EFFECT OF TYPE 2 DIABETES MELLITUS ON BONE MINERAL DENSITY (BMD) IN MIDDLE-AGED BLACK SOUTH AFRICAN WOMEN

by

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LIST OF ABREVIATIONS

AGEs	Advanced Glycation End Products
AP	Anteroposterior
ART	Anti-Retroviral Therapy
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BPA	Bone Projective Area
cAMP/cGMP	Cyclic Adenosine Monophosphate /Cyclic Guanosine Monophosphate
CI	Confidence Interval
cm	Centimeter
DM	Diabetes Mellitus
DXA	Dual-Energy X-ray Absorptiometry
DXA-BMD	Dual-Energy X-ray Absorptiometry-Bone Mineral Density

FNW	Femoral Neck Width
FODMAPs	Fermentable oligo-, di-, monosaccharides and polyols
FRAX	Fracture Risk Assessment Tool
g/cm^2	Gram per centimeter square
GCP	Good Clinical Practice
GIPR	Gastric Inhibitory Polypeptide Receptor
GTT	Glucose Tolerance Test
HAL	Hip Axis Length
HbA1c	Haemoglobin A1c
HIV/AIDS	Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome
HSREC	Ethics Committee of the University of the Free State
IBS	Irritable Bowel Syndrome
IDF	International Diabetes Federation

IGF-1	Insulin Growth Factor 1
IQR	Inter Quartile Range
IRS-1	Insulin Receptor Substrate 1
IRS-2	Insulin Receptor Substrate 2
ISCD	International Society for Bone Densitometry
kg	Kilogram
kg/m^2	Kilogram per square meter
LADA	Latent Autoimmune Diabetes in Adults
ml	Millilitre
mmom/l	Millimoles per litre
MTNR1B	Melatonin Receptor 1B
NHANES	National Health and Nutrition Examination Survey
NOFSA	National Osteoporosis Foundation of South Africa

OGTT	Oral Glucose Tolerance Test
PBM	Peak Bone Mass
PPP	Public-Private Partnerships
QC	Quality Control
QCT	Quantitative Computed Tomography
HR-pQCT	High-resolution Peripheral Quantitative Computed Tomography
ROI	Region of Interest
SD	Standard Deviations
SEMDSA	Society for Endocrinology Metabolism and Diabetes of South Africa
SHBG	Sex-hormone Binding Globulin
SNP	Single Nucleotide Polymorphisms
SSA	Sub-Saharan Africa
TBS	Trabecular Bone Score

TCF7L2	Transcription Factor 7-like 2
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
UFS	University of the Free State
UN	United Nations
USA	United States of America
USPSTF	United States Preventative Services Task Force
WHO	World Health Organization

IMPORTANT DEFINITIONS

Bone Mineral Density (BMD)	DXA calculates BMD (grams per square centimeter) as bone mineral content (BMC; in grams) divided by the projected bone area (square centimeters) (Leslie e*t al.*, 2012).
Dual-Energy X-ray Absorptiometry (DXA)	DXA is widely used for measurement of bone mineral density. DXA allows accurate diagnosis of osteoporosis, estimation of fracture risk and monitoring of patients undergoing treatment (El Maghraoui & Roux, 2008).
Diabetes Mellitus	Diabetes mellitus is a metabolic disorder with heterogeneous aetiologies which is characterised by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Amod *et al.,* 2012).
Femur	The femur is the longest and heaviest bone in the human body. The superior end of the femur consists of a head, and the greater and lesser trochanter. The head is attached to the femoral body or shaft by the neck of the femur (Cooper e*t al.,* 2015).

Lumbar Spine	The lumbar spine consists of 5 moveable vertebrae numbered L1-L5. Lumbar disks are well designed to sustain compression loads but rely on posterior elements to limit axial rotation (Bogduk, 2016).
Osteopenia	Osteopenia is referred to as decrease in bone mineral density which if continues leads to osteoporosis (Asif *et al*., 2015).
Osteopenia diagnosis	Osteopenia is defined as a bone density between 1.0 and 2.5 SD below the mean for young adult women (Syed & Khan, 2002).
Osteoporosis	Osteoporosis is often called the "silent disease," because bone loss usually occurs gradually over time without symptoms. Osteoporosis is defined as a combination of reduced bone mass and altered bone quality, with microarchitectural abnormalities, resulting in decreased bone strength with an increased risk of fractures (Jackuliak & Payer, 2014).
Osteoporosis diagnosis	The diagnosis of osteoporosis based on a T-score of ≤ -2.5 is and should remain one important way to identify an individual with an increased risk for fracture (Siris *et al*., 2014).

Peak Bone Mass	Wilken *et al*., (2010) mentioned in an article that although research varies on the age at which peak BMD is reached, most suggest peak is reached somewhere between the ages of 20 and 30 years. They also mentioned that some investigators suggest that 95% of peak BMD is reached by age 17 years for females and two to three years later for males, while others suggest PBM is reached by age 30 years for most bone sites (Wilken *et al.,* 2010).
Recommended sites	It is recommended that bone density at the lumbar spine be evaluated from the first to the fourth lumbar vertebrae. At the hip, the diagnosis of osteoporosis can be based on the T-score obtained at the femoral neck, total hip, or trochanteric regions (Syed & Kahn, 2002).
Region of Interest	The software marks regions of interest in the spine and hip. The spine region of interest consists of the L1 through L4 vertebrae. The hip regions of interest include the femoral neck, trochanter and total hip (El Maghraoui & Roux, 2008).

T-score	The T-score is defined as the number of standard deviations the patient's BMD is above or below the sex-matched mean reference value of young adults. The T-score thus provides a comparison of the patient's BMD to the mean peak bone mass (Syed & Khan, 2002).
Type 1 Diabetes Mellitus and Type 2 Diabetes Mellitus	The aetiological types of diabetes are type 1, type 2, other specific types and gestational diabetes. Patients with any form of diabetes may require insulin treatment at some stage of their disease. Such use of insulin does not, of itself, allow for aetiological classification. Type 1 diabetes results from pancreatic beta-cell destruction. Type 2 diabetes is the most common aetiological type and is predominated by disorders of insulin action (insulin resistance), and with insulin deficiency relative to a predominant secretory defect (i.e. disorders of insulin action and secretion) (Amod *et al.,* 2012).
Z-score	The Z-score is the number of standard deviations above or below the expected BMD for the patient's age and sex (Qaseem *et al.,* 2017*)*.

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SUMMARY

Diabetes mellitus is a serious medical condition that occurs when the body cannot utilise glucose normally. There are two main types of diabetes, known as type 1 diabetes mellitus and type 2 diabetes mellitus. Type 2 diabetes mellitus is the most common type of diabetes which can have serious complications if not treated.

Osteoporosis/osteopenia (low bone mass) are hidden, major health concerns for our society as a result of their quiescence nature of the bone loss process with no signs or symptoms until a fracture occurs.

Non-invasive bone densitometry utilising X-ray absorptiometry enables accurate evaluation of bone mass and the diagnosis of osteoporosis in asymptomatic individuals prior to fracture. Bone mineral density is a vital component in the diagnosis and management of osteoporosis regarding bone strength, since fracture risk increases exponentially as bone mineral density decreases. A DXA scan was performed on each subject. The sites used in this study included the femoral neck (left and right), total hip (left and right) and the AP lumbar spine.

This was a retrospective study. Data of 140 Black female subjects were obtained from a database. Ninety-one subjects were previously diagnosed with type 2 diabetes mellitus and acted as the test group, whilst 49 subjects had no diagnosis of diabetes mellitus and acted as the test group. The T-scores of the test group and the control group were compared according to the guidelines of the World Health Organization. These two groups were further divided into two subgroups according to age, as proposed by the National Osteoporosis Foundation of South Africa. Subjects ≥ 50 years were added in the calculations for the T-score, whilst subjects < 50 years were added in the calculations for Z-score.

The aim of this study was to address the effect of type 2 diabetes mellitus on bone mineral density in middle-aged Black South African women. A literature review was conducted to identify data sources for this ethnic group.

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Statistical analysis was used to determine whether numerical variables followed a normal distribution pattern. Numerical values used included age, height, weight, BMI, T-score, Z-score and BMD. Descriptive statistics (means and standard deviations and percentiles) were used to calculate for numerical data, whilst frequencies and percentages were calculated for categorical data. The test and control groups were compared with calculations from the independent T-test to test for differences between mean values, whilst the Mann Whitney-U test was used to identify differences between the median values. A significance level (α) of 0.05 was used, where $p \geq 0.05$ indicates no significant difference in the mean or median values of the two groups, and $p < 0.05$ indicates significant differences. Fisher's exact test was used to compare percentages of the T-score and Z-score in the two groups with the same significant level (α) p 0.05 to determine the difference between the proportions of the two groups.

The results of the test group compared with the control group according to the WHO definition of diagnosis with regards to the T-score showed no significant difference in the bone mineral density in the different areas. Osteopenia was more prevalent than Osteoporosis in both groups.

The results of the subjects ≥ 50 years' T-score diagnosis according to the NOFSA guidelines indicate no significant difference between the proportions of the two groups, accept at the right femoral neck.

There was no significant difference between the proportions of the two groups at the Z-score for women < 50 years.

Although the difference was not statistically significant between the T-score, Z-score and BMD between the two groups, there is evidence of low bone mass (osteopenia) in general for this population. It has been observed that type 2 diabetes mellitus negatively affects bone strength, regardless of bone mineral density. Furthermore, diabetes mellitus is a risk factor for osteoporosis and fractures, and that fractures can occur at higher bone mineral density levels in patients with diabetes mellitus.

CHAPTER 1

INTRODUCTION

1.1 Background

Diabetes mellitus (DM) is a metabolic disorder with heterogeneous aetiologies which is characterised by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The long-term effects of DM include the development of retinopathy, nephropathy and neuropathy. People with diabetes are also at increased risk of other diseases, which include cardiac, peripheral arterial and cerebrovascular diseases (Amod e*t al.,* 2012).

Zheng *et al.* (2018) state that genetic predisposition partly determines individual susceptibility to type 2 diabetes mellitus (T2DM). An unhealthy diet (inadequate protein, calcium and vit D intake, etc.) and a sedentary lifestyle are important drivers of the current global epidemic. Early developmental factors (such as intrauterine exposures) also have a role in susceptibility to T2DM later in life.

According to Amod *et al.* (2012), several pathogenic processes are involved in the development of DM. These processes destroy the function of the pancreatic beta cells, leading to consequent insulin deficiency (type 1 diabetes mellitus; T1DM), and others can result in resistance to insulin actions (insulin resistance/insulin insensitivity - T2DM). The abnormalities of

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carbohydrate, fat and protein metabolism are the result of inadequate insulin action within the target organs resulting in insensitivity to or the lack of insulin, or both.

It is important to note that it may only be possible to establish the aetiology of diabetes retrospectively. Diabetes, regardless of the aetiology, progresses through several clinical stages during its natural history, and an individual may progress from stage to stage in either direction. Persons who have, or who are developing, diabetes can be categorised in a specific clinical stage according to their clinical characteristics, even in the absence of information about the underlying aetiology (Amod *et al*., 2012).

DM adversely affects the skeleton and is associated with an increased risk of osteoporosis and fragility fractures (Hamann *et al*., 2012). The mechanisms underlying low bone strength are not fully understood but could include impaired accrual of peak bone mass (PBM) and diabetic complications, such as nephropathy. T1DM affects the skeleton more severely than T2DM, probably because of the lack of bone anabolic actions of insulin and other pancreatic hormones. Bone mass can remain high in patients with T2DM, but it does not protect against fractures, as bone quality is impaired. A physically active, healthy lifestyle and prevention of diabetic complications, along with calcium and vitamin D repletion, represent the mainstay of therapy for osteoporosis in patients with T1DM or T2DM. Assessment of bone mineral density (BMD) and other risk factors as part of the diagnostic procedure can help design tailored treatment plans for osteoporosis. Increased awareness of osteoporosis is needed in view of the growing and aging population of patients with DM.

Osteoporosis is often referred to as "the silent disease," as bone loss usually occurs gradually over time without symptoms. Osteoporosis is defined as a combination of reduced bone mass and altered bone quality, with microarchitectural abnormalities, resulting in decreased bone strength with an increased risk of bone fractures. Based on this definition, both bone density and quality, which encompass structural and material properties of bone, are important factors in determining bone strength (Jackuliak & Payer, 2014). As mentioned by Cadarette *et al*. (2000) osteoporosis frequently results in fractures that lead to pain, deformity and disability.

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Osteoporosis frequently results in fractures that lead to pain, deformity and disability. Wrist, spine and hip fractures are frequently seen and are associated with an economical burden not only on the individual but also on society (Cadarette *et al.*, 2000).

Along with the rising trend of DM, rapid urbanization has been observed, Kapoor *et al.* (2014) predict that this demographic transition will largely take place in developing countries, particularly in Asia and Africa. Throughout the process of development and urbanisation, national economies are shifting away from physically active economic activities like farming, mining, forestry, and so forth to more sedentary, often office-based, occupations (Kapoor *et al*, 2014).

In an article published by Padzys *et al.* (2015) in addition to sedentary lifestyles, diet also plays an important role in diabetes prevalence in Africa. Padzys *et al.* (2015) mention that population migration is leading to a nutritional transition in many African countries. People arriving in town abandon their traditional lifestyles to adopt a diet rich in saturated animal products, salt, sugars and fats. Nutrition transition that results from urbanization are recognized as the two main factors responsible for the development of diabetes and obesity in Africa, especially in the Sub-Saharan area. In addition to urbanisation, the social status of the individual may also be a factor related to the prevalence of diabetes (Padzys *et al.*, 2015).

Goedecke *et al.* (2017) stated that women in Sub-Saharan Africa (SSA) also have a greater risk factor burden for T2DM than men. The pathogenesis of diabetes differs between African and Caucasian women, with implications for risk assessment. It seems that African women are more insulin resistant than their Caucasian counterparts. Notably, women in SSA face the dual burden of T2DM and Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS). HIV-positive women in SSA are typically young and obese, with the latter being exacerbated by anti-retroviral therapy (ART). Cultural perceptions regarding

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weight loss and limited financial resources are the major limitations to the management of T2DM (Goedecke *et al*., 2017).

Ellis e*t al.* (2019) have done research on black South African women (n=542) aged 29-65 years. They investigated the differences between BMD of HIV-positive and HIV-negative women. They found that low BMD was more common among HIV-positive than HIV-negative women. When the groups were matched for age and body mass index (BMI), only spine BMD was marginally lower in HIV-positive women. Older HIV-positive women with low educational status showed particular risk for low BMD.

A study done by Hamill *et al.* (2017) suggested that, in urban, premenopausal, black South African women, HIV infection per se has no discernible effects on BMD status over a 12-month period, but that exposure to Tenofovir Disoproxil Fumarate-based ART is associated with loss of BMD and an increase in bone turnover.

Postmenopausal women with diabetes, who are a particularly fragile population because of the higher cardiovascular disease-related risk, are those at significantly higher risk for osteoporosis and its complications. Sex hormones play a central role in the physiology of bone by direct and indirect mechanisms, and the abrupt loss of estrogens at menopause onset is considered the major reason for primary osteoporosis in women (Russo *et al*., 2014).

Adolescents with T1DM may not reach potential PBM, putting them at greater fracture risk. In adults with T2DM, fracture risk is increased but not explained by the BMD measured by dual-energy X-ray absorptiometry (DXA), which is still considered the gold standard predictor of fracture (Sealand *et al*., 2013). DXA is widely used for the measurement of BMD. DXA allows accurate diagnosis of osteoporosis, estimation of fracture risk and monitoring of patients undergoing treatment. Additional features of DXA include measurement of BMD at multiple skeletal sites, safety of performance, short investigation time and ease of use (El Maghraoui & Roux, 2008).

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Recognition of various artefacts and pathologic processes that can falsely increase the measured BMD, is essential to accurate DXA scan analysis. Critical evaluation of the DXA scan image and careful appraisal of numeric data on the computer-generated printout by clinicians and radiology technologists are essential to ensure correct DXA scan interpretation (Theodorou & Theodorou, 2002).

Another independent contributor to the assessment of fracture risk is trabecular bone score (TBS) and the fracture risk assessment tool (FRAX). TBS is a grey-level textural measurement derived from lumbar spine DXA images. It appears to be an index of bone microarchitecture that provides skeletal information additional to the standard BMD measurement. Factors such as bone geometry, microdamage, mineralisation, bone turnover, age, family history of fracture, prior fracture, and fall risk contribute to the overall assessment of fracture risk. Several of these additional factors are captured by FRAX, which estimates the 10-year probability of hip and major osteoporotic fracture based on the individual's risk factor profile (McCloskey *et al.,* 2016).

In the United States of America (USA) today, the standard criterion for defining and diagnosing osteoporosis is the finding of a T-score of $\leq$ -2.5 at the lumbar spine, femur neck, or total hip by BMD testing (Siris *et al*., 2014).

Early diagnosis is the key for appropriate osteoporosis management. To date, DXA is the most commonly used and validated method for bone densitometry in clinical practice. Nevertheless, some important limitations (e.g. use of ionising radiation, large size of the equipment, high costs and limited availability) do not allow DXA to be the true "gold standard technique" and

make it unsuitable as a screening tool at the primary health care level for prevention purposes (Pisani *et al.,* 2013).

1.2 Purpose of the study

Osteoporosis is a major public health problem because of its cost implications. Thus, identifying and evaluating populations at increased risk of developing osteoporosis is critical for disease prevention. Although osteoporosis traditionally has not been listed as a complication of diabetes, patients with either type 1 or type 2 diabetes mellitus are among those at increased risk for this disease (Chau & Edelman, 2002).

Vertebral and hip fractures both substantially reduce quality of life, and although vertebral fractures are more common than hip fractures, hip fractures have the greatest health and economic impact. High-income settings have health and social systems that facilitate long-term care; sub-Saharan Africa does not. Thus, younger family members are likely to take responsibility for the care of older relatives, further affecting individuals of working age. To date, no studies have been published on the health costs of fractures within the SSA region. The predicted increase in fractures in SSA now means there is an urgent need to strengthen health-care systems (Gregson *et al*., 2019).

The true occurrence of osteoporosis may be significantly underestimated because many women who suffer minimal trauma fractures are still not being evaluated for osteoporosis (Chau e*t al.,* 2003). Prevention of osteoporosis requires not only recognition of populations who are at risk, but also screening programmes targeting high risk populations.

A review published by Wagner and Heyward (2000) examined the biological differences in body composition, including BMD between Blacks and Whites. They stated that in general,

Blacks have a greater BMD and whole-body protein content than Whites, resulting in a greater fat-free body density. The purpose of this study was to determine the effect of T2DM on BMD in middle-aged black South African women. This approach agrees with the Surgeon General Bone Health and Osteoporosis Report (2004), which suggests that more research needs to be conducted examining racial and ethnic minorities (Wilken *et al*., 2010).

1.3 Aim

The aim of this study was to determine the impact of T2DM on the BMD of Black middle-aged women in Central South Africa.

1.4 Objective

For the purpose of this study a DXA scan was done on all subjects participating in the study to determine the effect of T2DM on BMD in middle-aged Black South African women. The participants were between the ages of 40 – 60 years. The data of participants previously diagnosed with T2DM acted as the test group, and the data of volunteers not diagnosed with T2DM acted as the control group.

To date, literature provide limited information about the effect of DM on the BMD of Black South African women. Therefore, it could be beneficial for the Black South African women population be investigated to address the shortage of information and to optimize treatment.

CHAPTER 2

LITERATURE REVIEW

2.1   Introduction

The worldwide prevalence of diabetes has continued to increase dramatically and globally. As of 2011, an estimated 366 million people had diabetes mellitus (DM), with type 2 diabetes mellitus (T2DM) making up about 90% of all diabetes cases (Baynest, 2015). The number of people with T2DM is on the rise in every country, with 80% of people with DM living in low- and middle-income countries. However, according to Baynest (2015), limited data are available on the prevalence of T2DM in Africa. The studies that did investigate DM trends within Africa provide evidence of a dramatic increase in the prevalence of DM in both rural and urban settings, affecting both males and females proportionally (Kapoor *et al.,* 2014; Ogbera & Ekpebegh, 2014). According to Baynest *et al*. (2015), the prevalence of DM in Africa was (3.2%) and (2.0%) in Ethiopia.

Osteoporosis is a common complication seen in patients suffering from DM - more specifically T2DM (Sundararaghavan *et al*., 2017).

Bone fractures are the first symptoms of osteoporosis. Osteoporosis is as a disorder with bone mineral density (BMD) 2.5 or more standard deviations (SD) less than the mean BMD in healthy young adults [T-score −2.5 or less] (WHO, 1994). The peak bone mass (PBM) of

women is lower than that of men; therefore, as age advances, women are more likely to be at risk for osteoporosis. It is estimated that every three seconds one osteoporotic fracture occurs somewhere in the world. The universal burden of the low BMD almost doubled (0.12% vs. 0.21%) over the 20–year period from 1990 to 2010, and low BMD caused nearly one-third of the all fall-related deaths around the world (Naz e*t al*., 2016).

2.2   *Diabetes mellitus (DM)*

DM is a chronic metabolic disorder characterised by hyperglycemia that contributes to substantial morbidity and mortality. Pharmacotherapy, continuing medical care and education are crucial for preventing acute and chronic complications of DM (Abdulameer *et al.*, 2012).

This also reflects an increase in associated risk factors, such as being overweight or morbidly obese. Over the past decade, diabetes prevalence has risen faster in low- and middle-income countries than in high-income countries. In 2012 diabetes was the direct cause of 1.5 million deaths, and high blood glucose was the cause of another 2.2 million deaths worldwide (WHO, 2016).

According to the International Diabetes Federation (IDF) it was estimated that in 2017 there were 451 million (age 18–99 years) people with diabetes worldwide. These figures were expected to increase to 693 million by 2045. It was estimated that almost half of all people (49.7%) living with diabetes are undiagnosed (Cho *et al.,* 2018).

In 2006, the United Nations (UN) adopted a resolution on diabetes, urging member states to develop national policies for the prevention, treatment and care of diabetes. The designation of 14 November as World Diabetes Day was also endorsed. An African diabetes declaration, launched by the IDF held in Cape Town in 2006, aimed to raise community and political awareness of the disease (Butler, 2011).

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2.2.1   *Types of diabetes mellitus*

The aetiological types of diabetes are classified as type 1 (T1DM), type 2 (T2DM), other specific types and gestational diabetes (Sundararaghavan *et al.,* 2017). Patients suffering from any type of DM may require insulin treatment at some stage. The use of insulin by itself, does not allow for aetiological classification (Amod *et al.,* 2012).

T1DM results from pancreatic beta-cell destruction. These patients are prone to ketoacidosis, coma and death. Diabetes that is caused by an autoimmune process for which the aetiology of beta-cell destruction is unknown [which includes latent autoimmune diabetes in adults (LADA)] is also classified as T1DM (Amod *et al*., 2012).

T2DM is the most common aetiological type and is predominated by disorders of insulin action (insulin resistance), and with insulin deficiency relative to a predominant secretory defect (i.e. disorders of insulin action and secretion). The clinical distinction between T1DM and T2DM can sometimes be difficult, particularly in adolescents and young adults (Amod *et al*., 2012).

