetes have lower bone density and may need osteoporosis prevention education.

Because many things can affect bone density, we are asking that you also complete a short questionnaire about your diabetes, physical activity, health history, ethnicity, and medications. We will also ask to take your height and weight and wrist circumference as these measurements may be associated with the bone density screening.

The benefits of this research study are primarily to improve the diabetes education program, and to help establish if additional research concerning bone health and diabetes is needed. However, you will be given the results of your heel ultrasound and may share this with your health care provider. The heel ultrasound is not diagnostic of bone health or osteoporosis but is a first level screening method for bone health. You will also be given a recipe book related to either bone health or diabetes and a $5 gift card. You will also have the opportunity to attend a class on diabetes and bone health if you choose to. By signing a list expressing interest in the class you will be notified as to time and place.

You can withdraw from this research study at any time without prejudice - your participation is voluntary. All information you provide will be kept confidential, and any publication of the results of this project will only provide anonymous, grouped data. If you have any questions you can call Dr. Karen Chapman-Novakofski at 217-244-2852, or kmc@uiuc.edu, or the Institutional Review Board, collect if necessary, at 217-333-2670 or irb@uiuc.edu if you have questions about research subject rights. You will be given a copy of this form for your own reference.

I voluntarily agree to participate in this research project.

__________________________  __________________________
Subject                        Date                        Investigator                   Date

UNIVERSITY OF ILLINOIS
APPROVED CONSENT
VALID UNTIL

OCT - 4 2010
Consent Form 2

Informed Consent for Participation in the Research Project:
Bone Screening in those with Diabetes; DNA testing

You are being asked to participate in a research project conducted by Drs. Karen Chapman-Novakofski & Margarita Teran-Garcia, Department of Food Science at the University of Illinois. The research project is looking at DNA markers for osteoporosis in those who have diabetes.

The testing for makers for osteoporosis itself takes only a few minutes. We will ask you to spit a small amount of saliva into a cup. The saliva will then be put in a container to be tested for DNA markers for osteoporosis and diabetes. There are no known health risks. The saliva may be stored for future analysis.

There are no direct benefits to you for participating in this research. The benefits of this research study are to better understand how often those with diabetes may also have DNA markers for osteoporosis. This does not mean you may develop osteoporosis and is not diagnostic. You will not be given the results of the tests, and all samples will be coded so that the results are confidential to the investigators.

You will be given a $5 gift card.

You can withdraw from this research study at any time without prejudice - your participation is voluntary. Samples will be coded, but there is a data key that links the individual with their sample, and the researchers will keep the results confidential. Any publication of the results of this project will only provide anonymous, grouped data. Results will published in scientific journals and presented at scientific conferences. If you have any questions you can call Dr. Karen Chapman-Novakofski at 217-244-2852, or kmc@illinois.edu, or Dr. Margarita Teran-Garcia at 217-244-2025, or teranmd@illinois.edu, or the Institutional Review Board, collect if necessary, at 217-333-2670 or irb@illinois.edu if you have questions about research subject rights. You will be given a copy of this form for your own reference.

I voluntarily agree to participate in this research project.

______________________________
Subject

______________________________
Date

______________________________
Investigator

______________________________
Date

UNIVERSITY OF ILLINOIS
APPROVED CONSENT
VALID UNTIL

OCT - 4 2010

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APPENDIX E

SCREENING TEST

Do you have diabetes? □ Yes  □ No

Do you have any pins or metal in your feet/ankles? □ Yes  □ No

If yes:

• Which foot/ankle? □ Right  □ Left  □ Both

Do you usually have any swelling of your feet/ankles? □ Yes  □ No
APPENDIX F

QUESTIONNAIRE

Code:                                                                                                              Date:

Please take a few minutes to answer the following survey about yourself.

Gender: □ Male  □ Female

Date of Birth: -----------

Ethnicity:
□ White/Caucasian
□ African-American/Black
□ Asian or Pacific Islander
□ Latino/Hispanic
□ Native American/American Indian/Alaskan Native
□ Mexican-American
□ Other: -----------

Education:
□ Did not complete High school
□ High school/GED
□ Some college
□ College/Professional degree

Diabetes status:
What type of diabetes have you been diagnosed with? □ Type1  □ Type2  □ Pre-diabetes

How long have you had diabetes/pre-diabetes? -----------

Are you taking medication now? □ Yes  □ No
If you take medication now,
  • Do you take insulin? □ Yes  □ No
  • Do you take an oral/injectable medication other than insulin? □ Yes  □ No
    If yes, circle around all the medication you are taking in the attached chart. Also
    specify the length of time you’ve been taking each.