Ndisang *et al.* (2015) mentioned in their article that in T1DM, autoimmune-mediated destruction of pancreatic beta-cells results in insulin deficiency. Obesity is one of the major causes of T2DM. In T2DM, a combination of peripheral insulin resistance and aberrant production of insulin are amongst the paradox commonly encountered in the pathogenesis of the disease. However, both forms of diabetes are characterised by elevated inflammation/oxidative stress, glucotoxicity, lipotoxicity, endoplasmic reticulum-induced stress with increased apoptosis and necrosis that ultimately leads to destruction loss of beta cells, and related complications including cardiomyopathy, nephropathy, neuropathy, and hepatopathy (Ndisang *et al*., 2015).

2.2.2 Diagnosis of diabetes mellitus

DM is diagnosed quite simply by measuring the level of glucose in the blood. According to the 2017 Society for Endocrinology Metabolism and Diabetes of South Africa (SEMDSA) guidelines, the criteria for diagnosis is confirmed in patients with symptoms of hyperglycaemia (polyuria, polydipsia, blurred vision, weight loss) or metabolic decompensation (diabetic ketoacidosis or hyperosmolar non-ketotic state), when any one single test confirms that the:

i. Random plasma glucose is $\geq$ 11.1 millimoles per litre (mmol/L)
ii. Fasting plasma glucose is $\geq$ 7.0 mmol/L
iii. Haemoglobin A1c (HbA1c) is $\geq$ 6.5% ∘ 2-hour post-load glucose is $\geq$ 11.1 mmol/L. However, a GTT is rarely needed in this category of patient.

In an asymptomatic individual, when any one of the following tests, repeated on separate days within a 2-week period confirms that the:

i. Fasting plasma glucose is $\geq$ 7.0 mmol/L
ii. 2 hr-post load oral glucose tolerance test (OGTT) is $\geq$ 11.1 mmol/L
iii. HbA1c is $\geq$ 6.5%

If the diagnosis of diabetes is not confirmed with the repeated test, institute lifestyle modification and retest in 3 to 6 months is needed (Amod *et al*., 2017).

Table 2.1 highlights the clinical differences between T1DM and T2DM (Amod *et al*., 2017).

Table 2.1: Clinical differences between T1DM and T2DM (adapted from Amod *et al.,* 2017).

TIDM	T2DM
Usually < 30, but not always	Usually older, but prevalence in children, adolescents and young adults increasing
Usually lean weight	Mostly overweight or obese with acanthosis nigricans
Onset is acute	Onset is insidious/gradual
Almost always symptomatic (i.e., polyuria, polydipsia, weight loss)	Often asymptomatic
Prone to ketosis, often ketoacidosis at diagnosis	Not usually prone to ketosis, but ketoacidosis may be present at diagnosis
Diagnosis: usually has unequivocal hyperglycemia	Diagnosis often during routine screening
Insulin necessary, as of diagnosis, for survival	Usually controlled with non-insulin therapies, or may need insulin for symptom control
Otherwise normally healthy	Often have co-morbidities e.g., hypertension, dyslipidemia, sleep apnoea, fatty liver disease, and polycystic ovary syndrome or diagnosed after emergency admission for myocardial infarction or stroke

The study was conducted on T2DM volunteers.

2.3   Type 2 diabetes mellitus (T2DM)

2.3.1   The pathophysiology of T2DM

According to Khan *et al.* (2014) normal regulation of glucose metabolism is determined by a feedback loop involving the islet β-cell and insulin-sensitive tissues in which tissue sensitivity to insulin determines the magnitude of the β-cell response. When insulin resistance is present, the β-cell maintains normal glucose tolerance by increasing insulin output. It is only when the β-cell is incapable of releasing sufficient insulin in the presence of insulin resistance that glucose levels rise. While β-cell dysfunction has a clear genetic component, environmental changes play a vital role. Modern approaches have also informed regarding the importance of

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hexoses, amino acids and fatty acids in determining insulin resistance and β-cell dysfunction as well as the potential role of alterations in the microbiome. Khan *et al*. (2014) explained that like most endocrine systems, a feedback loop operates to ensure integration of glucose homeostasis and maintenance of glucose in a tight range. This feedback loop relies on crosstalk between the β-cell and the insulin sensitive tissues (Figure 2.1 A). Insulin released in response to β-cell stimulation mediates the uptake of glucose, amino acids and fatty acids by insulin-sensitive tissues. In turn, these tissues feedback information to the islet regarding their need for insulin, the mediator of which has not yet been identified but is likely to involve integration between the brain and humoral systems. When insulin resistance is present, as seen most commonly with obesity, the β-cell increases its insulin output to maintain normal glucose tolerance (Figure 2.1 B). However, when the β-cell is incapable of this task, the result is an elevation in plasma glucose (Figure 2.1 C) (Khan e*t al*., 2014).

| A | B | C |
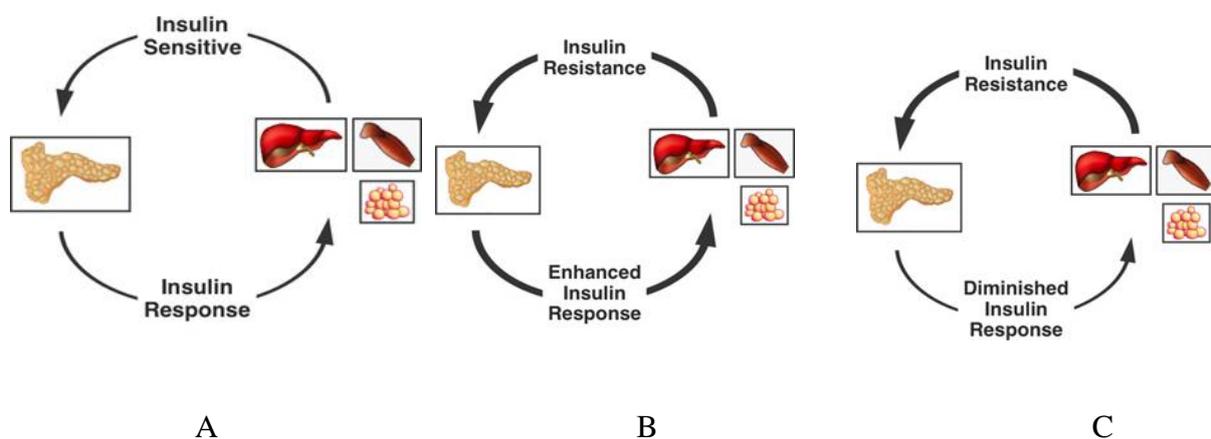
Figure 2.1:   **Feedback loop between the islet β-cell and the insulin-sensitive tissues**

(A) Insulin acts in the liver to suppress glucose production, and in the muscle and adipose tissue to stimulate the uptake of glucose, amino acids and fatty acids. The amount of insulin released to maintain normal glucose homeostasis is determined by the prevailing insulin sensitivity. This feedback is likely mediated through neuronal and humoral mechanisms, but the exact mediators are still not known. (B) When insulin resistance develops in the insulin-sensitive tissues, feedback to the β-cell ensures that it increases insulin output

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to maintain normal glucose tolerance. (C) When the β-cell is incapable of increasing insulin output in the presence of insulin resistance, the result is the development of elevated glucose levels, initially manifest as impaired glucose tolerance. As β-cell dysfunction progresses, further elevations in glycaemia occur and diabetes is the eventual result (Adapted from Khan *et al.,* 2014).

In addition to a considerable number of genetic components associated with T2DM, segregation analysis also suggests the polygenic nature of T2DM. Most identified diabetes loci have not been mechanistically tied to the disease. While loci are commonly referred to by the names of genes located close to them, only a few are close to strong biological candidates, e.g., the melatonin receptor 1B (MTNR1B) and the insulin receptor substrate-1 (IRS-1). For others, like Transcription factor 7-like 2 (TCF7L2*)* and Gastric Inhibitory Polypeptide Receptor *(*GIPR*)*, the evidence is quite strong that an intronic single polymorphisms (SNP) is the causal SNP (Prasad & Groop, 2015). MTNR1B has been associated with both fasting glucose and T2DM risk. Melatonin works as a chronobiotic factor, adjusting the timing of the biological clock. Its receptors are present in the pancreas and melatonin is proposed to contribute to the nocturnal lowering of insulin in humans. The MTNR1B risk genotype is associated with impaired early insulin release to both oral and intravenous glucose and insulin secretion deteriorates over time in the risk allele carriers. The proposed mechanism by which MTNR1B polymorphism could predispose to T2DM involves altered expression of MTNR1B in pancreatic β-cells, leading to decreased cyclic adenosine monophosphate /cyclic guanosine monophosphate (cAMP/cGMP) concentrations via G proteins and, thereby, impaired insulin secretion (Cöl *et al*., 2018; Prasad & Groop, 2015).

Like Prasad and Groop (2015), Dendup *et al.* (2018) also suggest a link between the environment and health outcomes closely related to T2DM such as obesity, cardiovascular diseases, hypertension, metabolic syndrome and physical activity. Obesity contributes to an increased production of glucose by the liver. This leads to a "prediabetes" condition, wherein the glucose levels are high but under the T2DM range. The metabolism of carbohydrate, fat,

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and protein is disturbed as the disease progresses. Hyperglycemia (high blood sugar levels) results when the β-cells fail to compensate insulin resistance with excess insulin output. The progressive decline of the β-cell function and mass over time with hyperglycemia marks the development of T2DM. Accumulation of fat in the liver, muscles, and pancreas from surplus calories and physical inactivity contributes to β-cell dysfunction and insulin resistance. Inflammation, oxidative and endoplasmic-reticulum stress, raised lipid levels, and amyloid accumulation also trigger β-cell dysfunction. Gastrointestinal tract hormones and the nervous system, including the brain, also act on β-cells and glucose metabolism. Early diagnosis and treatment with lifestyle interventions (physical activity, diet, and weight loss) and glucose-lowering medications can reduce complications and vascular diseases and prevent or delay disease progression. However, in type 2 diabetes of duration greater than 10 years, the cellular changes appear to pass a point of no return. This review summarizes the evidence that early type 2 diabetes can be regarded as a reversible β-cell response to chronic positive calorie balance. (Dendup *et al.* 2018; White *et al.,* 2016).

Lifestyle factors contributing to the development of T2DM, include a sedentary lifestyle, physical inactivity, smoking and alcohol consumption. As mentioned previously, obesity is the most important risk factor for T2DM, which may influence the development of insulin resistance and disease progression. Nearly 90% of diabetic patients develop T2DM mostly relating to excess body weight (Ley *et al.,* 2016). Furthermore, obesity is strongly inherited.

In addition, Wu e*t al*. (2014), stated that diet is considered as a modifiable risk factor for T2DM. They mentioned that a low-fiber diet with a high glycemic index is positively associated with a higher risk of T2DM, and they found that specific dietary fatty acids may affect insulin resistance and the risk of diabetes in varying degrees. Total and saturated fat intake is associated with an increased risk of T2DM independently of body mass index (BMI), but higher intake of linoleic acid has the opposite effect, especially among leaner and younger men. Frequent consumption of processed meat, but not other meats, may increase the risk of T2DM after adjustment for BMI, prior weight change, and alcohol and energy intake. Soft drinks have

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also been bounded up with increased risk of T2DM and metabolic syndrome, because they are directly associated with BMI (Wu *et al.*, 2014).

2.3.2 Complications associated with T2DM

The complications of DM can be divided into acute and chronic complications. Acute complications include diabetic ketoacidosis, hyperosmolar hyperglycemic nonketotic coma, Somogyi effect, Dawn phenomenon and hypoglycemia. Long-term complications can include cardiovascular complications both micro- and macrovascular, vision loss (retinopathy), renal damage, peripheral nervous system damage, autonomic neuropathy and metabolic syndrome (Chawla *et al*., 2016).

Despite the complications mentioned above, patients with DM can also sustain secondary complications and have an increased risk of falling because of primary complications like peripheral neuropathy, possible hypoglycemia, nocturia, and visual impairment. Because many T2DM patients are obese and sedentary, coordination and balance factors acting as protective mechanisms against falls may be absent (Chau & Edelman, 2002).

2.3.2.1 Factors contributing to falls in DM patients

a) Vision related

- Diabetes retinopathy
- Advanced cataracts (visual field deficits)
- Laser therapy for retinopathy (peripheral and night vision decreases)
- Hypoglycemia

b) <u>Gait/balance related</u>

   i. Peripheral

  ii. Foot ulcers

 iii. Polyuria and nocturia, urgent and frequent trips to the restroom, especially at night

 iv. Decreased reflexes

Another important complication associated with DM is low bone mass, thus contributing to osteoporosis (Abdulameer *et al.*, 2012). For the purpose of this study emphasis was placed on osteoporosis as a complication of T2DM.

2.3.3 Potential mechanisms contributing to low bone mass in T2DM

Lifestyle choices influence 20 – 40% of adult PBM (Weaver *et al.*, 2016). Therefore, optimisation of lifestyle factors known to influence PBM and strength is an important strategy aimed at reducing risk of osteoporosis or low bone mass later in life.

The ability of bone to resist fracture depends on several factors including bone mass, the shape and microarchitecture of the bone, and innate properties of the materials that comprise the bone (e.g., mineralisation and microdamage). Bone density measures grams of mineral per area or volume and is determined by PBM and the amount of bone loss. PBM is achieved between the ages of 18 and 25 years and is largely determined by genetic factors. Other determinants of PBM include nutrition, endocrine status, physical activity, and overall health during growth (Dempster, 2011).

Bone mass in older adults equals the PBM achieved by age 18–25 years minus the amount of bone subsequently lost due to menopause when remodeling becomes unbalanced and rapid. Each of the many remodeling transactions deposits less bone than it resorbed, producing

microstructural deterioration (Bjornerem *et al*., 2017). PBM is determined largely by genetic factors, with contributions from nutrition, endocrine status, physical activity, and health during growth. The process of bone remodeling that maintains a healthy skeleton may be considered a preventive maintenance programme, continually removing older bone and replacing it with new bone. Bone loss occurs when this balance is altered, resulting in greater bone removal than replacement. The imbalance occurs with menopause and advanced age. With the onset of menopause, the rate of bone remodeling increases, magnifying the impact of the remodeling imbalance. The loss of bone tissue leads to disordered skeletal architecture and an increase in fracture risk (Cosman *et al*., 2014).

High glucose levels in T2DM leads to the accumulation of advanced glycosylation end-products (AGEs) in the organic bone matrix by a process known as non-enzymatic glycation (the Maillard reaction) (Singh *et al*., 2014). Increased AGEs may weaken bone by decreasing bone formation. There is evidence suggesting that AGEs interfere with normal osteoblast development, function and attachment to the collagen matrix. Moreover, low bone formation also works in the opposite direction to further increase AGEs, as, for example, with high bisphosphonate dosages. Biochemical markers of bone formation have generally been reduced, although T2DM has not definitely been established to contribute to low bone formation (Leslie *et al*., 2012).

In contrast to T1DM with autoimmune ß-cell destruction and complete insulin and amylin deficiency, T2DM is characterised by peripheral insulin resistance with a variable degree of hyperinsulinemia and impaired insulin secretion after a metabolic challenge by glucose. Hyperglycemia may have several adverse effects on bone metabolism both in patients with poorly controlled T1DM and T2DM. Glucose is the principal energy source for osteoclasts and is able to dose-dependently enhance avian osteoclast activity *in vitro*. In addition, hyperglycemia leads to non-enzymatic glycosylation of various bone proteins, including type I collagen, which may impair bone quality (Hofbauer *et al*., 2007). Figure 2.2 represents a suggested model of potential deleterious effects of diabetes on bone based on *in vitro* findings, animal studies, and observational human data.
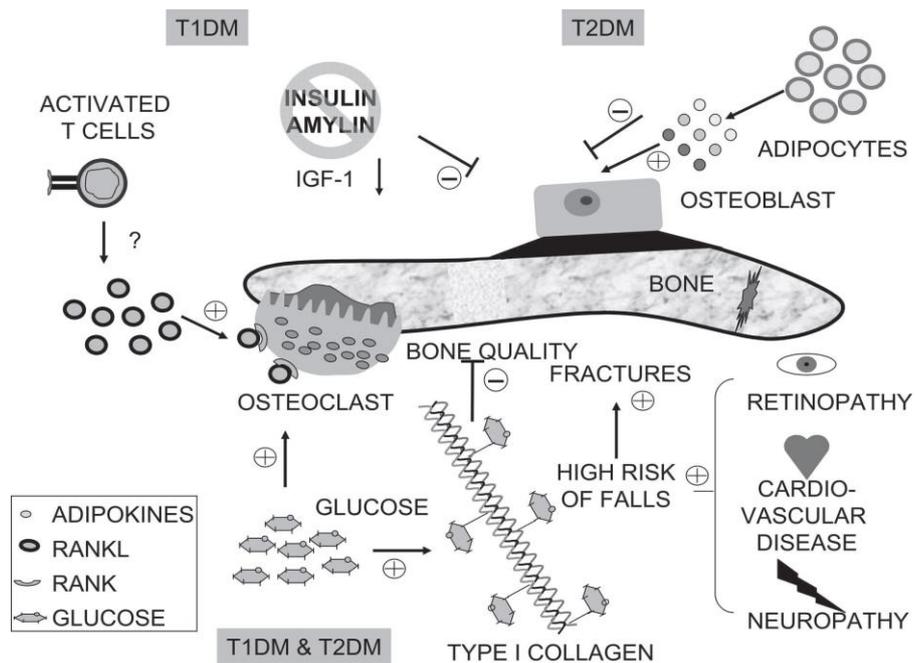
Figure 2.2: **Potential mechanisms contributing to low bone mass and increased fracture susceptibility in DM** (adapted from Hofbauer *et al*., 2007).

Currently, it can be concluded that T2DM compromises bone microstructure by inducing aberrant bone cell function (cellular failure) and abnormal matrix structure (matrix failure). Regarding the cellular effect, T2DM is associated with increased osteoblast apoptosis, diminished osteoblast differentiation, and enhanced osteoclast-mediated bone resorption, which, in part, resulted from hyperglycemia and insulin resistance. Prolonged accumulation of AGEs coexisting with a decrease in lysyl oxidase activity causes abnormal structure and alignment of collagen, leading to bone fragility. Several confounding factors in T2DM, particularly body weight gain, obesity, and dyslipidemia, can mask the detrimental effects of T2DM, and may delay diagnosis of diabetic osteoporosis. In other words, bone is already damaged in T2DM despite a relatively high BMD (Wongdee & Charoenphandhu, 2015).

2.4   *What is osteoporosis?*

Osteoporosis is the most common metabolic bone disorder in humans, and bone fractures are the hallmark of the disease. It constitutes an enormous socio-economic crisis with severe impact on patient morbidity and mortality (Abdulameer *et al*., 2012). The disease is characterised by low bone mass, deterioration of bone tissue and disruption of bone architecture, compromised bone strength, and an increase in the risk of fractures (Siris *et al*., 2014).

It can be caused by acceleration of bone resorption and/or deceleration of bone formation. Clinically, osteoporosis most often results from a combination of postmenopausal estrogen deficiency and age-related bone loss. Irreversible bone loss can result from an imbalance between osteoclast and osteoblast activities, i.e. enhanced bone resorption and/or suppressed bone formation, resulting in an uncoupling event that can prolong duration of the bone remodeling cycle (Wongdee & Charoenphandhu, 2011).

Although often thought of as a static support structure, the skeletal system is a dynamic organ with many functions, including giving us our human shape, allowing locomotion and motor function, facilitating respiration, protecting vital organs, producing marrow-derived cells, and playing a crucial role in homeostasis (Hossain, 2018). Bones are dynamic structures that are undergoing constant change and remodeling in response to the ever-changing environment. The human skeleton is completely transformed every four years. Bones can react and respond to environmental stimuli; they can become bigger or smaller, they can strengthen themselves when need be, and, when broken, they are among the few organs in the body with the ability to regenerate without scarring (Petre *et al*., 2013).

Bone must be stiff and able to resist deformation, therefore allowing loading capacity, but also be flexible and able to deform to allow energy absorption during impact loading. Bone must

also be light to allow movement. The balance between bone's material stiffness and its flexibility is achieved by varying its mineral content. The greater the mineral content, the greater the material stiffness and the lower the flexibility. Bone strength, one of its major determinants, is dependent both on bone mass, reflected by BMD, and on bone microarchitecture (Jackuliak & Payer, 2014).

The changes within cancellous bone as a result of bone loss is illustrated in Figure 2.3. Individual trabecular plates of bone are lost, leaving an architecturally weakened structure with significantly reduced mass. Increasing evidence suggests that rapid bone remodeling (as measured by biochemical markers of bone resorption or formation) increases bone fragility and fracture risk (Cosman *et al*., 2014).

Normal bone   Osteoporotic bone
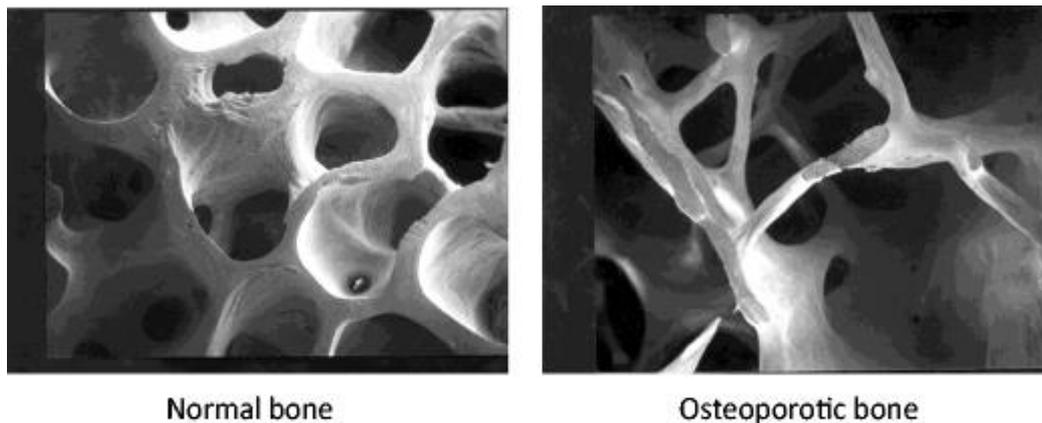
Figure 2.3: The changes within cancellous bone as a results of bone loss (adapted from Cosman *et al.,* 2014)

The main recognised functions of bone remodeling include preservation of bone mechanical strength by replacing older, micro-damaged bone with newer, healthier bone and calcium and phosphate homeostasis. Both cortical and trabecular bone are composed of osteons. The relatively low adult cortical bone turnover rate of 2 to 3% per year is adequate to maintain biomechanical strength of bone. The rate of trabecular bone turnover is higher, more than

required for maintenance of mechanical strength, indicating that trabecular bone turnover is more important for mineral metabolism (Clarke, 2008).

Most bones have a thick, well-organised outer shell (cortex) and a less dense mesh of bony struts in the center (trabecular bone) (Figure 2.4). The ratio of cortical bone to trabecular bone varies widely; in adults, this ratio is typically 80:20. The only bones that lack a true cortex are the vertebrae, which are covered by a compact condensation of trabecular bone (Spencer e*t al*., 2015). According to Clarke (2008) the vertebra is composed of cortical to trabecular bone in a ratio of 25:75, whilst the ratio is 50:50 in the femoral head. Trabecular bone is found on the interior of cortical bone and is less dense. Cortical bone is the dense, extremely strong bone that is found at the periphery of bones. It makes up 80% of the skeleton. Its primary function is mechanical, but it has a role in calcium homeostasis as well. Mature cortical bone is lamellar, meaning it has a distinct layered structure (Figure 2.5) (Petre *et al*., 2013).
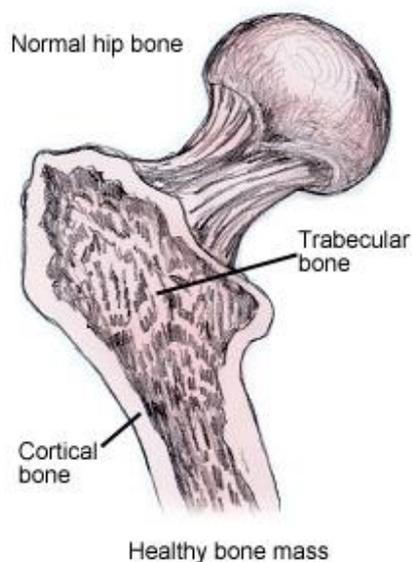
Figure 2.4: Trabecular and cortical bone (adapted from Petre *et al*., 2013)

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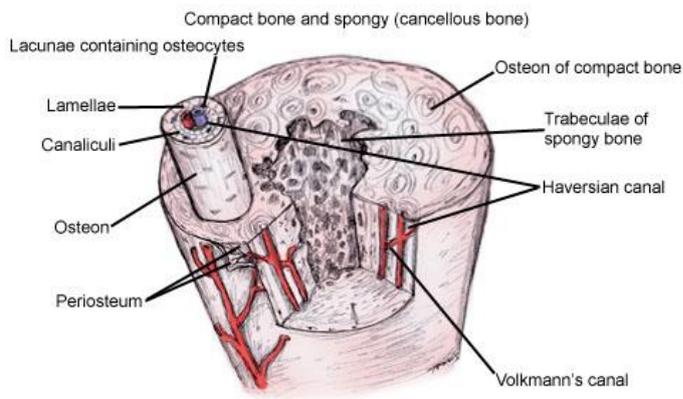
Figure 2.5: Anatomy of bone (adapted from Petre *et al*., 2013)

2.5 Osteoporosis and peak bone mass (PBM)

PBM in young adults is a major determinant of bone mass later in life. Environmental influences such as exercise, smoking, and nutrition, as well as genetic influences are mediated in part by hormonal regulation of bone accrual during growth and maturation. The major and most extensively studied hormonal systems implicated in this regulation are the somatotropic and the gonadal axes. Another hormonal determinant of PBM is thyroid hormone, known to have potentially marked effects on bone maturation and metabolism (Roef *et al*., 2011).

Wilken *et al*. (2010) stated that although research varies on the age at which peak BMD is reached, most suggest that peak BMD is reached somewhere between the ages of 20 and 30 years. These authors also mentioned that some investigators suggest that 95% of peak BMD is reached by age 17 years for females and two to three years later for males, while others suggest PBM is reached by age 30 years for most bone sites. It has been further suggested that osteoporosis is a pediatric disorder that manifests in old age. Furthermore, females can lose up to 20 percent of their bone mass in the five to seven years following menopause, making them more susceptible to osteoporosis (Wilken *et al*., 2010).

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2.6 Relationship between T2DM and osteoporosis

Though the relationship between T2DM and osteoporosis has been widely investigated, it remains controversial.

Patients with DM have various skeletal disorders, including osteopenia or osteoporosis, Charcot's arthropathy, and diabetic foot syndrome. According to Hofbauer *et al.* (2007), bone and mineral abnormalities in patients with DM may be caused by:

- direct effects of insulin deficiency or resistance and hyperglycemia on the bone and bone marrow micro-environment,
- AGEs of bone matrix proteins, abnormal cytokine and adipokine production and their detrimental effects on bone cells, and
- impaired neuromuscular/skeletal interactions.

Furthermore, several other diabetic mechanisms could influence bone, some of which may have contradictory effects. Obesity is strongly associated with higher BMD, probably through mechanical loading and hormonal factors, including insulin, estrogen, and leptin. Low levels of insulin and the progression of T2DM may cause reductions in BMD. Higher glucose levels in the blood are known to interact with several proteins to generate a higher concentration of AGEs in collagen that may reduce bone strength. Accumulated AGEs in the body may stimulate apoptosis of osteoblasts, thereby contributing to deficient bone formation. Another indirect effect of hyperglycemia is glycosuria, which causes hypercalciuria, leading to decreased levels of calcium in the body and poor bone quality, hastening bone loss. There is established evidence that low levels of vitamin D are not only associated with the incidence of DM but also that altered vitamin D metabolism leads to diabetic osteopenia (Abdulameer *et al.*, 2012).

DM contributes to the development of osteoporosis because DM leads to reduced metabolic activity of osteoblasts, increased osteoclastic activity due to diabetic acidosis, secondary hyperparathyroidism related to diabetic nephropathy, reduced sexual hormones secretion, increased secretion of glycocorticoids, and decreased blood supply to bones due to diabetic angiopathy (Al-Maatouq *et al.*, 2004).

Diabetes itself is associated with increased risk of bone fractures, although T2DM is often characterised by normal or high BMD. Thus, diabetes may be associated with a reduction of bone strength that is not reflected in the measurement of BMD. Diabetic osteopathy is a significant comorbidity of both forms of diabetes and is characterized by micro-architectural changes that decrease bone quality, leading to an increased risk of bone fractures in both types of diabetes. In T2DM, obesity, increased load on bone, and insulin resistance resulting in hyperinsulinemia contribute to increased bone formation (Jackuliak and Payer, 2014). Furthermore, bone loss has been observed to be greater in patients with poorly controlled diabetes than in those patients with well-controlled diabetes (Chau & Edelman, 2002).

Shan *et al.* (2011) studied 1 253 women with T2DM and 1 194 control subjects aged 40-80 years in mainland China. They investigated age-related BMD, bone projective area (BPA) and the prevalence of osteoporosis using DXA. These authors reported that the BMD of the lumbar spine and hip decreased with age. BMD of the lumbar spine was higher in T2DM patients than in the control subjects. The same was observed for BPA at some vertebral bodies, whereas no significant intergroup differences in BPA were observed at the hip. The prevalence of osteoporosis in the women with T2DM increased with age: 0.00-2.58% at age 40-49 e years; 6.94-28.40% at age 50-59 years, 32.70-76.70% at age 70-80 years, with the range reflecting differences between skeletal sites. In patients over 60 years, the rates of osteoporosis at the anteroposterior (AP) spine were significantly lower in T2DM patients than in the control subjects. They concluded that women with T2DM had higher BMD and a lower risk of developing osteoporosis compared to non-diabetic patients.

Al-Maatouq *et al*. (2004) studied the prevalence of osteopenia and osteoporosis among Saudi postmenopausal women with non-insulin dependent T2DM. BMD of the lumbar spine and femoral neck using DXA was performed on 104 postmenopausal Saudi women with T2DM, and 101 postmenopausal non-diabetic women (control). They concluded that osteoporosis is more common among T2DM postmenopausal females in this ethnic group. Since both groups are postmenopausal, having equal percentage of Vitamin D deficiency, multi-parity, non-exposure to sun, lack of exercise and negligible milk intake, one can conclude that the low BMD can be attributed to DM in the absence of other causes of osteoporosis (Al-Maatouq *et al*., 2004).

The American Iowa Women's Health Study (Anderson *et al*., 2001) reported that women with T2DM had a 1.7-fold higher risk for reporting hip fractures compared with women without T2DM. It has been suggested that long-standing T2DM may predispose to a higher incidence of falls, thus increasing the likelihood of suffering fractures despite higher average BMD values reported in these patients (Hofbauer *et al*., 2007).

2.7 Factors contributing to the risk of developing osteoporotic fractures

2.7.1 Gender and ethnicity associated with osteoporosis

There are several risk factors that increase the risk for fracture development, which include BMD, bone geometry, age, fall rates, fracture history, and medication used, to name but a few (Pisani *et al.,* 2013).

Cauley (2011) stated that gender and ethnicity is strong determinants of fracture risk. In general, White women experience hip fractures about twice as much as men, especially in countries with high incidence rates, but the gender difference in hip fracture risk in African Americans and Asians is negligible. Spencer *et al*. (2015) mentioned that clinical studies

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suggest bone also varies due to ethnicity or ancestry. He claims that it has been shown that people of African descent have higher BMD than Caucasians (Pollitzer & Anderson, 1989; Wang *et al*., 1997; Ortiz *et al*., 1992) and people of Hispanic origin have BMD similar to Caucasians, with Asians having lower bone mass than Caucasians (Pollitzer and Anderson, 1989; Barrett-Connor *et al*., 2005; Cundy *et al*., 1995). Rates of hip fractures are about 50% lower in African American and Asian women than in White women. Ethnic and race variability is much lower for men, although White men tend to have slightly higher hip fracture rates compared to Asian and African American men (Cauley, 2011). BMD is consistently higher in African American women than in White women at every level of body weight and could contribute to their lower fracture rates (Cauley, 2011). However, differences in hip geometry could also contribute. Longer hip axis lengths have been linked to an increased risk of hip fractures, and hip axis lengths are reportedly shorter among African Americans and Asians, even after adjusting for height.

The amount of bone mass a person has, along with bone structure, varies between individuals and populations due to ethnicity, sex, age, diet, or even behavior. According to Spencer e*t al.* (2015), people of African descent have greater bone mass than Caucasians.

There are substantial geographic and ethnic variations in fracture rates around the world. For both men and women, the highest age-adjusted hip fracture rates have been reported in North Europe and America, and the lowest rates in Africa. Caucasians have the highest rates of hip fracture compared to other ethnic groups. It is believed that the higher the BMD, the lower the risk for bone fractures (Chan *et al.*, 2018). Differences exist in the areal BMD between ethnic and racial groups and gender. Areal BMD integrates the size of the bone with its thickness and true volumetric density, while areal BMD is similar in White and Asian patients. Asians reportedly have higher trabecular volumetric BMD, at least in older men, which could contribute to their lower fracture rates (Cauley, 2011).

Ma *et al*. (2012) provide insights into the inconsistent reported relationship between T2DM and BMD. Their meta-analysis concluded that overall individuals with T2DM have

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approximately a 25–50% higher BMD SD compared to non-diabetic control subjects. Subjects with T2DM had elevated BMD at the femoral neck, hip, and spine. They reported no evidence suggesting there is gender-specificity in the observed BMD differences between diabetics and non-diabetics. BMD differences seem larger in women than in men, but according to Ma *et al.* (2012), power limitations such as considerable heterogeneity influence can also play a role.

2.7.2 Insulin

Insulin is an anabolic hormone, which acts on bone through insulin receptors expressed by osteoblasts— IRS-1 and insulin receptor substrate 2 (IRS-2) (insulin-like substrate). Stimulation of IRS-1 affects bone turnover, while stimulation of IRS-2 shifts the balance between bone formation and resorption towards the former. Insulin stimulates osteoblast proliferation, inactivates p27 (responsible for osteoblastogenesis), promotes collagen synthesis, and increases glucose uptake (Li *et al*., 2016). In T1DM, the deficiency of insulin and insulin growth factor 1 (IGF-1), which is present since the diagnosis of T1DM, leads to impaired bone formation, abnormal mineralisation, abnormal bone micro-architecture, increased fragility of the bone, and reduced PBM. In T2DM hyperinsulinism (the stimulatory effects of insulin on bone formation) coupled with insulin resistance increase bone mass through effects on bone formation via IRS-1 and IRS-2 surface receptors on osteoblasts, and by reducing the concentration of sex-hormone binding globulin (SHBG), which leads to increased concentrations of estradiol and testosterone (Jackuliak & Payer, 2014).

2.7.3 Protein

Nutrition is an important component of bone health. The value of nutrients such as calcium is well documented. Protein makes about 50% of bone volume and approximately one-third of its mass. It provides the structural matrix of bone, whereas calcium is the dominant mineral within that matrix. Collagen and a variety of non-collagenous proteins form the organic matrix of bone, so an adequate dietary protein intake would seem to be essential for optimal acquisition and maintenance of adult bone mass (Shams-White *et al.*, 2017). Previously dietary protein intake has been implicated in the loss of bone due to the acidification of blood. Wolfe (2015) indicates that, when net bone formation has been determined, higher rates of protein intake have been shown to have beneficial effects on bone health. He mentioned that bone strength is directly affected by the torque placed on the bones because of muscular contraction. Because higher levels of protein intake increase strength in the elderly, increased protein intake may have an indirect effect on bone strength by enabling the generation of greater muscular force (Wolfe, 2015).

2.7.4 Vitamin D

Maintaining adequate calcium intake during childhood and adolescence is necessary for the attainment of PBM, which may be important in reducing the risk of fractures and osteoporosis later in life. Approximately 99% of total body calcium is found in the skeleton, with only small amounts found in the plasma and extravascular fluid. The primary need for dietary calcium is for bone mineral deposition. Calcium and vitamin D can decrease postmenopausal bone loss and prevent fracture risk. However, there is still a high prevalence of calcium and vitamin D insufficiency in women aged 50+ years. Dietary sources of these nutrients are the preferred choice, and dairy products represent a valuable dietary source of calcium due to the high

content, high absorptive rate and relatively low cost (Rizolli *et al*., 2014). Overall calcium homeostasis is maintained by the actions of calcium-regulating hormones, which most notably include parathyroid hormone, calcitonin, and 1, 25-dihydroxy vitamin D. Calcium is absorbed in the intestine by both passive and active processes, the active process being more important in situations in which dietary calcium intakes are suboptimal. The active process requires vitamin D, which emphasises the fact that good bone health requires satisfactory intakes of both calcium and vitamin D. Optimising calcium intake is particularly important during adolescence. Peak calcium-accretion rate is attained at an average of 12.5 years of age in girls, and at 14.0 years of age in boys. During the third to fourth year period of increased bone mass acquisition that occurs during adolescence, 40% of total lifetime bone mass is accumulated. This emphasises the importance of establishing dietary practices in childhood that promote adequate calcium intake throughout life (Greer *et al*., 2006).

Most studies across a variety of geographic locations suggest that vitamin D insufficiency is more common in individuals with diabetes compared to the general population. Proposed mechanisms for vitamin D deficiency in diabetes include genetic predisposition (T1DM), increased BMI (T2DM), concurrent albuminuria (T1DM or T2DM), or exaggerated renal excretion of vitamin D metabolites or vitamin D-binding protein (T1DM, T2DM) (Jackuliak & Payer, 2014).

Gilani *et al. (2019)* have done a study on 69 females and 40 males. The mean age was 44.13 years. Their study showed a statistically significant difference in the vitamin D status in diabetic versus non diabetic patients. With regards to BMI and vitamin D status, the difference was also statistically significant. Caglar e*t al.* (2017) demonstrated a negative correlation between Vit D and BMI when they studied 31 Turkish women with BMI>25 kg/m^2. It seems that there is a high prevalence of obesity and DM and they are inversely related to low Vit D levels.

2.7.5 Barriers to adequate calcium intake

Mangano *et al*. (2014) suggest that dietary protein is beneficial to bones, and this may be most apparent when calcium intake is optimal. He mentioned that a higher protein diet increases IGF-1 (a key mediator of bone health), increases intestinal calcium absorption, suppresses parathyroid hormone, and improves muscle strength and mass, all of which may benefit the skeleton.

Vitamin D is another essential component for bone health. In addition to adequate calcium intake, maintaining an optimal vitamin D level is necessary for preventing bone loss (Kim *et al*., 2014). Suboptimal intakes of calcium in children and adolescents may be related to the replacement of milk intake by soft drinks and fruit juices and/or other fruit drinks. Soft-drink consumption peaks in adolescence, at which time milk intake is at its lowest level (Larson *et al*., 2015). Primary lactose intolerance may be a problem in some populations. It is more common in children of African, Mexican, American Indian, and Asian descent than in White children (Deng *et al.*, 2015). Many children with lactose intolerance can drink small amounts of milk without discomfort, especially when accompanied by other foods. Intolerance to the consumption of 250 millileter (ml) of milk or less is rarely seen in preadolescents, and the addition of small amounts of lactose-containing foods to the diet may decrease the severity of lactose intolerance (Greer *et al*., 2006). Other alternatives include the use of fermented dairy products such as hard cheese and yogurt, which may be tolerated better than milk. Lactose-free and low-lactose milks are available. Non-dairy food products (such as certain vegetables) or calcium-supplemented foods (including calcium-fortified soy milk) may be used as other calcium sources, especially for vegetarians who do not consume dairy products (Greer *et al*., 2006). Deng *et al.* (2015) suggested that treatment of lactose intolerance should not be primarily aimed at reducing malabsorption, but rather at improving gastrointestinal symptoms. In their experience, this approach is effective if symptoms are related only to dairy products; however, in irritable bowel syndrome (IBS) patients, lactose intolerance tends to be part of a

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wider intolerance to poorly absorbed, fermentable oligo-, di-, monosaccharides and polyols (FODMAPs).

According to Kim *et al*. (2014) low dietary calcium intake is associated with low bone density, and calcium supplementation can attenuate age-related bone loss. Therefore, they suggested that calcium supplementation is generally recommended for people who might be at risk of inadequate dietary calcium intake or osteoporosis, regardless of age, particularly to prevent deterioration in bone strength in postmenopausal women.

2.7.6 Weight-bearing exercise

Shanb and Youssef (2014) state that exercise training has many advantages such as improving mechanical properties of bone by changing its composition. They suggest that public health programmes should be designed to help prevent bone loss and promote osteogenesis, improve body composition, muscle strength and balance, and reduce recurrent falls and associated risks of fractures.

According to Greer *et al.* (2006), weight-bearing exercise also plays a role in achieving maximal PBM, but data to quantify the effect are limited. These authors indicate that there is evidence that childhood and adolescence may represent an important period for achieving long-lasting skeletal benefits from regular exercise. For example, regular weight-bearing activity had a greater influence on PBM than did dietary calcium intake in children. It is still unclear whether a given level of calcium intake influences the degree of benefit derived from physical activity on bone mass, or whether exercise alone, independent of calcium intake, improves bone mass. According to Greer *et al*. (2006) further studies needs to explore the combined effects of calcium and exercise on bone mass.

Different exercise techniques that benefit mechanical properties of bone are recommended. Some are resistive exercises in the form of strength training programmes, which are used to increase muscular strength, enhance bone mass, improve balance and mobility, and in turn lead to improved quality of life. There are also weight-bearing exercises which are most popular with children, adolescents, adults, and postmenopausal women, because these exercises generate the highest mechanical load on bones. Weight-bearing exercises are applied with different modes such as walking, running or jumping (Shanb & Youssef, 2014).

2.7.7 Muscle strength and bone mass

Chahal *et al*. (2014) explain that mechanical loading from physical activity is a key determinant of the growth and maintenance of the musculoskeletal system. They describe how bone and muscle can increase their mass and strength rapidly in response to mechanical loading during the early years through the process of modelling. Peak mass and strength are usually attained around the second and third decade. However, with the ageing process, there is a decline in musculoskeletal health in both men and women. The primary musculoskeletal changes reported with ageing include a decline in skeletal muscle mass, strength and size, together with a net loss of BMD, bone mineral content (BMC), bone structure and strength (Chahal *et al*., 2014).

Physical activity increases muscle strength and bone mass, while disuse causes muscle wasting and bone loss. Neither body weight nor physical activity is independent of muscle mass, but muscle forces place greater loads on bones than do gravitational forces associated with weight (Burr, 1997). The mechanostat propose that bone gain and loss are determined within ranges of mechanical stimulation that are bounded by hormonal or metabolically determined set points. The set points do not themselves determine whether bone will be gained or lost, only when the remodeling system will be activated above baseline levels or inactivated. Mechanical usage modulates an activated remodeling system and determines bone balance (Burr, 1997).

Pivonka *et al*. (2018) explain the conceptual model of the mechanostat proposed by Harold Frost in 1983. This model states that bone and other musculoskeletal tissues, including cartilage, tendon and muscle, respond to habitual exercise/loading, and that changes in the loading environment lead to adequate structural adaptation of (bone) tissue architecture. The analogy with a thermostat clearly indicates the presence of a physiological feedback system which can adjust bone mass and structure according to the engendered loads. Pivonka *et al.* (2018) recognise that in the bio-engineering community, the mechanostat has been mathematically formulated as a feedback algorithm using a set point criterion based on a particular mechanical quantity such as strain - strain energy density, among others. The belief that a single mechanostat set point exists in an individual is flawed by the fact that different bones throughout the skeleton require a specific strain magnitude to maintain bone mass. Consequently, different bones respond differently to increases or decreases in loading, depending on the sensitivity of the mechanostat. Osteocytes, i.e., cells embedded in the bone matrix, are believed to be the major bone cells involved in sensing and transduction of mechanical loads (Pivonka *et al*., 2018).

In an article published by Herrmann *et al.* (2015), high levels of physical activity have been found to optimise skeletal development early in life, thus preventing age-related bone loss and osteoporotic fractures. These authors concluded that muscle strength and muscle mass play an important role in bone development during growth after school-based interventions an osteogenic effect of weight bearing exercise such as jumping, or ballgames has been observed. Herrmann *et al*. (2015) stated that the effect of high-impact physical activity has been largely explained by the muscle force and strength acting on bone.

2.7.8 Bone remodeling

Bone quality is an amalgamation of all the factors that, in addition to bone mass, determine how well the skeleton can resist fracture, including micro-architecture accumulated microscopic damage, the quality of collagen, the degree of mineralisation, and the rate of bone turnover. According to Dempster (2011), bone remodeling, specifically the balance between the formation of new bone and bone resorption (breakdown of bone), is the biological process that maintains a healthy skeleton and mediates changes in the factors that influence bone strength. Remodeling does not change the shape of bone, but is vital for bone health, as it repairs skeletal damage that can result from repeated stresses by mending small damaged areas (Dempster 2011). Remodeling also serves to renew the cellular elements of bone, in particular, the osteocytes, which are derived from osteoblasts. Osteocytes play a key role in bone health by regulating the remodeling process, among many other functions. Dempster (2011) also stated that in addition, remodeling prevents the accumulation of too much old bone, which can lose its resilience and become brittle.

Zheng *et al*. (2016) explain that bone remodeling involves coordinated actions of osteoclasts (cells that break down bone) to remove bone matrix through resorption of old bone, followed by osteoblasts (cells that form bone) creating new bone through the secretion and mineralisation of new bone matrix. Both processes are important for the maintenance of bone volume and structure.

When the balance between the formation of new bone and bone resorption is impaired and there is greater bone breakdown than replacement, bone loss occurs. Therefore, disease processes and pharmacologic agents that impact bone remodeling will ultimately influence bone's resistance to fracture (Dempster, 2011).

Imbalances of remodeling can result in gross perturbations in skeletal structure and function, and potentially to morbidity and shortening of lifespan. Most adult skeletal diseases are due to excess osteoclastic activity, leading to an imbalance in bone remodeling which favors resorption. Such diseases would include osteoporosis, periodontal disease, rheumatoid arthritis, multiple myeloma and metastatic cancers (Boyle *et al*., 2003).

2.7.9 Bone geometry

The shape of bone could influence its propensity to fracture. Research has identified hip axis length as a characteristic of femur shape that could influence fracture risk. According to Aloia (2008), shorter hip axis length protects against osteoporotic fractures.