List your previous medications related to your diabetes: ----------------------

Do you have your Hemoglobin A1C tested regularly? □ Yes  □ No
  • If yes, do you remember when the last time was? □ Yes  □ No
    ▪ If yes, do you remember what it was? □ Yes  □ No

Current status:
Do you smoke cigarettes regularly? □ Yes  □ No
  • If yes, how many per day? ---------  And for how long? ---------
  • If no, have you ever smoked cigarettes in your life? □ Yes  □ No
    ▪ If yes, how many per day? ---------  And for how long? ---------

Do you drink alcohol regularly? □ Yes  □ No
  • If yes, how many drinks do you have per day? □ 1  □ 2  □ >2
Do you have more than 4 cups of coffee or tea per day? □ Yes □ No
Do you take Calcium/Vitamin D supplements? ---------------
  • If yes, how much daily? ---------------
Are you a vegetarian/vegan? □ Yes □ No

Physical activity:
Now, think about all the vigorous activities which take hard physical effort that you did in the last 7 days. Vigorous activities make you breathe much harder than normal and may include heavy lifting, digging, aerobics, or fast bicycling. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the last 7 days, on how many days did you do vigorous physical activities?
   ______ Days per week
   □ Don't Know/Not Sure
2. How much time did you usually spend doing vigorous physical activities on one of those days?
   ___ ___ Hours per day
   ___ ___ Minutes per day
   □ Don't Know/Not Sure

Now think about activities which take moderate physical effort that you did in the last 7 days. Moderate physical activities make you breathe somewhat harder than normal and may include carrying light loads, bicycling at a regular pace, or doubles tennis. Do not include walking. Again, think about only those physical activities that you did for at least 10 minutes at a time.

3. During the last 7 days, on how many days did you do moderate physical activities?
   ______ Days per week
   □ Don't Know/Not Sure
4. How much time did you usually spend doing moderate physical activities on one of those days?
   ___ ___ Hours per day
   ___ ___ Minutes per day
   □ Don't Know/Not Sure

Now think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?
   ______ Days per week
   □ Don't Know/Not Sure
6. How much time did you usually spend walking on one of those days?
   ___ ___ Hours per day
   ___ ___ Minutes per day
   □ Don't Know/Not Sure
Now think about the time you spent *sitting* on week days during the last 7 days. Include time spent at work, at home, while doing course work, and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you usually spend **sitting** on a week day?

   ___ ___ Hours per weekday
   ___ ___ Minutes per weekday
   □ Don't Know/Not Sure

**History:**

Have you lost >15 pounds in the last year?  □ Yes  □ No
Have you gained >15 pounds in the last year?  □ Yes  □ No

Have you fallen in the last 6 months or do you fall easily?  □ Yes  □ No
Have you had a bone fracture during your adult life (21 years or older)? □ Yes □ No
  - If so, what areas were fractured?  __________
  - What year did you have your fracture(s)?  __________

Have you ever had a Bone Mineral Density Scan of your whole body or hip/spine performed?  □ Yes □ No
  - If yes, where was the scan performed?  __________
  - And in what year(s)?  __________

The following conditions may have an impact on bone density/diabetes. Check all those you currently have or have had a history of.

□ Blood clots  □ Liver problems
□ Cancer  □ Osteopenia
□ Celiac  □ Osteoporosis
□ Chronic gastrointestinal disorders  □ Rheumatoid arthritis
□ Dyslipidemia (Lipid disorders)  □ Seizures/Epilepsy
□ High blood pressure  □ Stomach or colon surgery
□ Hyperparathyroidism  □ Thyroid condition
□ Kidney problems  □ None

The following medications may have an impact on bone density. Check all those you are currently taking or have taken for more than 3 months in the past 10 years?

□ Aluminum-containing antacids e.g. Aluminum hydroxide (Alu-Cap)
□ Anticoagulants e.g. Heparin
□ Anticonvulsants e.g. Phenytoin (Dilantin)
□ Antidepressants
□ Chemotherapy drugs
□ Glucocorticoids e.g. Prednisone
□ Loop diuretics e.g. Furosemide, Lasix, …
□ Thyroid medicine e.g. Levo-thyroxin
□ None

The following medications are specifically prescribed for bone health. Check all those you are currently taking or have taken before. Also specify for how long you have/had been taking them.
Biphosphonates:  
- Actonel (Risedronate)  
- Boniva (Ibandronate Sodium)  
- Didrocal (Etidronate)  
- Fosamix (Alendronate)  
- Reclast (Zoledronic acid)  

Calcitonin:  
- Calcimar (Miacalcin)  
- Fortical (Calcitonin-Salmon (Recombinant) Nasal)  

PTH:  
- Forteo (Teriparatide)  

SERMs:  
- Evista (Raloxifene)  

None

Family history:
Do you have a family history of:
- Diabetes:  
  - Yes  
  - No  
- High blood pressure:  
  - Yes  
  - No  
- Dyslipidemia (Lipid disorders):  
  - Yes  
  - No  
- Osteoporosis:  
  - Yes  
  - No  
- Bone fracture:  
  - Yes  
  - No  
- Loss of height as grown older:  
  - Yes  
  - No

Females only:
Have you gone through menopause?  
- Yes  
- No  
  - If yes, at what age?  
- Are you taking a Hormone Replacement Therapy now?  
  - Yes  
  - No  
  - If no, have you ever taken it before?  
  - Yes  
  - No

Have you had your ovaries removed before menopause?  
- Yes  
- No

Do you have any children?  
- Yes  
- No

Have you ever breast fed?  
- Yes  
- No
This sheet might help you identify which kind(s) of medication you take.