According to Knapen *et al*. (2007) Dual-Energy X-ray Absorptiometry-Bone Mineral Density (DXA-BMD) is still the method of choice used in the clinical evaluation of hip fracture risk; however, it has been stipulated that its uncritical use may lead to size-related artefacts in the estimation of bone strength and the identification of fracture risk. Therefore, the ultimate concern in studying bone status is bone strength. Holding other variables constant, strength will increase both as bone mass increases and as bone size increases. When estimating bone strength, two strategies that also compensate for bone dimensions have been proposed. First, it is encouraged that densitometric comparisons between groups are based on BMC rather than DXA-BMD. Second, bone dimensions are used as independent determinants for bone strength. Important geometric parameters are the hip axis length (HAL) and the femoral neck width (FNW). Patients with a low DXA-BMD, or who have experienced a hip fracture, had an increased FNW, suggesting an attempt to compensate for the increased fracture risk at this critical site. On the other hand, it seems obvious that at comparable DXA-BMD, a larger FNW will positively contribute to bone strength. In this way it is understandable that an increase in BMC may contribute to bone strength, although it should be kept in mind that it is not the mass per se, but the distribution of mass that is crucial for bone strength (Knapen *et al*., 2007).

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2.8 Diagnosis of osteoporosis

Under the auspices of the World Health Organization (WHO, 1994), a panel of experts has met periodically. They first convened as a group of experts in 1994 to assess fracture risk and its application to screening for postmenopausal osteoporosis. The scientific group defined osteoporosis based on BMD (WHO, 1994).

According to the WHO's diagnostic classification (1994), osteoporosis is defined by BMD at the hip or lumbar spine that is less than or equal to 2.5 SD below the mean BMD (T-score ≤-2.5) of a young-adult reference population. The National Osteoporosis Foundation of South Afrika (NOFSA, 2010) recommend that the T-score should be used when diagnosing postmenopausal women, and that the Z-score should be used in premenopausal women (Hough *et al.*, 2010). Z-score is the number of SD above or below the expected BMD for the patient's age and sex. A Z-score of –2.0 or lower is defined as either "low BMD for chronological age" or "below the expected range for age," and those above –2.0 are "within the expected range for age" (Qaseem e*t al.*, 2017).

Osteoporosis is regarded as a risk factor for fracture just as hypertension is for stroke. The risk of fractures is highest in those with the lowest BMD; however, most fractures occur in patients with low bone mass rather than those classified as having osteoporosis, because of the large number of individuals suffering from low BMD (Siris *et al.*, 2014).

As BMD has a Gaussian distribution, it is difficult to define a cutoff for osteoporosis diagnosis (T-score <-2.5). However, the majority of individuals who have low-trauma fractures do not have osteoporosis with DXA (i.e., T-score <-2.5), and some of them have no decreased BMD at all. Some medical conditions (spondyloarthropathies, chronic kidney disease and mineral bone disorder, diabetes, obesity) or drugs (glucocorticoids, aromatase inhibitors) are more prone to cause fractures with subnormal BMD. In the situation of fragility fractures with

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subnormal or normal BMD, clinicians face a difficulty, as almost all the pharmacologic treatments have proved their efficacy in patients with low BMD and in patients with a previous fragility fracture (especially vertebra or hip). It is recommended to treat patients with a major fragility fracture even if areal BMD T- score is above -2.5 (Lespessailles *et al.,* 2017).

Osteopenia is defined as a bone density between 1.0 and 2.5 SD below the mean for young adult women. The T-score is defined as the number of SD the patient's BMD is above or below the sex-matched mean reference value of young adults. The T-score thus provides a comparison of the patient's BMD to the mean PBM. The Z-score is defined as the number of SD the patient's BMD is above or below the sex-matched mean reference value for individuals of the same gender and age. The Z-score, therefore, enables a comparison of the patient's BMD to individuals of the same age (Syed & Khan, 2002).

Eriksson e*t al.* (2018) studied a group of 45 obese, non-diabetic, antipsychotic-treated patients. The mean age of the patients was 35.8 years. With one exception, all sex and age-adjusted BMD Z-score measurements were within the normal range. They noted that the presence of marked obesity might partly explain their findings.

A cross-sectional study that consisted of 5 892 consecutive non-institutionalised men and women who were referred to the Isfahan Osteoporosis Diagnosis and Body Composition Center was done by Salamat *et al*. (2016). Compared with men ≥50 years, and postmenopausal women with BMI <25 kg/m^2, the age-adjusted risk of femoral neck osteoporosis was more than four-fold lower in those with a BMI ≥30. They found that the association between BMI and osteopenia was similar. When a Z-score ≤–2.0 was used as alternative analysis to diagnose low bone mass in premenopausal women and men <50 years, the results were very similar to results of T-score ≤–2.5 comparisons. In men, premenopausal women, and postmenopausal women, there was a negative correlation between age and BMD indicators, and a positive correlation between BMI and BMD indicators; the strongest correlation coefficients were between age and BMD in the femoral neck, and the weakest correlations were between age and L1 to L4 BMD. Their study showed that the association between age and BMD was stronger in postmenopausal

women. In conclusion, in men, premenopausal women, and postmenopausal women, the correlation between BMI and BMD indicators remained after age-adjustment. In their study, obesity significantly decreased the risk for osteoporosis, osteopenia, and low bone mass in all participants. They did not identify any influence of gender and menopause on the obesity paradox in osteoporosis, despite significant differences in characteristics between both genders and menopause status.

Alarkawi *et al*. (2015) indicate that the femoral neck region is widely regarded as the optimum site for osteoporosis diagnosis and fracture risk assessment, because it has good predictive value for all major osteoporotic fractures, and because lumbar spine bone density is often spuriously elevated by degenerative changes. The diagnosis of osteoporosis based on a T-score of $\leq$ -2.5 should remain an important way to identify an individual with increased risk for fracture development. Bone density testing is recommended based on age and risk factor status in both men and women. According to Siris *et al.* (2014), only a small proportion of older men and women have a BMD test. Many who do receive the test may still not be recognised as having an elevated fracture risk, because their scores reflect "osteopenia," which in some instances does indicate a high risk based on elevated age or prior fracture history or other validated risk factors. Prior fracture affords the highest risk for future fracture, yet an older patient with a hip fracture may not be diagnosed as having osteoporosis, unless the patient has a BMD test with a T-score of $\leq$ -2.5, and the majority of hip fracture patients have T-scores that are better than −2.5. An incident vertebral fracture strongly predicts an increased risk of another vertebral fracture as soon as within the next year. Most fractures occur in people with low bone mass, not T-score osteoporosis, because a greater number of people have osteopenia than osteoporosis as defined by BMD. The failure to detect clinical osteoporosis when it is present likely contributes to the current lack of awareness of the consequences of this disease by both clinicians and patients (Siris *et al*., 2014).

2.9 Complications associated with osteoporosis

Osteoporosis is associated with an increased risk of fracture development. Vertebral fractures result in the development of dorsal kyphosis and height loss and can also result in chronic back pain (Syed & Khan, 2002). A significant proportion of vertebral fractures are not identified, and only one-third of vertebral fractures receive medical attention. Hip fractures are associated with a significant increase in morbidity, and approximately a 20% mortality rate within the first year following a hip fracture. Clinically, the diagnosis of osteoporosis is made in its advanced stages and usually following a bone fracture. As the presenting fracture is associated with an increased risk of subsequent fractures, it is important to diagnose and treat osteoporosis prior to the development of the first fracture (Syed & Khan, 2002).

Paolucci *et al*. (2016) stated that osteoporosis and sarcopenia are often associated in the elderly. The number and size of muscle fibers are reduced, and there is a preferential loss of type II fibers. They stated that age-related immobilisation also increases the risk of muscle atrophy and bone loss, boosting the risk of fractures. The elderly is at greater risk of debilitating postural changes due to several factors, particularly the involutional loss of functional muscle motor units and the higher prevalence of osteoporosis in these subjects. This muscle loss can contribute to osteoporosis-related skeletal changes. Muscle weakness has been suggested to be related to a progressive decline in bone mass, with consequent axial kyphosis, even in the absence of vertebral fractures.

Bone has trabecular and cortical components. Trabecular bone predominates in vertebrae and the proximal femur, whereas cortical bone is prominent in the long bone shafts. Trabecular remodeling occurs at a rate of approximately 25% per year, while the cortical rate is approximately 3% per year. Thus, changes in BMD occur more quickly and have greater clinical implications in trabecular bone, which is consistent with the prevalence of vertebral and femoral fractures in patients with osteoporosis (Lash *et al*., 2009).

In a study done by Petit *et al*. (2010) they suggested that in patients with T2DM, trabecular bone mass and structure are intact and perhaps even enhanced, whereas the cortical compartment is preferentially compromised. This is noteworthy because: (a) the cortex makes up 80% of the skeleton, (b) cortical bone is present primarily at non-vertebral sites, and (c) in T2DM, most of the fractures occur at sites that are rich in cortical bone. Increased cortical porosity has been reported at the radius in female diabetics who have fractured, as measured by intracortical pore volume fraction via high-resolution peripheral quantitative computed tomography. Although endosteal cortical remnants can be mistakenly interpreted as trabeculae, true increases in cortical porosity could be an important cause of increased fracture risk in T2DM patients because it reduces bone strength, yet is undetectable by DXA (Leslie *et al*., 2012).

In an observational study done by Paruk e*t al.* (2017) the incidence rates and relative risk ratios of osteoporotic hip fractures were calculated in the black population, aged 60 years and older, residing in the eThekwini region of South Africa. All subjects presenting with a minimal trauma hip fracture. Paruk's study represent the largest number of hip fractures recorded in black Africans. Although the incidence rate was approximately tenfold higher than previously recorded, it remains amongst the lowest globally.

2.10   Bone densitometry

Today, DXA is still considered the golden standard for the measurement of BMD because of its reproducibility, large normative data, non-invasive nature, short analysis time, and minimal radiation exposure (Garg & Kharb, 2013). In clinical practice, treatment decisions are based on BMD measurements obtained from DXA and the WHO classification for the diagnosis of osteoporosis (WHO, 1994). The WHO criterion applies to BMD of three skeletal sites, the hip,

lumbar spine or forearm. The hip and spine are the two most commonly used skeletal sites (Arabi *et al*., 2007).

2.10.1   What is DXA scanning?

Non-invasive bone densitometry utilizing X-ray absorptiometry enables accurate evaluation of bone mass and the diagnosis of osteoporosis in asymptomatic individuals prior to fracture. DXA can evaluate bone quality through indirect analysis of micro- and macro-architecture of the bone, which can improve the prediction of fracture risk (Choi, 2016). BMD testing is a vital component in the diagnosis and management of osteoporosis regarding bone strength. Instead of a specific threshold, fracture risk increases exponentially as BMD decreases.

2.10.2 Technical advantages

DXA is a quick method that is accurate (exact measurement of BMD), precise (reproducible), and flexible (different regions can be scanned), and is performed with a low radiation dose (Lorente-Ramos *et al*., 2011). These authors explained that a DXA scanner consists of a low-dose x-ray tube with two energies for separating mineral and soft-tissue components, and a high-resolution multi-detector array. The devices have one of two different systems: a fan-beam device that emits alternating high (140 kVp) and low (70–100 kVp) x-rays and sweeps across a scan area, or a constant x-ray beam with a rare-earth filter and energy-specific absorption, which separates photons of higher (70 keV) and lower (40 keV) energy (Lorente-Ramos *et al*., 2011).

Although different types of DXA systems are available, all of them operate on similar principles. A radiation source is aimed at a radiation detector placed directly opposite to the site to be measured. Early DXA systems used pencil beam geometry and a single detector, which was scanned across the measurement region. Modern full-table DXA scanners use a fan-beam source and multiple detectors, which are swept across the measurement region. DXA technology can measure virtually any skeletal site, but clinical use has been concentrated on the lumbar spine, proximal femur, forearm and total body. The patient is placed on a table in the path of the radiation beam. The source/detector assembly is then scanned across the measurement region. The attenuation of the radiation beam is determined and is related to the BMD. Because DXA scanners use two X-ray energies in the presence of three types of tissue (bone mineral, lean tissue and adipose tissue), there are considerable errors arising from the inhomogeneous distribution of adipose tissue in the human body (El Maghraoui & Roux, 2008). Syed and Khan (2002) stated that by using the dual energy beams, corrections for soft tissue are made, enabling the assessment of BMD.

2.10.3   Possible artefacts

Images should be assessed for artefacts, which should be excluded from the region of interest (ROI). Artefacts include dense objects such as piercings, catheters, surgical material, and contrast medium, such as barium and myelographic agents. Calcifications superimposed on the ROI should be noted as causes of increased BMD (Lorente-Ramos *et al*., 2011).

DXA measurement at the hip is the best predictor of future hip fracture risk. In postmenopausal women and men age 50 and older, the WHO (1994) diagnostic T-score criteria (normal, low bone mass, and osteoporosis) are applied to BMD measurement by central DXA at the lumbar spine and femoral neck (Cosman *et al*., 2014).

As with many other diagnostic examinations, DXA scans should be critically assessed by the interpreting physician and densitometrist for abnormalities that may affect BMD measurements. In clinical practice, recognition of diverse artefacts and disease processes that may influence BMD results can be of major importance in the optimal interpretation of DXA scans. Physicians not directly involved in the performance and interpretation of DXA should familiarise themselves with the procedure to be able to detect common positioning and scanning problems, to know what should appear on a report, what questions to ask if the necessary information is not on the report, how to apply the results in patient management, and when to do and how to interpret a second measurement to monitor treatment.

2.10.4 Indications and contraindications

The decision to perform bone density assessment should be based on an individual's fracture risk profile and skeletal health assessment. BMD is not recommended in children or adolescents and is not routinely indicated in healthy young men or premenopausal women unless there is a significant fracture history or there are specific risk factors for bone loss (Cosman *et al*., 2014). According to Cosman *et al*. (2014), BMD testing is important in the following individuals:

- Women aged 65 and older and men aged 70 and older, regardless of clinical risk factors.
- Younger postmenopausal women, women in the menopausal transition, and men age 50 to 69 with clinical risk factors for fracture.
- Adults who have a fracture at or after age 50.
- Adults with a condition (e.g., rheumatoid arthritis) or taking medication (e.g., glucocorticoids in a daily dose > 5 mg prednisone or equivalent for > 3 months) associated with low bone mass or bone loss.

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The International Society for Bone Densitometry (ISCD) guidelines recognise the need to identify individuals at risk for osteoporosis (ISCD, 2002). The United States Preventive Services Task Force (USPSTF) released guidelines for osteoporosis screening on 16 September 2002. The USPSTF is an independent panel of experts in primary care and prevention. This panel systematically reviews the evidence of effectiveness and develops recommendations for clinical preventive services.

Contraindications for bone densitometry include pregnancy, although radiation exposure with central DXA assessments is minimal (1–5 microsieverts per scan). In individuals who have recently had gastrointestinal contrast or a nuclear medicine test, BMD should be delayed by at least 72 hours, as these tests can affect the results of the scan (Syed & Khan, 2002).

2.10.5 Measured DXA sites

DXA calculates BMD (grams per square centimeter) as BMC (in grams) divided by the projected bone area (square centimeters). As a two-dimensional projectional (areal) technique, DXA does not fully account for skeletal size, as it cannot compensate for differences in the unmeasured third dimension (depth). Thus, a larger bone will tend to have higher BMD than a smaller bone, and comparison of BMD between individuals with different bone sizes can be misleading (Leslie *et al.*, 2012).

BMD assessments should include DXA evaluation of the hip and spine. Both sites are of value in global fracture prediction (Alarkawi *et al*., 2015). Spine scans are of benefit in the younger postmenopausal female, as the spine is a site rich in cancellous bone and is often the first site to reflect early postmenopausal bone loss. In the elderly population, as the spine assessments are more likely to be falsely elevated, it is particularly important to review the hip scan and consider intervention based on the bone density at the hip site. The spine assessment may be

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falsely elevated in the presence of extensive degenerative change, aortic calcification, or vertebral compression fractures (Figure 2.6) (Syed & Khan, 2002).
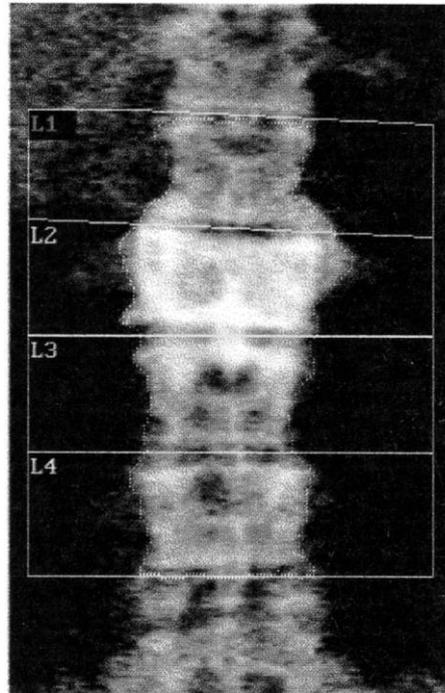
Figure 2.6: **Anteroposterior (AP) spine scan that illustrates abnormality at L2 with sclerosis, resulting in a falsely elevated lumbar spine bone density.** Sclerosis or other obvious skeletal abnormalities on the spine scan should be further evaluated with spinal X-rays. This patient had radiographic confirmation of Paget's disease at L2 (adapted from Syed & Khan, 2002).

In the spine, absent bone (laminectomy or spina bifida) or vertebral rotation (idiopathic scoliosis) will spuriously lower BMD. All evaluable vertebrae should be used, but vertebrae that are affected by local structural change should be deleted from the analysis. Most agree that decisions can be based on two vertebrae; the use of a single vertebra is not recommended. If all vertebrae are affected, the spine should be reported as 'invalid,' with no BMD or T-score results given. Figure 2.7 illustrates examples from the common spine, and Figure 2.8 illustrates hip scanning problems (El Maghraoui & Roux, 2008).

Figure 2.7 illustrates some pitfalls when scanning the lumbar spine.
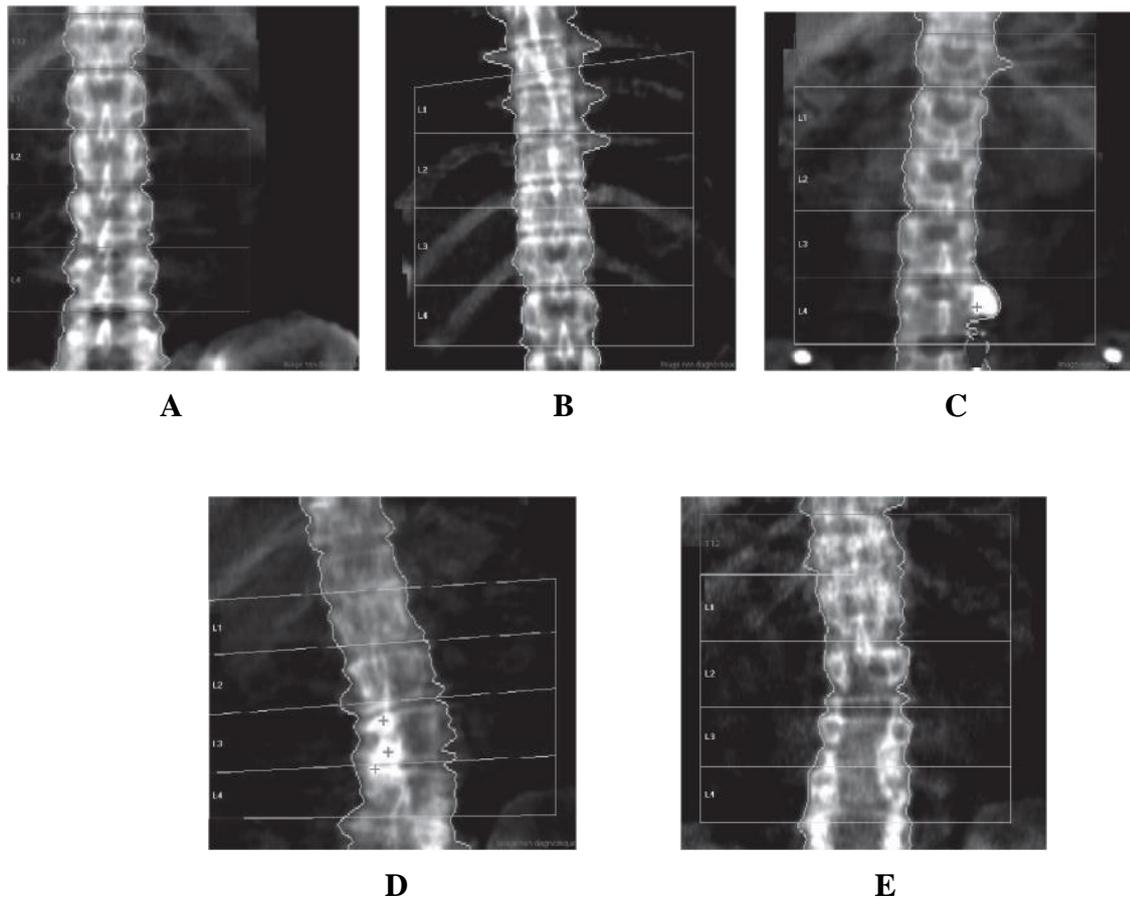
Figure 2.7: Examples of some common spine scanning problems: A) The spine is too close to the right side of the image B) Vertebral levels are misidentified C) Metal button over L4 D) Scoliosis and osteophytes at L3-L4 E) Laminectomy (adapted from El Maghraoui & Roux, 2008).

It is recommended that bone density at the lumbar spine be evaluated from the first to the fourth lumbar vertebrae. At the hip, the diagnosis of osteoporosis can be based on the T-score obtained at the femoral neck, total hip, or trochanteric regions. It is not recommended to base the diagnosis solely on Ward's region, as this area is too small to be adequately accurate or precise. The total hip bone density provides greater precision than the femoral neck, as a larger area of the skeleton is evaluated. The use of additional peripheral sites is of value in conditions such as primary hyperparathyroidism, in which preferential bone loss occurs at sites rich in cortical bone. The one-third radial site reflects the effect of primary hyperparathyroidism to a greater degree than BMD measurement at the lumbar spine or the total hip, as this site is essentially purely cortical bone (Syed & Khan, 2002).

One important way to describe bone quality is to assess its micro-architecture. Bone micro-architecture contributes to the mechanical strength of bone and, thus, to its ability to withstand fractures. Bone loss is often accompanied by deterioration in bone architecture, resulting from a decrease in the number of trabeculae or cancellous bone, increased intertrabecular distances, and a loss of trabecular connectivity. In addition, a reduction in the thickness of cortical bone and an increase in the porosity of trabecular bone can result in fragility of the femoral neck. Osteoporotic bone is, hence, called "porous" (Jackuliak & Payer, 2014).

The hip bone density can be falsely elevated in the presence of osteoarthritis due to the presence of increased bone mineral deposition along the medial aspect of the femoral neck. The presence of hardware such as Harrington spinal rods or hip replacements precludes a useful BMD assessment at the affected site. In situations where differing T-scores are obtained at the two skeletal sites, the diagnosis of osteoporosis is based on the lower T-score (Syed & Khan, 2002).

In hip scanning, it is important to avoid undesired bone. The anatomic landmark selected for femoral neck ROI placement is the greater trochanteric notch (Lorente-Ramos *et al*., 2011).