<table>
<thead>
<tr>
<th>Class of Medication</th>
<th>Generic Name</th>
<th>Brand Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonylureas (first and second generation)</td>
<td>Glimepiride</td>
<td>Amaryl®</td>
</tr>
<tr>
<td></td>
<td>Glyburide</td>
<td>DiaBeta®</td>
</tr>
<tr>
<td></td>
<td>Glyburide</td>
<td>Glynase®</td>
</tr>
<tr>
<td></td>
<td>Glyburide</td>
<td>Micronase®</td>
</tr>
<tr>
<td></td>
<td>Chlorpropamide</td>
<td>Diabinese®</td>
</tr>
<tr>
<td></td>
<td>Acetohexamide</td>
<td>Dymelor ®</td>
</tr>
<tr>
<td></td>
<td>Glipizide</td>
<td>Glucotrol®</td>
</tr>
<tr>
<td></td>
<td>Tolbutamide</td>
<td>Orinase®</td>
</tr>
<tr>
<td></td>
<td>Tolazamide</td>
<td>Tolinase®</td>
</tr>
<tr>
<td>Meglitinide</td>
<td>Repaglinide</td>
<td>Prandin®</td>
</tr>
<tr>
<td>Biguanides</td>
<td>Metformin</td>
<td>Glucophage®</td>
</tr>
<tr>
<td></td>
<td>Metformin hydrochloride</td>
<td>Glucophage XR ®</td>
</tr>
<tr>
<td>Alpha-glucosidase inhibitors</td>
<td>Acarbose</td>
<td>Precose®</td>
</tr>
<tr>
<td></td>
<td>Miglitol</td>
<td>Glyset®</td>
</tr>
<tr>
<td>Thiazolidine-diones</td>
<td>Rosiglitazone</td>
<td>Avandia ®</td>
</tr>
<tr>
<td></td>
<td>Pioglitazone</td>
<td>Actos ®</td>
</tr>
<tr>
<td></td>
<td>Troglitazone</td>
<td>Rezulin ®</td>
</tr>
<tr>
<td>D-Phenylalanine derivatives</td>
<td>Nateglinide</td>
<td>Starlix®</td>
</tr>
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</table>

Common Pill Shapes:

<table>
<thead>
<tr>
<th>Rounded rectangle</th>
<th>Round pill</th>
<th>Diamond pill</th>
<th>Oval pill</th>
<th>Two-tone capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Rounded rectangle" /></td>
<td><img src="image" alt="Round pill" /></td>
<td><img src="image" alt="Diamond pill" /></td>
<td><img src="image" alt="Oval pill" /></td>
<td><img src="image" alt="Two-tone capsule" /></td>
</tr>
<tr>
<td>Half rounded rectangle</td>
<td>Half round pill</td>
<td>Half diamond pill</td>
<td>Half oval pill</td>
<td>Square pill</td>
</tr>
</tbody>
</table>
APPENDIX G

QUS RESULTS FORM

Name: 
Date: 
Age: 

Foot: 

The Bone Ultrasound is a screening tool for osteoporosis. The bone ultrasound measures the speed of sound (SOS, in m/s) and broadband attenuation (BUA, in dB/MHz) of an ultrasound beam passed through the calcaneus or heel. It combines these results mathematically to obtain a Quantitative Ultrasound Index (QUI). The results are also expressed as a T-score and as an estimate of the Bone Mineral Density (BMD, in g/cm2) of the calcaneous as measured by Dual Energy X-ray Absorptiometry (DXA).

This is not a diagnosis of osteoporosis. These results may suggest that you talk to your doctor or health care provider about other clinical risk factors for osteoporosis that you may have and/or about having a BMD measured by DXA.

Your estimated BMD is: _______________ g/cm2. We have plotted that for your age on the graph below. Being between +1 and -1 on the graph (shaded areas) is associated with higher bone density.

![Graph showing BMD levels]

Your T-score is __________. Being between +1 and -1 is associated with higher bone density.

Your QUI/stiffness is __________. A normal range is 0 to 150, the higher numbers being associated with higher bone density.

Your BUA is __________. A range of 30 to 130 is normal, with the higher numbers associated with higher bone density.

Your SOS is __________. A range of 1450 to 1700 is normal, with the higher numbers associated with higher bone density.
APPENDIX H

GENETIC STUDY

INTRODUCTION

Genetics of Osteoporosis:

It has been shown in several heritability studies that more than half of the variance in BMD is due to genetics (Krall et al., 1993; Gueguen et al., 1995; Slemenda et al., 1996). In general, twin studies have reported higher estimates than family studies (Pocock et al., 1987; Seeman et al. 1989; Slemenda et al., 1991), in which generations have been compared. In a study by Arden et al., postmenopausal BMD and heel ultrasound were analyzed in relation to hip fracture to determine the genetic component of these important risk factors. In this twin study with identical and non-identical pairs aged 50 to 70 years, bone density was found to have a strong genetic component with estimates of heritability ranging from 0.46 to 0.84 at different sites. Other aspects of the heel ultrasound also had major genetic components, which remained significant after adjustment for BMD. This study suggested that the importance of family history as a risk factor for hip fracture could be explained by a combination of different genetic factors influencing the structure and density of bone (Arden et al., 1996).

Vitamin D regulates bone and calcium homeostasis through Vitamin D receptor (VDR) which belongs to a superfamily of nuclear hormone receptors and activates the transcription of target genes that are involved in calcium metabolism and bone formation (Walters, 1992; Bunce et al., 1997; Ferrari et al., 1997). VDR gene has been suggested as a potential candidate gene for regulation of genetic susceptibility to osteoporosis (Morrison et al., 1992; Stewart et al., 2000; Rizzoli et al., 2001). There are several allelic variants of the VDR gene, including ApaI (allele
A/a), BsmI (allele B/b), FokI (allele F/f; also known as allele C/T, which was analyzed in the present study) and TaqI (allele T/t) restriction endonucleases, all of which have been associated with low BMD and bone loss (Morrison et al, 1994; Ferrari et al, 1995; Graafmans et al, 1997). However, the results are conflicting (Lim et al, 1995; Looney et al, 1995; Vandevyver et al, 1997), which mostly arise from inadequately powered studies, genetic heterogeneity, different ethnic groups and gene-environment or gene-gene interactions (Lander et al, 1994; Gennari et al, 2005; Ralston et al, 2006).

The frequency of VDR alleles varies greatly by ethnic/racial distribution. The association between various allelic variants and BMD has been reported separately in Japanese (Kurabayashi et al, 1999; Kikuchi et al, 1999), Italian (Gennari et al, 1998), and Mexican-American populations (Tornout et al, 1997). Additionally, the frequency of VDR alleles ‘f’, ‘B’ and ‘t’, which are associated with low BMD, have been found to be highest in Caucasians followed by Africans and Japanese (Ortiz et al, 1992; Dvornyk et al, 2004).

Therefore, more studies need to be conducted in order to analyze the association between VDR genotypes and BMD in different ethnic groups.
Objectives:

In an effort to examine the vitamin D receptor gene marker of adult men and women with pre-, type 1 and type 2 diabetes, we aimed to:

1. determine the frequency distribution of Vitamin D receptor (VDR) genotypes by diabetes status.
2. determine the relationship between VDR genotype and/or polymorphism with risk of low BMD.

Hypotheses:

1. The frequency of CC and TT genotypes are significantly lower and higher, respectively, in individuals with type 1 diabetes with lower BMD values.
2. Vitamin D receptor genotype and/or polymorphism are significantly associated with an increased risk for low BMD.
METHODOLOGY

DNA extraction and PCR:

Saliva DNA was extracted using the manufacturer’s protocol: Laboratory Protocol for Manual Purification of DNA from 4.0 mL of Oragene® DNA/saliva (DNA Genotek, Ontario, Canada, 2009). Extracted DNA samples were re-suspended in 1.5 ml TE buffer and quantified using the Nanodrop spectrophotometer with an average yield of 112 µg (average 260/280 ratio of 1.78) and stored at -20°C at their original extracted concentrations. The quality of the DNA samples was further assessed through polymerase chain reaction (PCR) and confirmation that a PCR product was produced through electrophoresis.

Two predesigned assays for the single nucleotide polymorphisms, SNPs, (rs1805192 and rs1544410) were selected from the Applied Biosystems database available online (Applied Biosystems Database, 2011). SNP rs1805192 corresponds to PPARγ gene and SNP rs1544410 corresponds to the Vitamin D receptor (VDR) gene. Genotyping of the two selected markers was carried out using Taqman SNP Genotyping Assays (Applied Biosystems, Foster City, CA, 2006) following the manufacturer’s protocol for the dry-down method for the DNA template. Samples were prepared by adding 5ng of DNA to the plate and allowing to dry overnight. The master mix was added to each well and consisted of 2.5ul Taqman Universal Master Mix, 0.25ul 20x SNP genotyping assay probe, and 2.25ul molecular grade water. Initial PCR was performed with a 7900 Real Time PCR System (Applied Biosystems, Foster City, CA, 2006) with the following temperature settings: 95°C for 10 minutes and 40 cycles of 92°C for 15 seconds and 60°C for 1min. Allelic discrimination was used in the analysis of the samples and genotypes were called using the Sequence Detection Systems software (SDS) version 2.4 from Applied Biosystems.
SDS software called each genotype with 95% confidence and each assay consisted of six non-template controls.

Since PPARγ gene was not polymorphic (all CC alleles), the analysis was not further carried out. However, for the VDR gene the FokI polymorphism in exon 2 of the VDR gene was studied. This polymorphism is a T-C transition and is recognized by the FokI restriction enzyme. This transition results in a different translational initiation codon that creates a shorter version of the VDR gene (Ralston et al, 2010). FokI digested the first ATG and yielded the f allele, whereas the T to C transition destroyed the FokI site and created the F allele. The FF and ff genotypes are also named CC and TT, respectively. The alleles were labeled as CC for homozygous absence and TT for homozygous presence of the restriction site. Alleles labeled as CT were heterozygous.

**Statistical analysis:**

ANOVA test was also used to analyze the covariates in the association between BMD as the dependent variable and VDR genotype as the independent variable. Logistic regression was used to analyze unadjusted and adjusted odd ratios for BMD and T-scores, as well as for the association of low BMD (T-scores ≤ -1) vs. high BMD (T-scores > -1) as the nominal dependent variable with independent variables including VDR genotype and VDR polymorphism. P-values were set at alpha = 0.05 for statistical significance.
RESULTS

Of the total 54 subjects, 51 individuals agreed to participate in the DNA testing and provided a saliva sample. The frequency of VDR alleles and their association with BMD is subject to ethnic distribution. In our study, analysis of the VDR genetic marker was done only on the Caucasian/White ethnicity group because only the frequency of VDR alleles for this ethnic group was available (n = 45). Table 1 illustrates age, BMI, BMD and T-scores in relation to VDR genotypes of CC, CT, and TT (CC = homozygous for absence of restriction site; TT = homozygous for presence of restriction site; CT = heterozygous). No significant associations were detected for any of the variables (p>0.05).