Figure 2.8 illustrates some pitfalls when scanning the proximal femur.
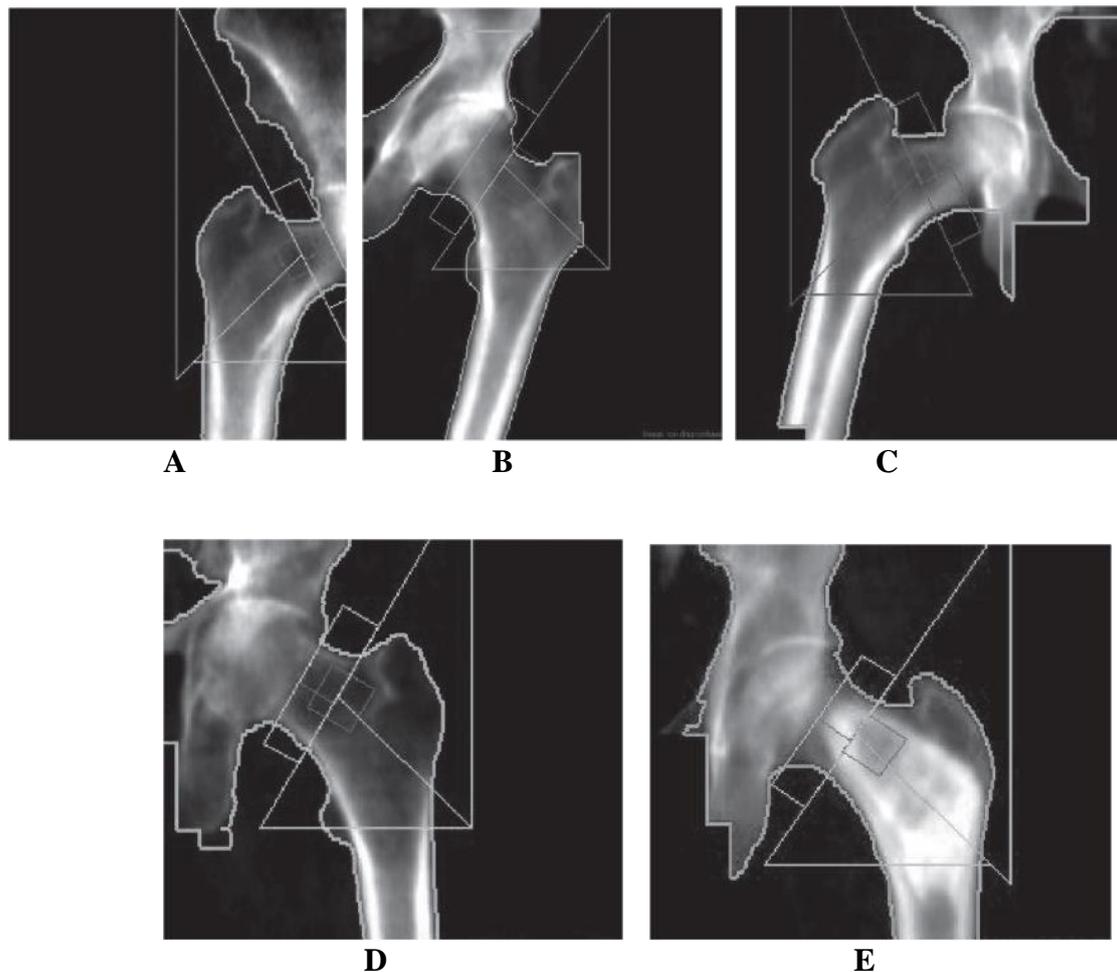
Figure 2.8: Examples among some common hip scanning problems: A) The scan did not
go far enough laterally, and part of the femoral head is missing. B) The femur is
adducted. C) The femur is abducted. D) Suboptimal internal rotation (too much
of the lesser trochanter is showing). E) Abnormal bone (history of hip fracture
and osteosynthesis). (Adapted from El Maghraoui & Roux, 2008).

The rate of bone loss differs according to the age of the patient and the skeletal site. In the
peri-menopausal period and in the early post-menopausal period, bone loss occurs mainly at
the spine reflecting the effect of estrogen deficiency on trabecular bone, thus by measuring the
hip only, the diagnosis of osteoporosis may be missed in this group of patients (Arabi *et al*.,
2007).

The ISCD recommends obtaining BMD measurements of the AP spine and hip. The lateral
spine and Ward's triangle region of the hip should not be used for diagnosis, as these sites

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overestimate osteoporosis, and results can therefore be falsely positive. Evidence suggests that the femur (neck or total hip) is the optimum site for predicting the risk of hip fracture, and the spine is the optimum site for monitoring response to treatment. Thus, many authors recommend hip measure alone for the fracture risk assessment. In very obese patients, those with primary hyperparathyroidism, or those in whom the hip or the spine, or both, cannot be measured or interpreted, BMD may be measured in the forearm, using a 33% radius on the no dominant forearm (El Maghraoui & Roux, 2008).

2.10.6 Patient positioning during BMD analysis

An important component of DXA interpretation involves scrutinising the skeletal images to assess patient positioning, correctness of edge detection, potentially confounding artefacts, and placement of margins to delineate ROIs (Lewiecki *et al*., 2016).

Tuna *et al.* (2017) state that, when there is inaccuracy in the labeling of vertebral bodies, bone edges, and ROI, these errors are defined as analysis errors. A non-optimal process may lead to over diagnosis or under diagnosis (Tuna *et al.,* 2017).

Appropriate patient positioning is essential for optimising BMD measurement. The patients are placed in the supine position for AP imaging of the lumbar spine and proximal femur (Lorente-Ramos *et al*., 2011).

A scan with the correct positioning of the spine is illustrated in Figure 2.9. The patient lies straight on the table (spine is straight on the image), not rotated (spinous processes are centered), and centered in the ROI (roughly equal soft tissue fields on either side of the spine). The scan should include part of the lowest vertebra with ribs (which is usually T12) and low enough to show the pelvic brim (which is usually the level of the L4–L5 interspace). Most

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testing centers will elevate the patient's knees with a foam block (hip at a 90º angle to the spine) to try to partially flatten the normal lumbar lordosis (El Maghraoui & Roux, 2008).
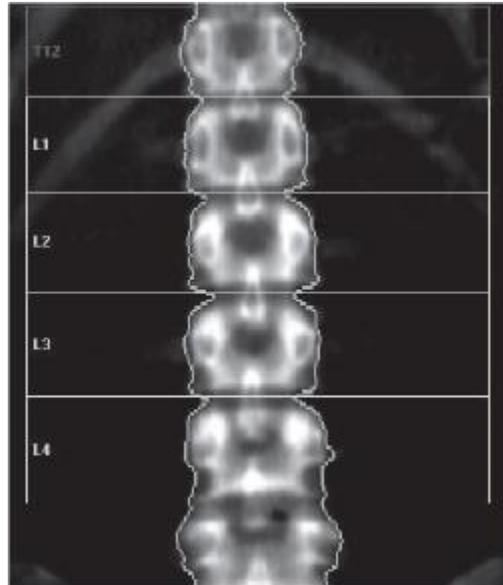
Figure 2.9: A scan with correct positioning of the spine (adapted from El Maghraoui & Roux, 2008)

For proper positioning of the hip (Figure 2.10), the patient should have the femur straight on the table (shaft parallel to the edge of the picture), with 15–25º of internal rotation, which can be achieved using positioning devices (Baniak *et al.,*2014). Internal rotation may be improved by having the patient flex the foot before doing the internal rotation, and then relaxing the foot after the strap is in place. This amount of internal rotation presents the long axis of the femoral neck perpendicular to the X-ray beam, providing the greatest area and the lowest BMC (and the lowest BMD), and is confirmed on the scan by seeing little or none of the lesser trochanter (Baniak *et al.*, 2014).

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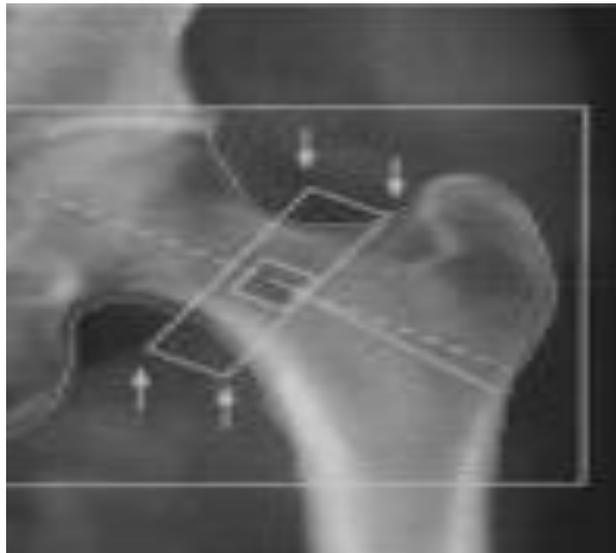
Figure 2.10: Proper positioning of the hip (adapted from El Maghraoui & Roux, 2008)

2.10.7 Scan analysis

The software marks regions of interest in the spine and hip, but the technologist can and should adjust if needed. The spine region of interest consists of the L1 through L4 vertebrae. Correct placement of the top and bottom of the spine 'box' is critical. The intervertebral lines can be moved or angled, if necessary. There must be sufficient soft tissue on both sides of the spine; otherwise BMD will be underestimated. The hip regions of interest include the femoral neck, trochanter and total hip (Figure 2.11) (Baniak *et al.,* 2014). The default hip analysis includes a midline that must be placed correctly for the other sites to be identified correctly. The preferred position for the rectangular femoral neck box differs for different manufacturers. For Discovery QDR series (HOLOGIC, USA) the box is on the distal part of the femoral neck (El Maghraoui & Roux, 2008).

The correct numbering of the vertebral bodies is the main goal in DXA of the lumbar spine. The indicators of correct positioning are as follows: the ribs appear at T12, the largest

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transverse processes are L3, the vertebral area values increase from L1 to L4, BMD increases from L1 to L3, and the BMD of L4 is similar to or slightly less than that of L3. Sometimes radiographs are necessary for correlation. Altered vertebrae (deformed or with lesions or artefacts in them) should be excluded from the analysis. If only one vertebral body is left, the region is not useful for diagnosis (Lorente-Ramos *et al.*, 2011).

The left proximal femur shows four ROIs and include the femoral neck, trochanter, intertrochanteric region, and Ward's triangle. The total hip comprises four RIOs. The image includes the entire femoral head and at least 1 cm under the region of the lesser trochanter, which should not be seen owing to rotation. As seen in Figure 2.11, the femoral axis is straight. Figure 2.12 indicates the wrong positioning of the patient showing the lesser trochanter (Lorente-Ramos *et al.*, 2011).
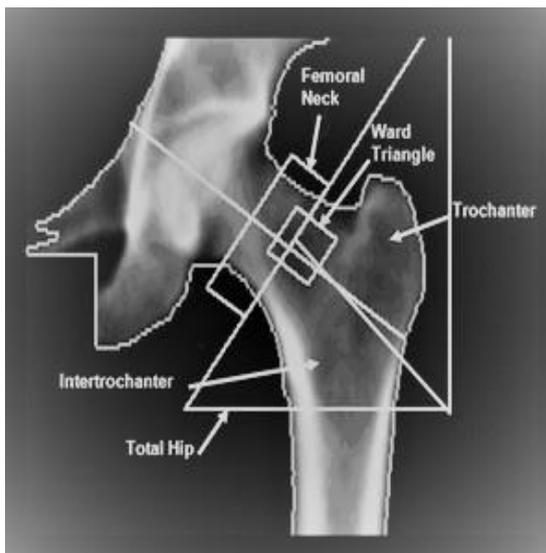
Figure 2.11: ROI of the proximal femur (adapted from Lorente-Ramos, 2011)
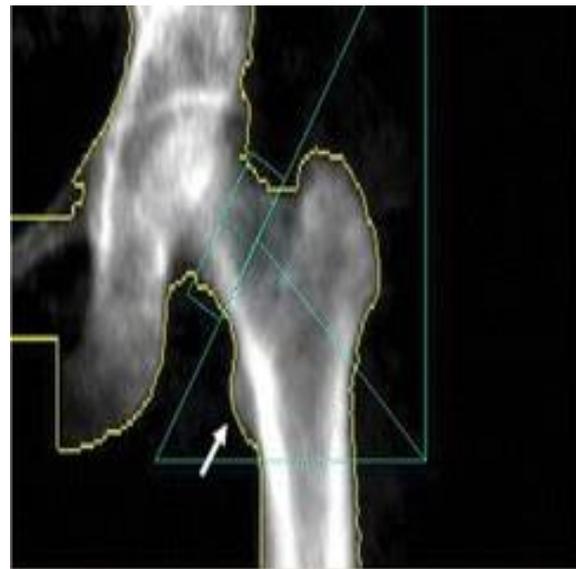
Figure 2.12: ROI of the proximal femur showing the lesser trochanter (adapted from Lorente-Ramos, 2011)

2.10.8 Interpretation of results and classification

DXA measurement of the hip and spine is the technology used to establish or confirm a diagnosis of osteoporosis, predict future fracture risk, and monitor patients. Areal BMD is expressed in absolute terms of grams of mineral per square centimeter scanned (g/cm^2), and as a relationship to two norms: compared to the BMD of an age-, sex-, and ethnicity-matched reference population (Z-score) or compared to a young-adult reference population of the same sex (T-score). The difference between the patient's BMD and the mean BMD of the reference population, divided by the SD of the reference population, is used to calculate T-scores and Z-scores (Baniak *et al.,* 2014). PBM is achieved in early adulthood, followed by a decline in BMD. The rate of bone loss accelerates in women at menopause and continuous to progress at a slower pace in older postmenopausal women and in older men. An individual's BMD is presented as the SD above or below the mean BMD of the reference population. The BMD diagnosis of normal, low bone mass (osteopenia), osteoporosis, and severe or established osteoporosis is based on the WHO diagnostic classification (1994), as seen in Table 2.2 (Cosman *et al*., 2014).

Bone density classification according to the Z-score is tabulated in Table 2.3.

Table 2.2: Defining osteoporosis by BMD T-score (adapted from Cosman e*t al*., 2014).

WHO definition of osteoporosis based on T-score		
Classification	**BMD**	**T-score**
Normal	Within 2 SD of the mean level for a young adult reference population	T-score at -1.0 and above
Low bone mass (osteopenia)	Between 1.0 and 2.5 SD below that of the mean level for a young-adult reference population	T-score between -1.0 and -2.5
Osteoporosis	2.5 SD or more below that of the mean level for a young-adult reference population	T-score at or below -2.5
Severe or established osteoporosis	2.5 SD or more below that of the mean level for a young-adult reference population with fractures	T-score at or below -2.5 with one or more fractures

Table 2.3: Defining osteoporosis by BMD Z-score (adapted from Qaseem *et al*., 2017)

NOFSA definition of expected range for age based to Z-score		
Classification	**BMD**	**Z-score**
Within the expected range for age	The number of SD above the expected BMD for the patient's age and sex	Z-score above -2.0
Below the expected range for age	The number of SD below the expected BMD for the patient's age and sex	Z-score below -2.0

The most important information to check is the correct identification of the patient, his date of birth and the sex and ethnicity which are mandatory to calculate T-scores. Sex is used by all manufacturers to calculate T-scores (i.e. T-scores for women are calculated using a female normative database, while T-scores for men are calculated using a male normative database). Although all manufacturers use race in calculating Z-scores, there is inconsistency in the way race is handled when calculating T-scores. Hologic is using race in calculating T-scores (i.e. T-scores for Caucasians are calculated using a Caucasian normative database, T-scores for Blacks are calculated using a normative database for Blacks); however, GE Lunar (GE Health care, USA) and recent Hologic machines use the database for young normal Caucasians to calculate T-scores, regardless of the race of the subject (Lo *et al.,* 2016). The ISCD recommends the latter approach for use in North America, since using race-adjusted T-scores results in a similar prevalence of 'osteoporosis' in every racial group, even though age-specific fracture rates can be very different (El Maghraoui & Roux, 2008).

Proper positioning of the hip is necessary for appropriate interpretation of the scan. The hip should be positioned such that the femoral shaft is straight, and the lesser trochanter is barely visible. The femoral neck region of interest box should not overlap portions of the ischium or the greater trochanter, as this can result in a falsely elevated assessment of BMD. As illustrated in Figure 2.13, the hip has not been adequately internally rotated, and the lesser trochanter is visible. Inadequate internal rotation results in a higher bone density than achieved with proper positioning of the hip. In this scan, the femoral neck region of interest box has also been incorrectly placed, overlapping a portion of the ischium (Syed & Khan, 2002).
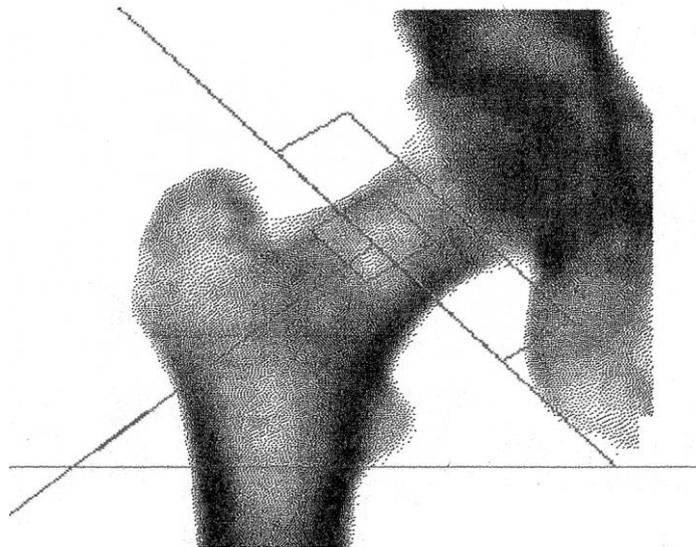
Figure 2.13: Poor positioning of a hip scan (adapted from Syed & Khan, 2002)

2.10.9 T-score and Z-score

The scanner calculates BMD in g/cm^2. A reference database is consulted, and values and curves are obtained. The main parameters are T-scores, which represents the SD by which the BMD differs from the mean BMD of a young adult reference population of the same ethnicity and

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sex, and Z-score, which is the SD by which the BMD differs from the mean BMD of a healthy population of the same ethnicity, sex and age as the person undergoing DXA (Lorente-Ramos *et al.*, 2011).

The T-score is defined as the number of SD the patient's BMD is above or below the sex-matched mean reference value of young adults. The T-score thus provides a comparison of the patient's BMD to the mean PBM (Baniak *et al*., 2014). The Z-score is defined as the number of SDs the patient's BMD is above or below the sex-matched mean reference value for individuals of the same gender and age. The Z-score, therefore, enables a comparison of the patient's BMD to individuals of the same age (Carey & Delaney, 2010).

2.10.10 Risk factors influencing DXA results

Low BMD has been recognised as a good predictor of osteoporotic fracture risk. Nevertheless, although widely used, a major limitation of BMD measurement is that a substantial degree of BMD overlap exists between subjects with and without subsequent fractures. An additional explanation for this is that BMD does not capture all the factors that contribute to bone strength. Among these factors is trabecular bone micro-architecture, which also appears to be a significant determinant of bone strength and is complementary to bone density. Another limitation of BMD measurement is that they disproportionately evaluate cortical bone depending on the skeletal site measured, which has a relatively slow rate of turnover (Jackuliak & Payer, 2014).

Failure to follow standard procedures may result in invalid data, which can be misleading and potentially harmful for patient care. Examples of DXA errors abound. These include incorrect patient positioning and/or analysis, failure to consider confounding artefacts that affect BMD values, and inappropriate reference database use for T-score derivation. Additional errors

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include failure to recognise densitometer drift or shift that could lead to reporting an inappropriate BMD change, thus leading to alteration of therapy, failure to change therapy, and/or unnecessary diagnostic studies. Another common error is failure to perform precision assessment, resulting in inability to distinguish between an apparent BMD difference that is simply within the range of error of the test versus one that is statistically significant (Lewiecki *et al*., 2016).

Physiologic discordance is related to the skeleton's natural adaptive reaction to normal external and internal factors and forces. Mechanical strain especially related to weight bearing plays a key role in this kind of discordance. An example of this type of discordance is the difference observed between the dominant and non-dominant total hip. The explanation is that weight bearing can cause rise in bone density, especially in the hip and femur regions. Moreover, the spine and hips usually start out with different T-scores (the spine is said to reach peak at least 5 years before the hip). Finally, another observation is that bone loss observed with age in an individual may be more rapid and important in trabecular than cortical bone. Trabecular bones (typical of lumbar area) are known to have a more rapid rate of deprivation in early postmenopausal state in comparison with cortical bone (typical of proximal femur) (Milovanovic *et al.,* 2017).

Another type of discordance is described as pathophysiologic discordance. Common examples observed in the elderly include vertebral osteophytosis, vertebral end plate and facet sclerosis, osteochondrosis, and aortic calcification (Lu *et al.,* 2016). Another important cause in younger patients is ankylosing spondylitis syndesmophytes. The abnormal calcium deposition within the field of the DXA ROI leads to the falsely elevated spine T-score. A second subtype is a true discordance resulting from a more decreased BMD in the lumbar spine than the hips. Indeed, most of the aetiologies of secondary osteoporosis (such as glucocorticoid excess, hyperthyroidism, malabsorption, liver disease and rheumatoid arthritis) affect the spinal column first. This will lead to higher prevalence of lumbar osteoporosis (Sheu & Diamond, 2016).

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Anatomic discordance is the result of differences in the composition of bone envelopes tested. An example is the difference in T-scores found for the AP lumbar spine and the supine lateral lumbar spine in the same patient (Lee e*t al.,* 2017).

The premenopausal period is important for bone health and prevention of future fractures but measuring BMD at only one site may not be sufficient to determine therapeutic strategies for low BMD in premenopausal women due to the presence of Z-score discordance (Park e*t al.,* 2016). They investigated Z-score discordance in addition to contributing factors of idiopathic low BMD in healthy premenopausal Korean women aged 18-50 years. Low BMI, low vitamin D level, and low body muscle mass were associated with low BMD even in these women. They found that low BMI and a low vitamin D level were risk factors for low femoral neck BMD, but not for low lumbar spine BMD, and suggested that BMD discordance in premenopausal women should be considered to provide information on correctable factors affecting low BMD in younger populations.

Artefactual discordance occurs when dense synthetic man-made substances are within the field of ROI of the test: e.g. barium sulphate, metal from zipper, coin, clip, or other metallic objects (Doroudinia & Colletti, 2015).

Errors in patient positioning, skeletal site, artefacts removal, and demographic data are considered improper acquisition. According to Tuna e*t al.* (2017) when there is inaccuracy in the labeling of vertebral bodies, bone edges, and ROI, these errors are defined as analysis errors.

Alavizadeh *et al.* (2014) stated that the bone loss after spinal cord injury is different from what happens during a normal aging process, and that it is mainly because of particular mechanical, neurovascular and hormonal changes that occur in these patients. According to them, bone loss happens at a greater pace in trabecular bones, resulting in T-score discordance. T-score discordance might be troublesome for the physicians, and may result in negative outcomes in

the patients, as a true osteoporotic patient must not go undiagnosed, and a healthy patient must not be falsely diagnosed as osteoporotic.

2.10.11 Ethnic differences in bone density also increase risk of fracture rate

A study done by Leslie *et al.* (2012) found that the available data highlighted the complex ethnic variations in BMD, which only partially accounted for observed variations in fracture rates. Factors contributing to ethnic differences include genetics, skeletal size, body size and composition, lifestyle, and social determinants. Despite BMD differences, the gradient of risk for fracture from BMD and other clinical risk factors appears to be similar across ethnic groups. Furthermore, BMD variation is greater within an ethnic population than between ethnic populations. New imaging technologies have identified ethnic differences in bone geometry, volumetric density, micro-architecture, and estimated bone strength that may contribute to a better understanding of ethnic differences in fracture risk. The conclusion was that factors associated with ethnicity affect BMD and fracture risk through direct and indirect mechanisms (Leslie *et al.*, 2012).

Racial differences in BMD values have been well recognised. African-Americans have a higher bone density than Caucasians. It is thus important to compare women to the appropriate ethnic normative reference data. The relationship between BMD and fracture risk is not well defined in the non-Caucasian population. Although Asians have a lower bone density than Caucasians, data from the National Health and Nutrition Examination Survey (NHANES) study in fact have demonstrated that Asian women have a lower risk of hip fractures. This may be explained based on differences in skeletal size between Asians and Caucasians. Areal BMD measured by DXA does not adjust for vertebral depth. Wider and larger vertebrae are deeper, thus not adjusting for depth will result in overestimation of BMD in individuals with larger skeletons. Similarly, BMD is underestimated in individuals with smaller skeletons. Correcting for the differences in

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skeletal size significantly reduces the differences in BMD seen among Asians and Caucasians (Syed & Khan, 2002).

It is important for the technologist to ensure that the appropriate race is identified when scanning a non-Caucasian patient, as misidentification will affect the results of the study (Figures 2.14 and 2.15). Standards and guidelines for the practice of densitometry have been developed by the ISCD, a non-profit global organisation addressing continuing medical education and certification for physicians and technologists (Syed & Khan, 2002).
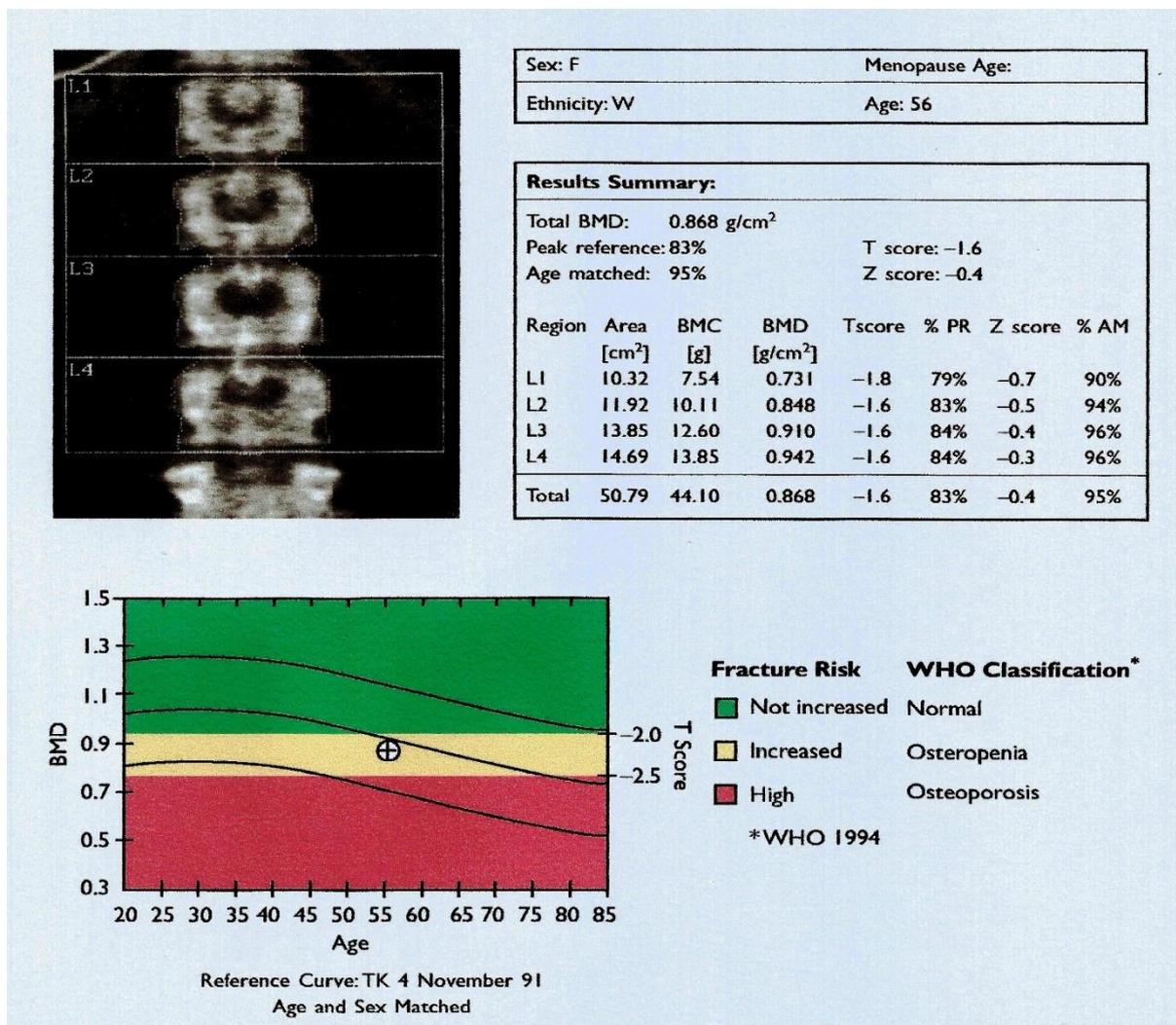
Figure 2.14:   An illustration of an African-Canadian female who was mistakenly identified as Caucasian (adapted from Syed & Khan, 2002)

Upon comparison to the Caucasian young adult normative data, she was identified as having osteopenia with a T-score of -1.6 (Syed & Khan, 2002).
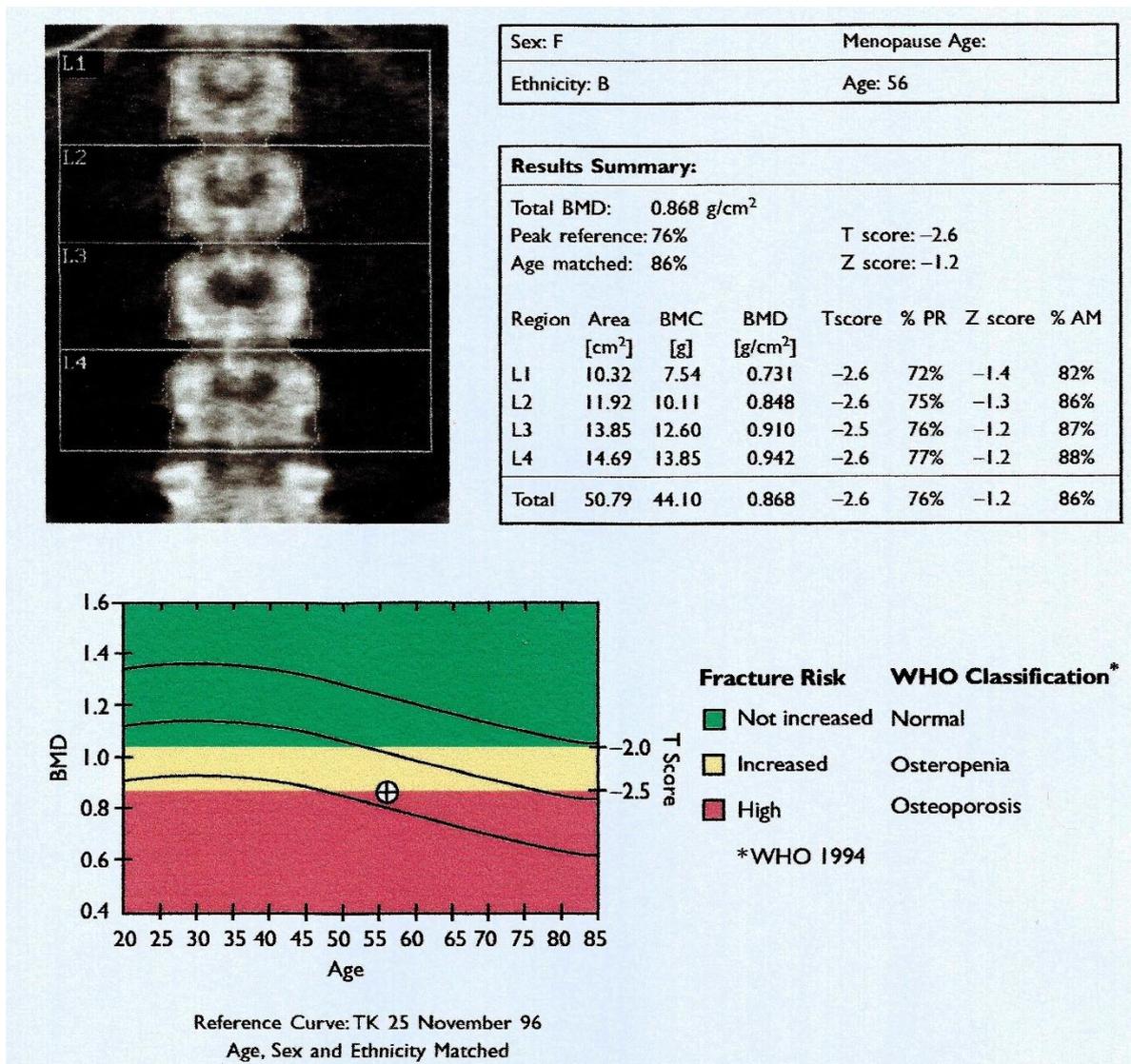
Figure 2.15: **An illustration of an African-Canadian female who was correctly identified as Caucasian (adapted from Syed & Khan, 2002)**

Upon comparison to the use of race-appropriate normative data, the patient was identified as having osteoporosis with a T-score of -2.6.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This research project forms part of a larger parent study entitled: Genetic polymorphisms in black South Africans with type 2 diabetes mellitus (T2DM) from the central Free State area (ECUFS No. 162/2012).

Permission was granted by the principle investigator of the above-mentioned study to access the data recorded. All the data collected was blinded and delinked from all personal information to ensure that patient confidentiality was maintained.

The objective of this study was to determine the effect of T2DM on bone mineral density (BMD) in middle-aged Black South African women. The bone density of 91 patients previously diagnosed with T2DM (test group) and 49 non-diabetic volunteers (control group) were compared during the study. Dual-energy X-ray absorptiometry (DXA) scans were performed by using the Discovery W QDR Hologic Densitometer (model S/N 70494) evaluating the anteroposterior (AP) lumbar spine, left hip and right hip on everyone.

3.2 Study location

The Free State is a country in South Africa which covers an area of 129,825 km^2. The estimated population was 2,954,300 in 2018, which consists of Blacks (87,6%), Whites (8.7%), Coloureds (3,1%) and Indians or Asians (0,4%) (www.freestateonline.fs.gov.za).

The study was conducted at Universitas Academic Hospital, Bloemfontein, involving the Department of Endocrinology. The history of the Universitas Academic Hospital is interwoven with the history of the University of the Free State - a multi-campus public university in Bloemfontein, the capital of the Free State and the judicial capital of South Africa.

The Universitas Academic Hospital was one of the first public-private partnerships (PPP) in health care in South Africa when it opened its doors in 2003. This was the first ever public-private healthcare partnership of its kind in South Africa, and it is managed by Netcare.

The Endocrine Clinic provides care to patients with a variety of endocrine conditions involving hormone function, such as Diabetes Mellitus (DM). DXA is one of the general tests done on the patients attending the clinic. DXA scans on both the test group as well as the control group were performed at this location.

3.3 Study population

In order to compare the patients previously diagnosed with T2DM with the patients not diagnosed with T2DM they were classified as the test group and the control group.

The study population consisted of 91 urban Black female patients with a confirmed diagnosis of T2DM. They attended the diabetes clinic at Universitas Academic Hospital, Bloemfontein, South Africa, from May 2013 to July 2014. These patients were referred to as the test group in this study.

Forty-nine volunteers with no confirmed diagnosis of T2DM (HbA1c% were <6%) were recruited from a local church by word of mouth acted as the control group. All participants included in the control group were informed about the benefits of a DXA scan and educated about osteoporosis as well as DM.

Subjects for the test group were numbered from T1-T91, and subjects for the control group were numbered from C1-C49.

All the individuals were aged ≥ 40 and ≤ 60 years and weight ≤ 130 kg.

3.3.1 Study cohorts

Haemoglobin A1c (HbA1c) % of all the participants were tested to confirm their diagnosis for DM.

Test group: Patients suitable for the test group were recruited from the diabetes clinic at Universitas Hospital, with confirmation of the T2DM from medical files as diagnosed with T2DM by an endocrinologist according to the 2012 guidelines of the Society for Endocrinology Metabolism and Diabetes of South Africa (SEMDSA).

According to Amod *et al*. (SEMDSA, 2012) the diagnosis of diabetes is confirmed in patients with symptoms of hyperglycaemia (polyuria,

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polydipsia, blurred vision, weight loss) or metabolic decompensation (diabetic ketoacidosis or hyperosmolar non-ketotic state), when any one single test confirms that the:

- random plasma glucose is ≥ 11.1 mmol/L;
- fasting plasma glucose is ≥ 7.0 mmol/L;
- haemoglobin A1c (HbA1c) is ≥ 6.5%;
- two-hour post-load glucose is ≥ 11.1 mmol/L.

However, a Glucose Tolerance Test (GTT) is rarely needed in this category of patient.

These patients' HbA1c% were all known to be > 6.5%.

Control group: Patients suitable for the control group were recruited by word of mouth and adhere to the following:

Amod *et al.* (SEMDSA*, 2012)* stated that HbA1c of < 6.5% is recommended as the cut-point for diagnosing diabetes. A value of less than 6.5% does not exclude diabetes diagnosed using glucose tests. A glucose-based measurement is desirable in individuals with HbA1c values close to the diagnostic cut-point (e.g. 6.0 to 6.4%).

HbA1c% of all the patients recruited for the control group was
< 6%. The HBA1c% was done by the National Health Laboratory Service, Universitas, Bloemfontein according to the standard operating procedure of the laboratory.

3.3.2 Inclusion and exclusion criteria

To address the objectives of the study, the selection criteria for the patients were based on the inclusion and exclusion criteria stipulated below to ensure a homogeneous population. Subjects were included in the study if they met the following criteria:

3.3.2.1 Inclusion criteria

The following inclusion criteria applied:

- Black females (as indicated in patient file),
- subject's weight ≤ than 130 kg, due to the weight limit of the DXA;
- age, only subjects ≥40 and ≤ 60 years;
- subjects with HbAc1% > 6.5% included in the test group; and
- subjects with HbAc1% < 6% included in the control group.

3.3.2.2 Exclusion criteria

- Patients not able to perform the physiological test to the satisfaction of the researcher.
- Patients with prosthesis (e.g. hip replacements) and surgical implants in any area analysed.

- Patients that received gastrointestinal contrast or a nuclear medicine test ≤ 72 hour prior to DXA scan.
- Pregnant subjects.

3.4 Study design

A retrospective analytical cohort study was performed including 91 Black South African females previously diagnosed with T2DM, and 49 Black South African volunteered females not diagnosed with T2DM that underwent a BMD test between May 2013 and July 2014.

Figure 3.1 represents a summary of the study layout. The test group consists of 91 subjects which were subdivided into two groups according to age: 37 subjects were <50 years and 54 subjects were ≥50. The control group consists of 49 subjects: 26 subjects were <50 years and 23 subjects were ≥50. The subjects <50 in both groups were diagnosed according to z-score and the subjects ≥50 were diagnosed according to T-score.

Subject recruitment for the parent study

May 2013 – July 2014

Test group

Control group

T2DM patients

91 subjects

Age <50: n=37 subjects

Age ≥50: n=54 subjects

Age <50 : Z-score

Age ≥50 : T-score

Non-diabetic volunteers

49 subjects

Age <50: n=26 subjects

Age ≥50: n=23 subjects

Data collection

- Demographic data
- Anthropometric data
- DXA measurements

Comparison between the

test group and the control group

Figure 3.1: Summary of the study layout

Advanced age is known to affect the quality of bone. The aim of the study was to determine the effect of T2DM on BMD. Thus, age was considered only as an independent variable of the same age (40 – 60 years) between the test group and the control group.

3.5 Data collection

3.5.1 Demographic data

Age (years)

All the subjects included in the study were ≥ 40 years and ≤60 years.

3.5.2 Clinical data

Patients were included in the test group based on their HbA1c%, which was above 6.5%. The criteria for the diagnosis of DM are ≥ 6.5% (SEMDSA, 2012).

Patients were included in the control group based on their HbA1c%, which was below 6%. According to Amod *et al,* (2012) the diagnostic cut-point is 6.0% to 6.4%. No other clinical data were recorded on both the study groups.

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3.5.3 Anthropometric data

The following anthropometric data were collected during the trial.

Weight (kg)

Patients were asked to remove their shoes, socks and hair ornaments when necessary for the weight and height measurement. Patients' weights were measured using a Nagata electronic platform scale. Weight was measured in kilogram (kg) and rounded to one digit after the decimal (e.g., 121.6 kg). Due to the weight limit of the DXA none of the patients weighed >130kg.

Height (cm)

Each patient's height was measured using a stadiometer. The stadiometer was mounted against a wall. All patients were instructed to stand up straight with their heads and heels against the wall. The height was measured in centimeter (cm) and rounded to one digit after the decimal (e.g., 121.6 cm).

BMI (kg/m^2)

Body mass index (BMI) were calculated by using each patient's height and weight. The formula to calculate BMI: BMI $=$ kilogram per square meter (kg/m^2). Kg is the person's weight in kg and m^2 is their height in meters squared (Harvey *et al.,* 2018).

3.6 Bone density measurement

A bone density test was performed on each participant in the test and control groups. Patients were asked to remove any metal objects such as buttons, cellular phones, coins, etc. None of the patients had hip replacements or any surgical implants near the spine and both hips.

After a new biography was created on the computer, the patients were instructed to lay down on their backs on the table, with their heads on the right side of the table.

3.6.1 Bone density procedure

The bone density of both hips and the lumbar spine of each patient was measured with the Discovery W QDR Hologic Dual Energy Absorptiometer (HOLOGIC, USA, model S/N70494).

The Discovery W QDR Hologic was a DXA. It was a third generation QDR densitometer that employed multiple detectors and a dual energy X-ray fan beam. The arm moved in a single direction decreasing scan times from minutes to seconds with improved image quality and equivalent precision. The beam swept across a region of interest on the scan area in a fan-shaped pattern and was detected by a high-resolution multi-detector array to form a high-quality image (Hologic Inc., 2006).

Daily quality control procedures ensured that the QDR system functions properly.

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3.6.2 Quality control

The spine phantom was scanned during daily quality control (QC). The system added the results of the scan to a database and plotted it on a graph. This recorded a daily comparison to 10 separate measurements taken at the time of the system's installation and provided the basis for the system calibration.

The QC Spine Phantom contained a humanlike spine segment made of a material called hydroxyapatite which was enclosed in a block of water-simulant epoxy.

To position the spine phantom, the phantom was placed on the table with the white dot to the left (foot end) of the table, and aligned with the laser cross-hair, and it was verified that it was centred on the white dot and parallel with the phantom. Once the phantom was properly positioned, the system was ready to perform the daily calibration. The QDR system automatically performed a system test to verify proper operation of its X-ray subsystem prior to scanning the spine phantom. If the automatic system test succeeded, the system ran an auto QC. When both the system test and the QC passed the BMD, values were plotted along the y-axis of the QC plot.

3.6.3 Positioning aids

The QDR series included several aids to help the operator position patients for specific examinations. Positioning aids maintained the correct patient position during the acquisition of a scan. They included:

    a) Knee positioner
    b) Hip positioner

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a) Knee positioner

The large knee positioner was placed under the patient's lower legs. This allowed positioning of the femurs so that they were as close to 90 ° to the spine as possible to flatten the back. The operator rotated the pillow to one of three sides to adjust for the height of the patient and length of their legs.

b) Hip positioner

The hip positioner (foot restraint) maintained the correct position of the femur, and minimised movement during the acquisition of a hip scan. It was placed between the feet. The foot and leg of the side being examined were rotated inwards, with the foot against the fixture. A strap was placed around the foot to secure the correct position.

3.6.4 Hip scan

a) Performing the hip scan

Positioning the patient for a hip scan involved using the foot positioner. This positioner helped to align the patient's hip, and to hold the foot firmly in place. The foot positioner was placed under the patient's legs, and the centre of the positioner was aligned with the patient's midline. The patient's entire leg was rotated (from hip socket to foot) 25 ° inward, and the radial edge of the foot was placed against the triangle. The foot was flexed towards the ceiling. The velcro strap was adjusted to hold the foot in the correct position.

The femur was positioned to be parallel with the table edge to provide adequate space for the neck box. By abducting the leg from the midline of the body the femur was straightened. The

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cross hair of the laser was placed three inches below the greater trochanter, and 1 inch medial to the shaft of the femur. [To help identify the greater trochanter, the thumb was placed on the iliac crest, and the fingers were spread. With the little finger directed toward the knee, the greater trochanter was located under the little finger. The greater trochanter is at the same level than the symphysis pubis].

Once the patient and C-arm were positioned correctly, the scan begun. The image was displayed on the screen as the scan progressed. The scan was completed if positioned correctly, or repositioned if necessary.

After scanning both hips, analysis was performed.

b) Analysis of the hip scan

The "Analysis step" buttons maximised image quality and accuracy, preventing the need for re-scanning.

Defining the Region of Interest (ROI)

The global ROI referred to the defined boundaries of the image that was analysed. The ROI appeared on the image as a box and included the proximal femur in its entirety, the lesser trochanter, the top of the femoral head and the lateral side of the greater trochanter. The pelvis (ischium) was deleted to prevent it from interfering with the placement of the neck box.

Positioning the Neck Box

The neck box was positioned by using the "Auto position" button, or by manual positioning. The neck box covered the femoral neck and did not include any area of the ischium, femoral head, or the greater trochanter. The upper outer corner was positioned at the notch of the greater trochanter. The remaining three corners of the neck box remained in soft tissue.

3.6.5 AP lumbar spine

a) Performing the AP lumbar spine scan

The goal for positioning the patient on the table was to ensure that the spine was as straight as possible for the scan. The patient's lower legs were positioned on the knee positioner to ensure that the spine is kept flat. The patient's pelvis and shoulders were aligned straight on the table pad and centred to the marks on the table pad. The cross hair of the laser was positioned 1" to 2" below the iliac crest and centred in the mid-line of the patient.

An acceptable AP lumbar spine scan included the following:
- The scan started in the middle of L5.
- The iliac crest was evenly displayed in both lower corners of the image area.
- The AP lumbar spine was centred in the middle of the scan window.
- There were even amounts of soft tissue on each side of the spine.
- The scan stopped where ribs were attached to T12 (usually the middle of T12).

b) Analysis of the AP lumbar spine scan

The "Analysis step" buttons, located on the left side of the window, allowed the operator to proceed through each task maximising image quality and accuracy, and preventing the need for re-scanning.