Frequency distribution and odds ratio of the three VDR polymorphism genotypes within each of the three diabetic groups is presented in Table 2. The total genotype distribution was CC, 0.29 (n = 12); CT, 0.42 (n = 17); and TT, 0.29 (n = 12). The data suggested that the VDR genotype did not differ significantly between the diabetic groups (p=0.3422, Chi-square = 4.5031, 4 df).

Genotype distributions in the whole sample and in the three diabetic groups of type 1 diabetes, type 2 diabetes and pre-diabetes were in Hardy-Weinberg equilibrium (p=0.267, p=0.445, p=0.193, p=0.083, respectively). On the other hand, the frequency of CC genotype was lower in individuals with type 1 diabetes (n=2, 25%) than in subjects with type 2 diabetes (n=9, 31%; OR = 2.00; 95% CI 0.15–26.18), however this difference was not statistically significant (p>0.05).

Data on the relationship between bone mineral density (BMD) as the dependent variable and VDR genotype as the independent variable, and the effect of covariates such as age, gender, BMI, duration of diabetes, type of diabetes, insulin use, and history of bone fracture are shown in Table 3. Since the covariance analysis only shows standard errors of the estimates, means ± standard errors are shown instead of standard deviations. BMD values were higher in CC
genotype than CT and TT genotypes, but the difference was not statistically significant (p>0.05). When adjusted for covariates, only age, gender, and duration of diabetes showed significant effects on the association between BMD and VDR genotype, but adjusting did not result in the relationship to become significant in any of the cases. Neither covariate had significant interaction with VDR genotyping.

Frequency distribution and odds ratio of the three VDR polymorphism genotypes were assessed between subjects who had diabetes for less than 7 years (median value) compared to those that had diabetes for 7 or more years. Data is presented in Table 4. Genotype distributions in the two duration groups were in Hardy-Weinberg equilibrium (p=0.096, p=0.906, respectively). VDR genotype did not differ significantly between the two duration groups (p=0.3037, Chi-square = 2.3832, 2 df). On the other hand, the frequency of CC genotype was higher in individuals with more than 7 years of diabetes (n=6, 35%) than in subjects who had diabetes for less than 7 years (n=5, 29%; OR = 2.80; 95% CI 0.46-16.93), however this difference was not statistically significant (p>0.05).

Multiple regression analysis of BMD as the dependent variables and VDR genotype, age, gender, duration of diabetes, type of diabetes and BMI as the independent variables, showed that only age and duration of diabetes contributed significantly to the variance in BMD. VDR genotype had no significant contribution. The multiple regression equation is as follows:

\[ BMD = 0.786 – 0.003 \text{[Age]} – 0.004 \text{[Duration]} \] (data not shown; p=0.0025)

Demographic and clinical data of subjects classified by C-T polymorphism genotypes are shown in Table 5. Duration of diabetes was significantly lower in individuals with TT genotype compared to those with the CC+CT genotype (p = 0.0162). Additionally, subjects with TT
genotype had higher BMI when compared with those with CC+CT genotype, and the p-value was close to the significance level (p=0.059). No other statistically significant relationships were found.

Table 6 shows the univariate analysis of VDR polymorphism in the two groups divided by T-scores into low BMD (T-scores ≤ -1) and high BMD (T-scores > -1). The frequency of TT polymorphism (homozygous for presence of restriction site) was higher in the group with low T-scores, but the difference was not statistically significant.

In order to find risk factors for the lower values of BMD, a logistic regression analysis was conducted using T-score as a dependent nominal variable (Low BMD: T-score ≤ -1 vs. high BMD: T-score > -1 as the reference) and those variables which had shown significant statistical differences in the multivariate regression analysis by BMD as well as VDR genotype and VDR polymorphism (TT/CT+CC) as independent variables. Data is shown in Table 7. Age, duration of diabetes and menopause were the only significant independent risk factors for low BMD. However, VDR genotype or VDR polymorphism were not associated with an increased risk for low BMD (p>0.05).
DISCUSSION

Vitamin D has been known as a regulator of bone and calcium metabolism and homeostasis. The active form of vitamin D, 1,25-dihydroxyvitamin D3 \([1,25(\text{OH})_2\text{D}_3]\) also functions in regulation of target gene expression through a specific receptor, called VDR. VDR is a transcriptional activator belonging to a superfamily of steroid receptors and can be found in almost all body cells (Soldati et al, 2004). The effects of vitamin D are mediated through transcriptional or posttranscriptional modifications of the \(1,25(\text{OH})_2\text{D}_3\)–VDR complex (Soldati et al, 2004).

The significance of VDR gene has not been clearly elucidated. VDR gene polymorphisms have been associated with multiple traits and diseases. In animal models, \(1,25(\text{OH})_2\text{D}_3\) has been shown to have a protective effect against autoimmune diseases (Cantorna et al, 1998). An important autoimmune disease that is thought to be genetically determined, at least in part, by VDR gene polymorphisms, is type 1 diabetes. In a study by Ban et al on a Japanese population, it was found that vitamin D receptor polymorphism has an impact on genetic susceptibility to type 1 diabetes (Ban et al, 2001). Similar finding was reported in a study by Pani and colleagues in a German population (Pani et al, 2000). In a more recent study, Zella and Deluca also showed the effectiveness of vitamin D in type 1 diabetes (Zella et al, 2003). Additionally, Ortlepp et al found that the VDR genotype and physical activity can predict fasting glucose levels in young healthy Caucasian men (Ortlepp et al, 2003).