Defining the Region of Interest (ROI)

The global ROI referred to the defined boundaries of the image that was analysed. The ROI appeared on the image as a box. A properly positioned global ROI included the spine centred

within the ROI and the top line of the ROI positioned between T12 and L1, and the bottom line of the ROI positioned between L4 and L5.

<u>Marking invertebral spaces</u>

Marking each invertebral space with a line allowed each individual vertebrae to be analysed separately. By choosing the "Invertebral lines" button, three lines appeared on the image that marked the spaces. Each line was evenly spaced between vertebrae in the space between L1/L2; L2/L3 and L3/L4.

<u>Point mode</u>

"Point mode" was used to mark invertebral spaces of the scoliotic space when the line between the vertebral bodies was not straight.

<u>Labelling the vertebral bodies</u>

The "Results" button automatically labelled the marked vertebral bodies. Vertebral labels were automatically assigned numbers starting at the top with L1 and down to L4.

3.7 Data recorded

The World Health Organization (WHO, 1994) defined osteoporosis by BMD at the hip, lumbar spine or forearm. The hip and spine are the two most commonly used skeletal sites.

Different bones and skeletal sites within bones have different ratios of cortical to trabecular bone. The vertebrae are composed of cortical to trabecular bone in a ratio of 25:75. This ratio is 50:50 in the femoral head (Clarke, 2008).

The difference between the test group and the control group on the spine, left hip and the right hip was evaluated on the BMD, T-score and the Z-score of specific site areas (Table 3.1).

Table 3.1   Scan sites and the area measured on each site

SCAN SITE	SITE AREA	CHART REFERENCES
Left Hip	Left Femoral Neck Bone Mineral Density	LFN BMD
	Left Femoral Neck T-score	LFN T-score
	Left Femoral Neck Z-score	LFN Z-score
	Left Total Hip BMD	LTH BMD
	Left Total Hip T-score	LTH T-score
	Left Total Hip Z-score	LTH Z-score
Right Hip	Right Femoral Neck Bone Mineral Density	RFN BMD
	Right Femoral Neck T-score	RFN T-score
	Right Femoral Neck Z-score	RFN Z-score
	Right Total Hip BMD	RTH BMD
	Right Total Hip T-score	RTH T-score
	Right Total Hip Z-score	RTH Z-score
AP Lumbar Spine	Bone Mineral Density	BMD
	T-score	T-score
	Z-score	Z-score

[LFN: Left Femoral Neck; LTH: Left Total Hip; RFN: Right Femoral Neck; RTH: Right Total Hip; BMD: Bone Mineral Density; T-score: Comparison of the patient's BMD to the mean peak bone mass]

3.8 Statistical analysis

Data from the data collection sheet was captured electronically in Microsoft Excel by the researcher, and double checked for accuracy. Any further analysis was done using SAS Version 9.2. The Shapiro-Wilk test was performed to determine if numerical variables followed a normal distribution or not. Descriptive statistics namely means and standard deviations (SD), or median and percentiles were calculated for numerical data, whilst frequencies and percentages were calculated for categorical data. To compare the test and control groups, analytical statistics were calculated, namely the independent T-test to test for differences between mean values, and the Mann Whitney U-test to test for differences between the median values. A significance level (α) of 0.05 was used, where $p \geq 0.05$ indicates no significant difference in the mean or median values of the two groups, and $p < 0.05$ indicates significant difference in the mean or median values of the two groups.

By interpreting the p-value from the Shapiro-Wilk test a conclusion could be made.

Interpretation of the p-value:
- If $p < 0.05$ then the distribution of the variable does not follow a normal distribution.
- If $p \geq 0.05$ then the distribution of the variable does follow a normal distribution.

To compare the age distribution between the test group and the control group, the Mann Whitney U-test was performed to determine the median and quartile values.

Interpretation of the p-value:
- If $p < 0.05$ then there is a significant difference between the median values in the two groups.
- If $p \geq 0.05$ then there is no significant difference between the median values in the two groups.

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<u>Interpretation of the 95% Confidence Interval (CI) of the difference between the means:</u>

- If 0 is included in the CI, then there is no significant difference in the mean values of the two groups.
- If 0 is excluded in the CI, then there is a significant difference in the mean values of the two groups.

To differentiate between the T-score and the Z-score, the subjects in the test group and the control group were divided into two subgroups according to their age: T-score values for subjects ≥50 years and Z-score values for subjects <50 years of age were used in the calculations. Analytical statistics, namely the Fisher's exact test, was used to compare percentages in the two groups. A significance level (a) of 0.05 was used.

<u>Interpretation of the p-value:</u>

- If $p < 0.05$ then there is a significant difference between the proportions of the two groups.
- If $p \geq 0.05$ then there is no significant difference between the proportions of the two groups.

3.9 Ethical aspects

3.9.1 Ethical clearance

The study only commenced after ethical approval was granted by the Ethics Committee of the University of the Free State (ECUFS 162/2012E). The project was presented to the Health Sciences Research Ethics Committee (HSREC) at the University of the Free State (UFS) as a sub-study of ECUFS162/2012E: Genetic polymorphisms in Black South Africans with Type 2 Diabetes Mellitus from the central Free State area (see Appendix A).

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A letter for permission to use the DXA data was obtained from the principle investigator of ECUFS162/2012E (see Appendix B).

3.9.2 Good clinical practice (GCP) / Quality assurance

All clinical work conducted during this research project was subjected to the Good Clinical Practice (GCP) guidelines. The Declaration of Helsinki's basic principle number 3 states that research should be conducted only by scientifically qualified people and under the supervision of adequately qualified employees involved (South African Good Clinical Practice Guidelines, 2006; World Medical Association Declaration of Helsinki, 2013). Fundamentally GCP requires oversight of the local ethics committee, verification of the investigator's qualifications, a study protocol, informed consent and essential documentation needed to undertake the study, monitoring, submission of reports and maintenance of records. By applying GCP guidelines in this research study provides public assurance that the rights, safety and well-being of the participants are protected, and that the research data are credible.

3.9.3 Confidentiality

Personal details of patients participating in this study were kept confidential as far as possible. At no time during the research was the identity of any of the patients revealed to persons that were not part of the research team. The parent data were delinked from all personal information, thus preventing disclosure of the patients' personal details, always ensuring patient confidentiality.

3.9.4 Consent

Patients signed a written consent form at the commencement of the parent study, which included permission to use data of the DXA analysis for research purposes.

CHAPTER 4

RESULTS

4.1 Introduction

This chapter represents the demographic, clinical, anthropometric and bone mineral density (BMD) measurements recorded for the 140 women receiving dual-energy X-ray absorptiometry (DXA) scans, and who participated in the research study. Ninety-one subjects were previously diagnosed with type 2 diabetes mellitus (T2DM), and acted as the test group, and 49 subjects had no diagnoses of T2DM, and acted as the control group. Diagnosis was based on haemoglobin A1c (HbA1c) %. Subjects where it was known that the HbA1c% was >6.5% were included in the test group, and subjects with a known HbA1c% <6% were included in the control group.

The test group and the control group were compared in the calculations for the T-score according to the guidelines of the World Health Organization (WHO): Normal ~ T-score at -1.0 and above; low bone mass (osteopenia) ~ T-score between -1.0 and -2.5; Osteoporosis ~ T-score at or below -2.5.

To accommodate the National Osteoporosis Foundation of South Africa (NOFSA, 2017), the test group and control groups were further divided into two subgroups according to age. Subjects ≥50 years were added in the calculations for T-score, and subjects <50 years were

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added in the calculations for Z-score: within the expected range for age~ Z-score above -2.0; below the expected range for age ~ Z-score below -2.0.

No medical and disease progression history were captured during this study.

Data from the data collection sheet were captured electronically in Microsoft Excel by the researcher, and double checked for accuracy. Any further analysis was done using SAS Version 9.2. The Shapiro-Wilk test was performed to determine if numerical variables (age, height, weight, body mass index (BMI), T-score, Z-score and BMD) followed a normal distribution, or not. Descriptive statistics namely means and standard deviations (SD), or median and percentiles were calculated for numerical data, and frequencies and percentages were calculated for categorical data (mean difference, 95% CI for mean difference, p-value, W-statistics). To compare the test and control groups, analytical statistics were calculated, namely the independent T-test to test for differences between mean values, and the Mann Whitney U-test to test for differences between the median values. A significance level (α) of 0.05 was used where $p \geq 0.05$ indicates no significant difference in the mean or median values of the two groups and $p < 0.05$ indicates significant difference in the mean or median values of the two groups. The Fisher's exact test was used to compare percentages of the T-score and Z-score in the two groups. A significance level (a) of 0.05 was used, where $p \geq 0.05$ indicates no significant difference between the proportions of the two groups, and $p < 0.05$ indicates a significant difference between the proportions of the two groups.

4.2 Demographic, clinical, anthropometric and bone density measurements

4.2.1 Demographic data

Considering that this was a retrospective study, the data of all the subjects (n=140) were used in the appropriate groups. The sample population consists of a test group (n=91) and a control group (n=49), as represented in Figure 4.1. The ratio of participants in the test and the control groups was 65% versus 35% respectively.
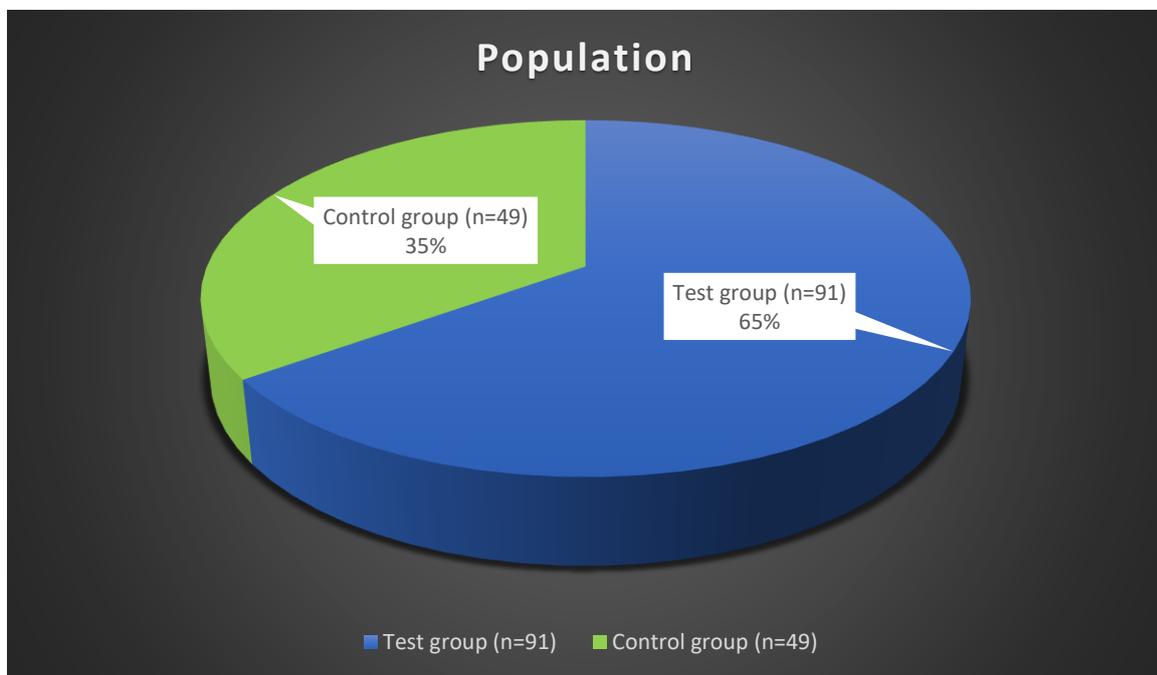
Figure 4.1: Schematic presentation of the sample distribution: Test group (n=91: T2DM) vs. control group (n=49: non-diabetics)

The demographic and anthropometric data of each subject are contained in Table 4.1 (test group) and Table 4.2 (control group).

Table 4.1: **The demographic and anthropometric data of the test group (n=91)**

SUBJECT NO.	AGE (years)	WEIGHT (kg)	HEIGHT (cm)	BMI (kg/m^2)
T1	53	64,2	153,5	27,2
T2	54	109,9	153,9	46,4
T3	53	117,4	156,8	47,8
T4	55	86,6	159,5	34
T5	55	108,2	162	41,2
T6	45	116,1	164,5	42,9
T7	52	83	158	33,2
T8	51	82	149	36,9
T9	43	120,6	165	44,3
T10	43	82	162,2	31,2
T11	58	104	152	45
T12	55	94,4	161,2	36,3
T13	41	106,4	167,6	37,9
T14	57	94,2	152,5	40,5
T15	52	114,9	156,7	46,8
T16	59	73,5	151,3	32,1
T17	54	80,7	153,2	34,4
T18	57	125,5	156,6	51,2
T19	52	53,4	141,5	26,7
T20	60	118,3	156	48,6
T21	40	53,1	152,9	22,7
T22	48	105,6	155	44
T23	46	87,6	160,5	34
T24	54	84,7	161	32,7
T25	57	122,9	158,6	48,9
T26	49	56,3	158,6	22,4
T27	51	96,8	165,7	35,3
T28	60	82,5	157,2	33,4
T29	59	87	155,5	36
T30	59	74,8	151,5	32,6
T31	46	52,3	155,9	21,5
T32	56	128,1	166	46,5
T33	60	121,4	162	46,3
T34	52	79,4	157,2	32,1
T35	50	60,9	152,3	26,3
T36	50	130,5	161,2	50,2
T37	51	101,4	151,1	44,4
T38	49	105,9	159,6	41,6
T39	56	84,8	172	28,7
T40	59	93,3	152	40,4
T41	58	92	153	39,3
T42	44	79,2	151,8	34,4
T43	48	82,8	158,1	33,1
T44	59	80	149,5	35,8

T45	53	101,9	160	39,8
T46	46	86,1	162,3	32,7
T47	41	60	153	25,6
T48	51	93,9	147	43,5
T49	57	81,5	153	34,8
T50	47	73,3	164	27,3
T51	60	88,8	150	39,5
T52	40	127,1	156,1	52,2
T53	52	66,5	159	26,3
T54	41	117,5	163	44,2
T55	60	121,2	156,7	49,4
T56	56	76,7	145	36,5
T57	44	52,3	148	23,9
T58	55	98,4	158	39,4
T59	54	121,5	169	42,5
T60	60	82	145	39
T61	49	96,2	157,7	38,7
T62	55	74,7	163,5	17,8
T63	45	83,8	163	31,5
T64	57	70,8	150	31,5
T65	54	78	154	32,9
T66	59	89,2	162,5	33,8
T67	49	84	159	33,2
T68	41	73,5	152	31,8
T69	49	60,5	152	26,2
T70	55	63	146	29,6
T71	41	69,5	164	25,8
T72	46	100,4	156	41,3
T73	47	66,9	161	25,7
T74	58	90,3	155,5	37,3
T75	60	73,7	151	32,3
T76	41	123,6	159	48,9
T77	47	89	155	37
T78	53	72,8	170	25,2
T79	46	69,8	156	28,7
T80	41	79,3	157	32,2
T81	44	98	160	38,3
T82	41	69,8	160	27,3
T83	56	59,9	155	24,9
T84	59	125,2	162	47,7
T85	44	82	162,2	31,2
T86	48	100,1	166	36,3
T87	49	74,5	158	29,8
T88	50	83,5	156	34,3
T89	51	79,9	154	33,7
T90	47	100,9	167	36,2
T91	42	75,4	149	34

Table 4.2: **The demographic and anthropometric data of the control group (n=49)**

SUBJECT NO.	AGE (years)	WEIGHT (kg)	HEIGHT (cm)	BMI (kg/m^2)
C1	47	67,2	160	26,2
C2	45	76,8	157	31,2
C3	49	85,5	157	34,7
C4	52	80,8	156	33,2
C5	58	83,2	167	29,8
C6	43	83,7	160	32,7
C7	59	51,7	148	23,6
C8	52	86,9	152	37,6
C9	52	67,5	146	31,7
C10	44	76,3	157	31
C11	53	93,7	154	39,5
C12	52	102,2	157	41,5
C13	53	94,5	166	34,3
C14	57	83,9	149	37,8
C15	46	105	180	32,4
C16	59	102	169	35,7
C17	44	73,1	157	29,7
C18	45	80,7	150	35,9
C19	41	73,9	161	28,5
C20	56	111,9	162	42,6
C21	46	97,3	152	42,1
C22	50	77,4	158	31
C23	51	66	150	29,3
C24	43	107,7	153	46
C25	58	79,2	158	31,7
C26	53	85,9	151	37,7
C27	43	75,3	158	30,2
C28	54	87,2	162	33,2
C29	41	109,8	151,5	47,8
C30	52	81,3	170	28,1
C31	41	91,1	154	38,4
C32	45	88,9	157	36,1
C33	45	72,2	163,5	27
C34	43	65,9	161	25,4
C35	40	52,2	161	20,1
C36	49	112,7	163	42,4
C37	49	67	164	24,9
C38	41	59,7	149	26,9
C39	41	114	162	43,4
C40	46	96,6	156	39,7
C41	49	71,1	154	30
C42	48	69,5	154	29,3
C43	57	76,4	153	32,6
C44	59	109,6	164	40,7

C45	53	95,8	163	36,1
C46	54	60,6	162	23,1
C47	59	99,5	151,5	43,4
C48	46	75,8	166	27,5
C49	56	85,7	164	31,9

The Shapiro-Wild test was used to investigate the normality of the numerical demographic variables (age, weight, height and BMI) within the two groups.

Table 4.3: Test of normality for the age (years) distribution within the test group, control group and the total group

Group	W-statistics	p-value
Test (n=91)	Wdm: 0.943313	0.00006
Control (n=49)	Wc: 0.941292	0.0166
Total (n=140)	Wtot: 0.946445	< 0.00001

Wdm: W-statistics of DM group; Wc: W-statistics of control group; W-statistics of the total group.

According to the Shapiro-Wilk test of normality, the distribution of the age of the participants for the test group, control group and the total group did not follow a normal distribution (Wdm = 0.943; p = 0.0006: Wc = 0.941; p = 0.0166: Wtot = 0.946; p = <0.000 respectively), where p < 0.05 for all groups. Since the distribution of the age of the participants was skewed, the median and inter-quartile range were reported on, and the Mann Whitney U-test was used to compare median values in the two groups.

The age for subjects included in the test group varied, from the youngest being 40 years, and the oldest 60 years of age, as seen in Table 4.4 below. Subjects included in the control group varied from the youngest being 40 years, and the oldest 59 years of age. The median age of the test group was 52 years, and the median age for the control group was 49 years. There was no significant difference between the median age values of the two groups (p = 0.0954).

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Table 4.4: Descriptive statistics of the age (years) of the test group and the control group

Group	Median	Inter-Quartile Range (IQR)	Minimum	Maximum	p-value
Test (n=91)	52.00	46.00 – 56.00	40.00	60.00	0.0954
Control (n=49)	49.00	45.00 – 53.00	40.00	59.00	

4.2.2 Clinical data

HbA1c% levels of all the subjects for the parent study were tested according to the guidelines provided by Amod *et al.* (SEMDSA, 2017). These guidelines require HbA1c% ≥6.5% to be diagnosed with diabetes mellitus (DM). The subjects in the test group were all known to be >6.5% HbA1c%. The guidelines indicate that the cut-point of HbA1c% is 6.0% to 6.4%. The subjects HbA1c% in the control group was known to be <6%.

Unfortunately, no data were collected regarding medical history, e.g. insulin use, time since diagnosis, possible previous fractures, menopause, alcohol intake, smoking history, etc.

4.2.3 Anthropometric data

The Shapiro-Wilk test of normality was used to investigate the distribution of the variables (weight, height and BMI) in the test group, control group and the total group, and the results are tabulated in Table 4.5 below.

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Table 4.5: Test of normality for the weight, height and BMI distribution within the test group, control group and the total group

Variable	Group	W-statistics	p-value
Weight (kg)	Test (n=91)	0.96466	0.0143
	Control (n=49)	0.976239	0.4197
	Total (n=140)	0.974267	0.0095
Height (cm)	Test (n=91)	0.996854	0.9992
	Control (n=49)	0.965849	0.1647
	Total (n=140)	0.990386	0.4522
BMI (kg/m^2)	Test (n=91)	0.981975	0.2406
	Control (n=49)	0.984408	0.7564
	Total (n=140)	0.985931	0.1635

According to the Shapiro-Wilk test of normality, the weight distribution of the control group followed a normal distribution, whilst the weight distribution for the test group and the total group was skewed. Therefore, the median and IQR were reported on, and the Mann Whitney U-test was used to compare the median values of the test and control groups.

The distribution for the height and the BMI was both normal for the test group, control group and the total group ($p > 0.05$). The mean and SD were reported on, and the independent T-test was used to compare the mean values of the test and control groups.

The descriptive statistics of the weight are captured in Table 4.6. The median weight of the test group was 84.7kg, and the median weight of the control group was 83.2kg. There was no significant difference between the mean weight values of the two groups (p = 0.2107).

Table 4.6: Descriptive statistics of the weight (kg) of the test group and the control group

Group	Median	(IQR)	Minimum	Maximum	p-value
Test (n=91)	84.70	74.50 – 101.90	52.3	130.5	0.2107
Control (n=49)	83.20	73.10 – 95.80	51.7	114.0	

The descriptive statistics regarding height and BMI for the test and control groups are tabulated in Table 4.7. The mean height of the test group was 157.07cm, and the mean height of the control group was 158.17cm. There was no significant difference between the mean height values of the two groups (p = 0.3191). The mean BMI of the test group was 35.81kg/m^2, and the mean BMI of the control group was slightly lower at 33.58kg/m^2. There was no significant difference between the mean BMI values of the two groups (p = 0.0882).

Table 4.7: Descriptive statistics of the height and BMI of the test group and the control group

Variable	Group	Mean	SD	Minimum	Maximum	p-value
Height (cm)	Test (n=91)	157.07	5.97	141.5	172.0	0.3191
	Control (n=49)	158.17	6.63	146	180	
BMI (kg/m^2)	Test (n=91)	35.81	7.78	17.8	52.2	0.0882
	Control (n=49)	33.58	6.38	20.1	47.8	

The BMI of the participants were classified into four classes, namely obese (BMI ≥ 30 kg/m^2), overweight (BMI between 25 kg/m^2 and 29.9 kg/m^2), normal (BMI between 18.6 kg/m^2 to 24.9 kg/m^2) and underweight (BMI ≤ 18.5 kg/m^2). The BMI distribution of the test group and the control group is demonstrated in Figure 4.2. This figure indicates that 77% of the test group was obese, and 16% was overweight. The control group consisted of 69% obese participants and 27% overweight participants.
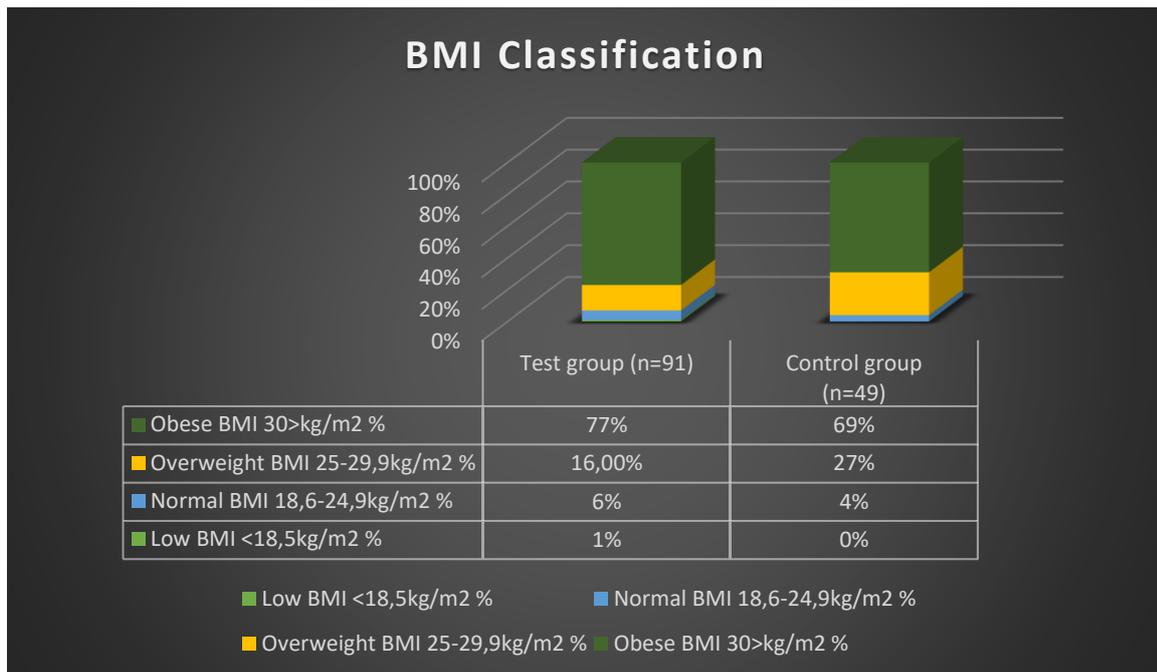
Figure 4.2: Health distribution according to BMI classification

4.2.4 Bone density measurements

Areal BMD is expressed in absolute terms of grams of mineral per square centimeter scanned (g/cm^2).