In the present study, the total genotype distribution was CC, 0.29 (n = 12); CT, 0.42 (n = 17); and TT, 0.29 (n = 12). This is in agreement with the minor allele (TT) frequency (0.28) in Caucasians specified by TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, 2006), which was used for genotyping of the marker.
Additionally, we did not find any statistically significant association between VDR genotype and bone mass density (BMD). However, several studies have shown that VDR gene polymorphisms are associated with BMD and perhaps, influence some determinant of bone metabolism. In a study by Mitra et al in 2006 in postmenaopausal Indian women, it was found that the average BMD at spine and hip of women with genotypes CC (absence of restriction sites for $FokI$) was more than 10% higher than those with genotypes TT (presence of restriction sites for $FokI$). This finding was significant at both bone sites ($p<0.05$). Additionally, compared to women with osteoporotic bone mass, the frequency of CC genotype was significantly higher in women with normal bone mass, whereas genotypes TT was more prevalent in women with osteoporotic bone mass (Mitra et al, 2006).

Similar findings of the effects of polymorphisms in $VDR$ gene on genetic susceptibility to type 1 diabetes have also been reported in several Caucasian groups, including: German (Pani et al, 2000), Romanian (Guja et al, 2002), Dalmatian population of South Croatia (Skrabic et al, 2003), and French (Taverna et al, 2002).

On the other hand, a large-scale meta-analysis of the association between VDR alleles with BMD and fracture risk conducted by the GENOMOS consortium on 26,000 subjects, no relationship was found between $FokI$ polymorphism of VDR gene and either BMD or fracture (Ralston et al, 2006). This suggests that allelic variation of VDR does not necessarily play an important role in regulation of bone mass density or bone fracture in osteoporosis (Ralston et al, 2010).
CONCLUSION

In the present study, no statistically significant association was found between VDR genotype and bone mass density. When adjusted for covariates, only age, gender, and duration of diabetes showed significant effects on the association between BMD and VDR genotype, but adjusting did not result in the relationship to become significant. Duration of diabetes was significantly lower in individuals with TT genotype compared to those with the CC+CT genotype. Additionally, subjects with TT genotype had higher BMI when compared with those with CC+CT genotype. In another multiple regression analysis using BMD as the dependent variable, age, duration of diabetes and menopause were the only significant independent risk factors for low BMD, but VDR genotype or VDR polymorphism were not associated with an increased risk for low BMD.
**Table H.1.** Age, Body Mass Index (BMI), Bone Mineral Density (BMD) and T-scores in Relation to Vitamin D Receptor (VDR) Genotypes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>VDR Genotype CC</th>
<th>VDR Genotype CT</th>
<th>VDR Genotype TT</th>
<th>P-value&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>12 (0.29)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>17 (0.42)</td>
<td>12 (0.29)</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.08 ± 18.51&lt;sup&gt;2&lt;/sup&gt;</td>
<td>64.93 ± 20.77</td>
<td>60.66 ± 9.55</td>
<td>0.4275</td>
</tr>
<tr>
<td>BMI</td>
<td>32.18; 29.45; 40.67&lt;sup&gt;3&lt;/sup&gt;</td>
<td>29.46; 26.70; 30.88</td>
<td>35.79; 28.33; 46.55</td>
<td>0.0954</td>
</tr>
<tr>
<td>BMD</td>
<td>0.5419 ± 0.1623</td>
<td>0.4723 ± 0.1896</td>
<td>0.4938 ± 0.1055</td>
<td>0.5390</td>
</tr>
<tr>
<td>T-score</td>
<td>-0.23 ± 1.48</td>
<td>-0.97 ± 1.69</td>
<td>-0.55 ± 1.10</td>
<td>0.4301</td>
</tr>
</tbody>
</table>

<sup>1</sup> Frequency (Percentage)
<sup>2</sup> Mean ± Standard deviation for normal variables
<sup>3</sup> Median; Lower quartile; Upper quartile for non-normal variables
<sup>4</sup> ANOVA test for normal variables; Kruskal-Wallis test for non-normal variables; α=0.05
**Table H.2.** Frequency Distribution and Odds Ratio of Vitamin D Receptor (VDR) Polymorphism Genotypes between Diabetic Groups

<table>
<thead>
<tr>
<th>VDR Genotype</th>
<th>Total ( n = 45 )</th>
<th>Type 1 Diabetes ( n = 8 )</th>
<th>Type 2 Diabetes ( n = 29 )</th>
<th>Pre-Diabetes ( n = 3 )</th>
<th>Odds Ratio(^2)</th>
<th>95% CI(^2)</th>
<th>( P)-value(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>12 (0.29)(^1)</td>
<td>2 (0.25)</td>
<td>9 (0.31)</td>
<td>1 (0.33)</td>
<td>2.00</td>
<td>0.15 – 26.18</td>
<td>0.5974</td>
</tr>
<tr>
<td>CT</td>
<td>17 (0.42)</td>
<td>5 (0.63)</td>
<td>11 (0.38)</td>
<td>0</td>
<td>4.09</td>
<td>0.40 – 41.66</td>
<td>0.2341</td>
</tr>
<tr>
<td>TT</td>
<td>12 (0.29)</td>
<td>1 (0.12)</td>
<td>9 (0.31)</td>
<td>2 (0.67)</td>
<td>1 (Ref)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Number of individuals (Frequency)

\(^2\) Odds ratio and 95% confidence interval for VDR polymorphism in type 1 vs. type 2 diabetes (Ref)

\(^3\) \( \alpha = 0.05 \)

All groups were in Hardy-Weinberg equilibrium (\( P > 0.05 \))

CC = homozygous for absence of restriction site

TT = homozygous for presence of restriction site

CT = heterozygous

TT genotype as reference (OR = 1.0)
Table H.3. Covariates for the Association between Bone Mineral Density (BMD) as the Dependent Variable and Vitamin D Receptor (VDR) Genotype as the Independent Variable

<table>
<thead>
<tr>
<th>VDR Genotype</th>
<th>BMD</th>
<th>P-value$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unadjusted</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.5419 ± 0.0486</td>
<td>0.5390</td>
</tr>
<tr>
<td>CT</td>
<td>0.4723 ± 0.0391</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.4938 ± 0.0465</td>
<td></td>
</tr>
<tr>
<td><strong>Adjusted for Gender</strong></td>
<td></td>
<td>0.4345$^*$</td>
</tr>
<tr>
<td>CC</td>
<td>0.5668 ± 0.0482</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>0.4884 ± 0.0383</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.5212 ± 0.0466</td>
<td></td>
</tr>
<tr>
<td><strong>Adjusted for Age</strong></td>
<td></td>
<td>0.6283$^*$</td>
</tr>
<tr>
<td>CC</td>
<td>0.5255 ± 0.0436</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>0.4694 ± 0.0373</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.4904 ± 0.0414</td>
<td></td>
</tr>
<tr>
<td><strong>Adjusted for Age and Gender</strong></td>
<td></td>
<td>0.5477$^*$</td>
</tr>
<tr>
<td>CC</td>
<td>0.5458 ± 0.0439</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>0.4835 ± 0.0371</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.5123 ± 0.0421</td>
<td></td>
</tr>
<tr>
<td><strong>Adjusted for Duration of diabetes</strong></td>
<td></td>
<td>0.4206$^*$</td>
</tr>
<tr>
<td>CC</td>
<td>0.5646 ± 0.0439</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>0.4985 ± 0.0393</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.4895 ± 0.0455</td>
<td></td>
</tr>
</tbody>
</table>

1 Mean ± Standard error
2 ANOVA test
*p<0.05 for the covariate
Table H.3. (cont.)

<table>
<thead>
<tr>
<th>VDR Genotype</th>
<th>BMD</th>
<th>P-value$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted for BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.5698 ± 0.0496</td>
<td>0.3171</td>
</tr>
<tr>
<td>CT</td>
<td>0.4786 ± 0.0391</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.4853 ± 0.0470</td>
<td></td>
</tr>
<tr>
<td>Adjusted for Type of diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.5025 ± 0.0559</td>
<td>0.5162</td>
</tr>
<tr>
<td>CT</td>
<td>0.4278 ± 0.0528</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.4556 ± 0.0529</td>
<td></td>
</tr>
<tr>
<td>Adjusted for Insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.5375 ± 0.0528</td>
<td>0.5654</td>
</tr>
<tr>
<td>CT</td>
<td>0.4695 ± 0.0415</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.4904 ± 0.0494</td>
<td></td>
</tr>
<tr>
<td>Adjusted for Bone fracture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.5306 ± 0.0480</td>
<td>0.5651</td>
</tr>
<tr>
<td>CT</td>
<td>0.4650 ± 0.0385</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.4869 ± 0.0457</td>
<td></td>
</tr>
</tbody>
</table>

1 Mean ± Standard error
2 ANOVA test; $\alpha=0.05$
*p<0.05 for the covariate
**Table H.4.** Frequency Distribution and Odds Ratio of Vitamin D Receptor (VDR) Polymorphism Genotypes between Duration of Diabetes Groups

<table>
<thead>
<tr>
<th>VDR Genotype</th>
<th>Duration &lt;7 years&lt;sup&gt;2&lt;/sup&gt; n = 17</th>
<th>Duration ≥ 7 years n = 17</th>
<th>Odds Ratio&lt;sup&gt;3&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;3&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>5 (0.29)</td>
<td>6 (0.35)</td>
<td>2.80</td>
<td>0.46 – 16.93</td>
<td>0.2621</td>
</tr>
<tr>
<td>CT</td>
<td>5 (0.29)</td>
<td>8 (0.47)</td>
<td>3.73</td>
<td>0.64 – 21.58</td>
<td>0.1411</td>
</tr>
<tr>
<td>TT</td>
<td>7 (0.42)</td>
<td>3 (0.18)</td>
<td>1 (Ref)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Number (Frequency)
<sup>2</sup> Median value
<sup>3</sup> Odds ratio and 95% confidence interval for VDR polymorphism in duration of diabetes of 7 or more years vs. less than 7 years (Ref)
<sup>4</sup> α=0.05

All groups were in Hardy-Weinberg equilibrium (P > 0.05)
CC = homozygous for absence of restriction site
TT = homozygous for presence of restriction site
CT = heterozygous
TT genotype as reference (OR = 1.0)
Table H.5. Demographic and Clinical Data in Subjects with Vitamin D Receptor (VDR) C-T Polymorphism Genotypes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>VDR polymorphism TT</th>
<th>VDR polymorphism CC + CT</th>
<th>P-value&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>12 (29.3)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>29 (70.7)</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.6 ± 9.5&lt;sup&gt;2&lt;/sup&gt;</td>
<td>61.0 ± 19.9</td>
<td>0.9442</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>3/9 (25/75)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>10/19 (34.5/65.5)</td>
<td>0.5527</td>
</tr>
<tr>
<td>BMI</td>
<td>35.8; 28.3; 46.5&lt;sup&gt;3&lt;/sup&gt;</td>
<td>30.0; 27.2; 31.2</td>
<td>0.0593</td>
</tr>
<tr>
<td>BMD</td>
<td>0.493 ± 0.105</td>
<td>0.499 ± 0.179</td>
<td>0.9176</td>
</tr>
<tr>
<td>T-score</td>
<td>-0.55 ± 1.11</td>
<td>-0.68 ± 1.63</td>
<td>0.7984</td>
</tr>
<tr>
<td>Type of diabetes (1/2)</td>
<td>1/9 (8/75)</td>
<td>7/20 (25/71)</td>
<td>0.2120</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>3.8; 1.0; 7.0</td>
<td>8.5; 3.5; 17.0</td>
<td>0.0162&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>HbA1c value (%)</td>
<td>0; 0; 6.5</td>
<td>6.2; 0; 7.0</td>
<td>0.0931</td>
</tr>
<tr>
<td>Insulin use (No/Yes)</td>
<td>9/3 (75/25)</td>
<td>22/7 (76/24)</td>
<td>0.9534</td>
</tr>
<tr>
<td>Smoking (No/Yes)</td>
<td>11/1 (91.5/8.5)</td>
<td>28/1 (96.5/3.5)</td>
<td>0.5088</td>
</tr>
<tr>
<td>Calcium/Vitamin D use (No/Yes)</td>
<td>4/5 (44.5/55.5)</td>
<td>11/15 (42.58)</td>
<td>0.9111</td>
</tr>
<tr>
<td>Vigorous physical activity (No/Yes)</td>
<td>8/3 (73/27)</td>
<td>13/14 (48/52)</td>
<td>0.1670</td>
</tr>
<tr>
<td>Moderate physical activity (No/Yes)</td>
<td>4/6 (40/60)</td>
<td>9/19 (32/68)</td>
<td>0.6530</td>
</tr>
<tr>
<td>Bone medication use (No/Yes)</td>
<td>10/1 (91/9)</td>
<td>17/7 (71/29)</td>
<td>0.1892</td>
</tr>
<tr>
<td>Osteoporosis (No/Yes)</td>
<td>11/0 (100/0)</td>
<td>23/6 (79/21)</td>
<td>0.1018</td>
</tr>
</tbody>
</table>

<sup>1</sup> Frequency (Percentage)  
<sup>2</sup> Mean ± Standard Deviation for normal variables  
<sup>3</sup> Median; Lower Quartile; Upper Quartile for non-normal variables  
<sup>4</sup> ANOVA test for normal variables, Kruskal-Wallis test for non-normal variables; α=0.05  
* p<0.05
Table H.6. Univariate Analysis of Vitamin D Receptor (VDR) Polymorphism between Two Groups Divided by T-scores

<table>
<thead>
<tr>
<th>VDR Polymorphism (TT/CC+CT)</th>
<th>Low T-score ($\leq -1$) $n = 16$</th>
<th>High T-score ($&gt; -1$) $n = 24$</th>
<th>P-value$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>8 / 16</td>
<td>4 / 12</td>
<td>0.5731</td>
</tr>
<tr>
<td>Percentage</td>
<td>(33 / 67)</td>
<td>(25 / 75)</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ Chi-square test; $\alpha=0.05$

All groups were in Hardy-Weinberg equilibrium ($P > 0.05$)

CC = homozygous for absence of restriction site

TT = homozygous for presence of restriction site

CT = heterozygous
Table H.7. Odds Ratios and 95% CI of T-score of Bone Mineral Density (BMD) Distribution in a Logistic Regression

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.05</td>
<td>1.007 – 1.106</td>
<td>0.0233*</td>
</tr>
<tr>
<td>Gender (F vs. M)</td>
<td>2.33</td>
<td>0.53 – 10.27</td>
<td>0.2623</td>
</tr>
<tr>
<td>Diabetes Duration</td>
<td>1.09</td>
<td>0.99 – 1.21</td>
<td>0.0481*</td>
</tr>
<tr>
<td>Type of diabetes (type 1 vs. type 2)</td>
<td>1.909</td>
<td>0.399 – 9.141</td>
<td>0.4609</td>
</tr>
<tr>
<td>Calcium/vitamin D use</td>
<td>2.42</td>
<td>0.62 – 9.43</td>
<td>0.2030</td>
</tr>
<tr>
<td>Family history of loss of height</td>
<td>2.96</td>
<td>0.80 – 10.87</td>
<td>0.1016</td>
</tr>
<tr>
<td>Menopause</td>
<td>6.00</td>
<td>1.50 – 23.99</td>
<td>0.0113*</td>
</tr>
<tr>
<td>VDR Genotype</td>
<td>0.99</td>
<td>0.98 – 1.00</td>
<td>0.5824</td>
</tr>
<tr>
<td>VDR polymorphism (TT/CT+CC)</td>
<td>1.50</td>
<td>0.36 – 6.17</td>
<td>0.5742</td>
</tr>
</tbody>
</table>

* p<0.05
CC = homozygous for absence of restriction site
TT = homozygous for presence of restriction site
CT = heterozygous
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