Bone density of the left hip and the right hip were evaluated on the following parameters: the femoral neck BMD, total hip BMD, femoral neck T-score, femoral neck Z-score, total hip T-score, and total hip Z-score.

Bone density at the lumbar spine was evaluated from the first to the fourth lumbar vertebrae on the test group as well as the control group. The regions of interest were: Anteroposterior (AP) Spine (L1-L4) BMD, AP Spine (L1-L4) T-score, and AP Spine (L1-L4) Z-score.

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The Shapiro-Wilk test of normality was used to investigate the distribution of the BMD at each site in the test group, control group and the total group, and the results are tabulated in Table 4.8 below. Normality was rejected if the p-value was p <0.05.

Table 4.8: Test of normality for the BMD distribution of each site within the test group, control group and the total group

Site	Group	W-statistics	p-value
LFN	Test (n=91)	0.970077	0.0342
	Control (n=49)	0.966339	0.1724
	Total (n=140)	0.971483	0.0050
RFN	Test (n=91)	0.980695	0.1961
	Control (n=49)	0.982625	0.6787
	Total (n=140)	0.986476	0.1865
LTH	Test (n=91)	0.985695	0.4230
	Control (n=49)	0.971935	0.2886
	Total (n=140)	0.987622	0.2448
RTH	Test (n=91)	0.98822	0.5927
	Control (n=49)	0.978289	0.4958
	Total (n=140)	0.993333	0.7602
AP Spine	Test (n=91)	0.983904	0.3248
	Control (n=49)	0.972636	0.3073
	Total (n=149)	0.993411	0.7684

[LFN: Left Femoral Neck; RFN: Right Femoral Neck; LTH: Left Total Hip; RTH: Right Total Hip]

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According to the Shapiro-Wilk test of normality, the BMD distribution at each site followed a normal distribution, except for the BMD distribution of the LFN of the test group and the total group, which was skewed. The mean and SD of the BMD at each site were reported on. The mean differences in BMD and 95% CI for the mean difference in BMD between the test and control groups for each site were calculated. The independent T-test was used to compare the mean BMD values of the test and control groups.

The descriptive statistics of the BMD of the different sites are summarised in Table 4.9. The mean LFN BMD of the test group was 0.8725 g/cm^2, and the mean LFN BMD of the control group was 0.8787g/cm^2. The mean RFN BMD of the test group was 0.8839 g/cm^2, and the mean RFN BMD of the control group was 0.8783/cm^2. The mean LTH BMD of the test group was 1.0603 g/cm^2, whilst the mean LTH BMD of the control group was 1.0443 /cm^2. The mean RTH BMD of the test group was 1.0616 g/cm2, and the mean RTH BMD of the control group was 1.0240 /cm^2. The mean AP Spine BMD of the test group was 0.9811 g/cm^2, whilst the mean AP Spine BMD of the control group was 0.9948/cm^2. To conclude, none of the mean BMD values of the different sites between the test group and the control group showed a significant difference.

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Table 4.9: Descriptive statistics of the BMD of the test group and the control group

Variable	TEST GROUP (n=91)				CONTROL GROUP (n=49)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
LFN BMD (g/cm^2)	0.8725	0.1612	0.4370	1.3730	0.8787	0.1696	0.5450	1.3170
RFN BMD (g/cm^2)	0.8839	0.1571	.05750	1.3200	0.8783	0.1589	0.5600	1.2410
LTH BMD (g/cm^2)	1.0603	0.1491	0.7450	1.3800	1.0443	0.1626	0.7230	1.4210
RTH BMD (g/cm^2)	1.0616	0.1444	0.7260	1.4160	1.0240	0.1612	0.6990	1.4940
AP Spine BMD (g/cm^2)	0.9811	0.1536	0.6630	1.3590	0.9948	0.1516	0.5340	1.2820

[LFN: Left Femoral Neck; RFN: Right Femoral Neck; LTH: Left Total Hip; RTH: Right Total Hip; BMD: Bone Mineral Density]

Figure 4.3 below is a presentation of only the mean BMD values of the different sites for the test group and the control group.
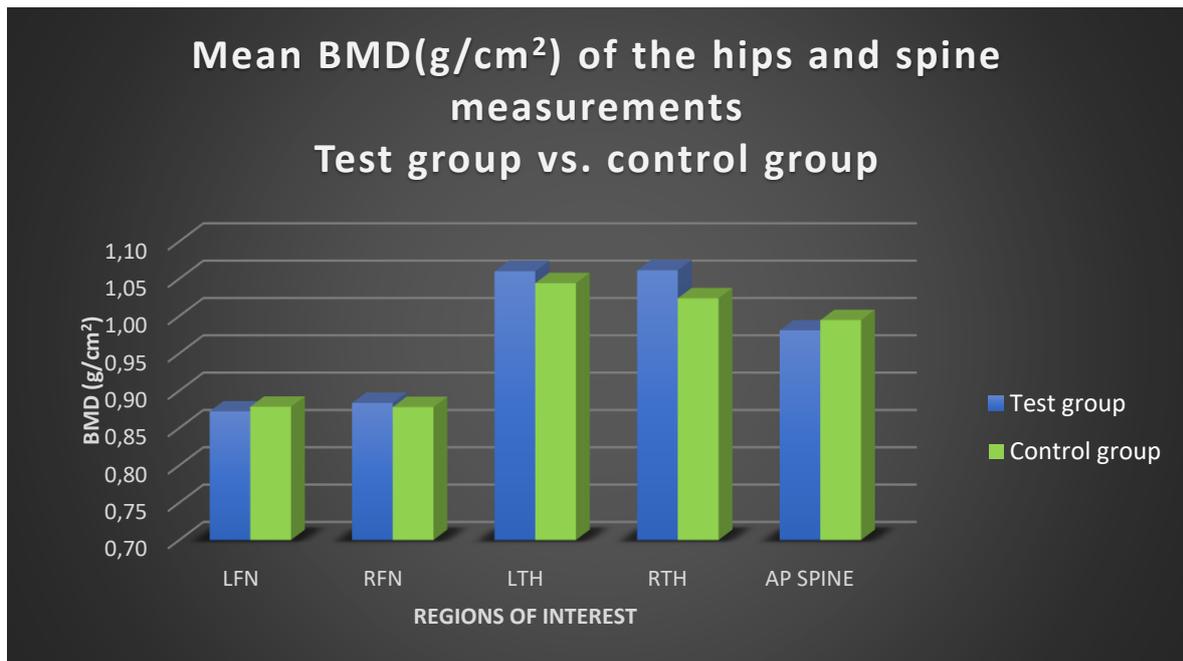
[BMD: Bone mineral density; g/cm^2: gram per centimeter square; LFN: Left Femoral Neck; RFN: Right Femoral Neck; LTH: Left Total Hip; RTH: Right Total Hip]

Figure 4.3: Mean BMD values of the hips and AP spine measurements for the test group and the control group

The mean differences in BMD (test – control) and 95% CI for the mean difference in BMD between the test and control groups for each site are shown in Table 4.10 below. The independent T-test was used to compare the mean BMD values of the test and control groups.

Table 4.10: Difference in BMD between the test group and the control group

Variable	Mean difference	95% CI for mean difference	p-value
LFN BMD (g/cm^2)	0.0061	-0.0514; 0.0636	0.8336
RFN BMD (g/cm^2)	0.0056	-0.0609; 0.0496	0.8408
LTH BMD (g/cm^2)	-0.0376	-0.0699; 0.0379	0.1606
RTH BMD (g/cm^2)	-0.0376	-0.0903; 0.0151	0.1606
AP Spine BMD (g/cm^2)	0.0137	-0.0399; 0.0673	0.6140

[BMD: Bone Mineral Density; g/cm^2; LFN: Left Femoral Neck; LTH: Left Total Hip; RFN: Right Femoral Neck; RTH: Right Total Hip]

As seen in Table 4.10, none of the mean differences in the BMD values between the test group and the control group of the different sites showed a significant difference (all p > 0.05).

The T-score represents the SD by which the BMD differs from the mean BMD of a young adult reference population of the same ethnicity and sex (Lorente-Ramos, 2011). Hough *et al*. (2010) explained that, when SD units are used in relation to the young adult population, this is referred to as the T-scores. The classification of BMD is normal when the T-score is at -1.0 and above; osteopenia (low bone mass) occurs when the T-score is between -1.0 and -2.5; and osteoporosis is present when the T-score is ≤-2.5 (WHO, 1994).

The Shapiro-Wilk test of normality was used to investigate the distribution of the T-score at each site in the test group, control group and the total group, and the results are tabulated in Table 4.11 below.

Table 4.11: Test of normality for the T-score distribution for the test group, control group and the total group: Overall T-score approach

Site	Group	W-statistics	p-value
LFN	Test (n=91)	0.968561	0.0267
	Control (n=49)	0.965787	0.1637
	Total (n=140)	0.970477	0.0040
RFN	Test (n=91)	0.980157	0.1797
	Control (n=49)	0.982461	0.6715
	Total (n=140)	0.986031	0.1675
LTH	Test (n=91)	0.985347	0.4023
	Control (n=49)	0.970641	0.2568
	Total (n=140)	0.986809	0.2020
RTH	Test (n=91)	0.987934	0.5720
	Control (n=49)	0.980083	0.5687
	Total (n=140)	0.992822	0.7054
AP Spine	Test (n=91)	0.983931	0.3261
	Control (n=49)	0.971738	0.2836
	Total (n=149)	0.992848	0.7082

[LFN: Left Femoral Neck; RFN: Right Femoral Neck; LTH: Left Total Hip; RTH: Right Total Hip]

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According to the Shapiro-Wilk test of normality the T-score distribution at each site followed a normal distribution, except for the T-score distribution of the LFN of the test group and the total group, which was skewed. The mean and SD of the T-score at each site were reported on. The mean differences in T-scores and 95% CI for the mean difference in T-scores between the test and control groups for each site were calculated. The independent T-test was used to compare the mean T-score values of the test and control groups.

Descriptive statistics of the T-score of the different sites between the test group and the control group are summarised in Table 4.12. The mean LFN T-score of the test group was 0.2077, and the mean LFN T-score of the control group was 0.2633. The mean RFN T-score of the test group was 0.3143, and the mean RFN T-score of the control group was 0.2673. The mean LTH T-score of the test group was 0.9692, compared to the mean LTH T-score of the control group, which was 0.8449. The mean RTH T-score of the test group was 0.9802, and the mean RTH T-score of the control group was 0.6816. The mean AP Spine T-score of the test group was -0.600, whilst the mean AP Spine T-score of the control group was -0.4796. To conclude, none of the mean T-score values of the different sites between the test group and the control group showed a significant difference.

Table 4.12: Descriptive statistics of the T-score of the test group and the control group

Variable	TEST GROUP (n=91)				CONTROL GROUP (n=49)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
LFN T-score	0.2077	1.4542	-3.7000	4.7000	0.2633	1.5275	-2.7000	4.2000
RFN T-score	0.3143	1.4135	-2.5000	4.2000	0.2673	1.4273	-2.6000	3.5000
LTH T-score	0.9692	1.2267	-1.6000	3.6000	0.8449	1.3290	-1.8000	3.9000
RTH T-score	0.9802	1.1839	-1.8000	3.9000	0.6816	1.3244	-2.0000	4.5000
AP Spine	-0.600	1.3943	-3.5000	2.8000	-0.4796	1.3847	-4.7000	2.1000

[LFN: Left Femoral Neck; RFN: Right Femoral Neck; LTH: Left Total Hip; RTH: Right Total Hip; T-score: Comparison of the patient's BMD to the mean PBM; g/cm^2: gram per centimeter square]

Figure 4.4 below is a presentation of the mean T-score of the different sites for the test group and the control group.
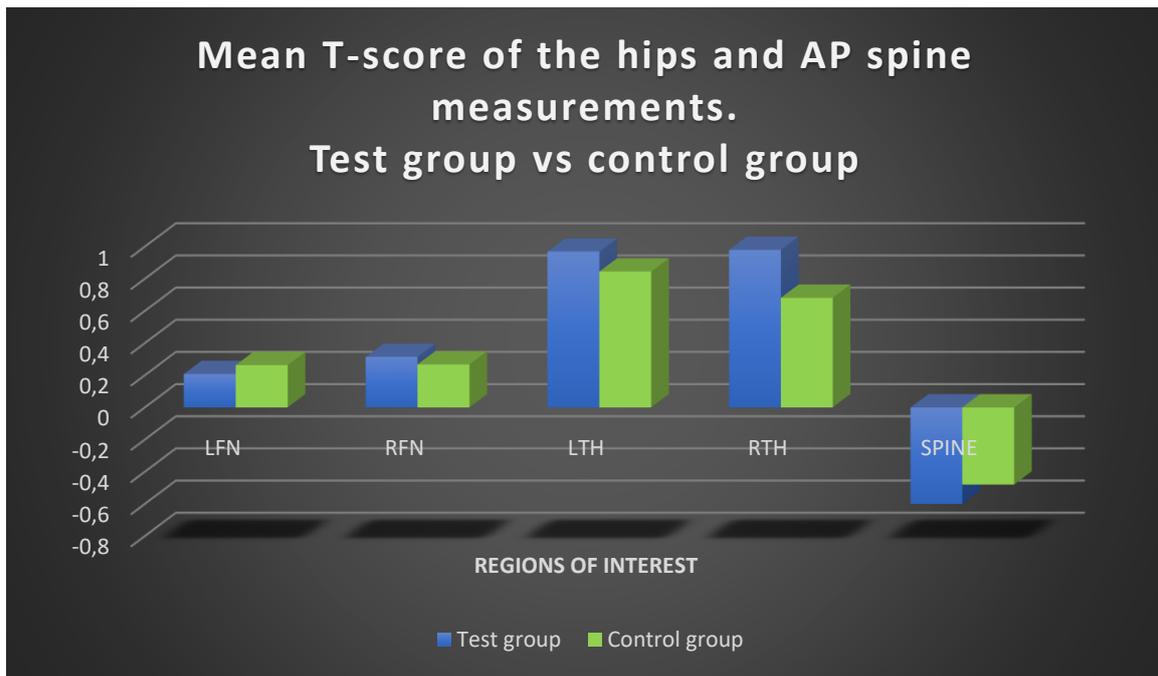
[LFN: Left Femoral Neck; RFN: Right Femoral Neck; LTH: Left Total Hip; RTH: Right Total Hip]

Figure 4.4: Mean T-score of the hips and AP spine measurements for the test group and the control group

The mean differences in T-score (test – control) and 95% CI for the mean difference in T-score between the test and control groups for each site are shown in Table 4.13 below. The independent T-test was used to compare the mean T-score values of the test and control groups.

Table 4.13: Difference in T-score between the test group and the control group

Variable	Mean difference	95% CI for mean difference	p-value
LFN	0.0556	-0.4630; 0.5741	0.8325
RFN	-0.0469	-0.5439; 0.4500	0.8521
LTH	-0.1243	-0.5669; 0.3183	0.5795
RTH	-0.2986	-0.7311; 0.1340	0.1745
AP Spine	0.1204	-0.3669; 0.6078	0.6259

[LFN: Left Femoral Neck; LTH: Left Total Hip; RFN: Right Femoral neck; RTH: Right total hip; T-score – comparison of the patient's BMD to the mean peak bone mass]

As seen in Table 4.13, none of the mean differences in the BMD values between the test group and the control group of the different sites showed a significant difference (all $p > 0.05$).

The T-score is an expression of SDs of current BMD relative to young adult BMD. The T-score is primarily used to diagnose osteoporosis in postmenopausal women. T-score was defined according to WHO (1994) criteria for osteopenia: T-score of <-1 and >-2.5 and osteoporosis: T-score of ≤-2.5.

The prevalence of osteoporosis and osteopenia in the test group and the control group is graphically presented in Figure 4.5.
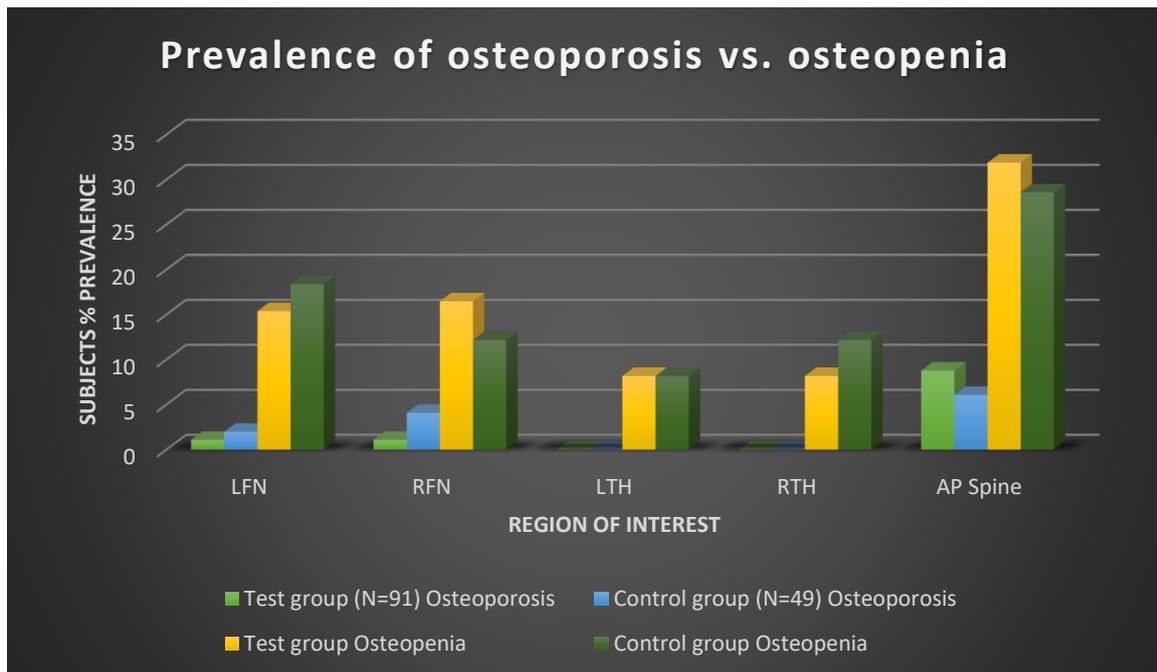
[LFN: Left Femoral Neck; RFN: Right Femoral Neck; LTH: Left Total Hip; RTH: Right Total Hip]

Figure 4.5: Prevalence of osteoporosis vs. osteopenia in the test group and the control group in different regions

Taking into consideration that osteoporosis is a major health disease, Figure 4.5 indicates that osteopenia is a higher risk factor than osteoporosis for the middle-aged Black South African population. Osteopenia was more prevalent than osteoporosis in the test group as well as in the control group. The femoral neck and the AP spine were more susceptible for bone weakening, which indicate that the quality of bone would deteriorate more in the femoral neck and spine regions. It seems that subjects diagnosed with T2DM have similar risks for osteopenia than subjects not diagnosed with T2DM.

With regards to the NOFSA (2017) guidelines the results of the T-score and Z-score classification for osteoporosis according to age were compared between the test group and the control group. The following data were captured after dividing the test group and the control group according to age: T-score ≥50 years and Z-score <50 years.

The results for women ≥50 years according to the T-score as recommended by the NOFSA (2017) guidelines are tabulated in Table 4.14.

Table 4.14: Diagnosis for women ≥50 years between the test group and the control group according to T-score

Variable	Test group (n=54)			Control group (n=23)			P-value
	Normal T ≥-1	Osteopenia T > -1 ≤ 2.5	Osteoporosis T > -2.5	Normal T ≥-1	Osteopenia T > -1 ≤ 2.5	Osteoporosis T > -2.5	
LFN	42 77.78%	11 20.37%	1 1.85%	19 82.61%	3 13.04%	1 4.35%	0.5685
RFN	42 77.78%	12 22.22%	0	19 82.61%	2 8.70%	2 8.70%	**0.0491**
LTH	52 96.30%	2 3.70%	0	22 95.65%	1 4.35%	0	1.0000
RTH	51 97.44%	3 5.56%	0	20 86.96%	3 13.04%	0	0.3557
AP SPINE	31 57.41%	18 33.33%	5 9.26%	13 56.52%	9 39.13%	1 4.35%	0.7421

[LFN: Left Femoral Neck; RFN: Right Femoral Neck; LTH: Left Total Hip; RTH: Right Total Hip]

The Fisher's Exact Test showed no significant difference between the proportions of the different groups, except at the RFN, where $p < 0.05$.

NOFSA (2017) recommend diagnosis for women <50 years according to the Z-score, as seen in Table 4.15.

Table 4.15: Diagnosis for women < 50 years between the test group and the control group according to the Z-score

Variable	Test group (n=37)		Control group (n=26)		P-value
	Normal: Z-score ≥-2	Below expected range: Z-score < -2	Normal: Z-score > -2	Below expected range: Z-score < -2	
LFN	36 97.30%	1 2.70%	26 100.%	0	1.000
RFN	36 97.30%	1 2.70%	26 100.%	0	1.000
LTH	37 100%	0	26 100%	0	
RTH	37 100%	0	26 100%	0	
AP SPINE	31 83.78%	6 16.22%	21 80.77%	5 19.23%	0.7500

[LFN: Left Femoral Neck; RFN: Right Femoral Neck; LTH: Left Total Hip; RTH: Right Total Hip]

The results in Table 4.15 show no significant difference between the proportions of the different groups (p≥0.05) when analysed with Fisher's Exact Test.

5.1 Introduction

Diabetes Mellitus (DM) is a metabolic disorder with heterogeneous aetiologies which is characterised by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. These include processes that include or destroy the function of the pancreatic beta cells, with consequent insulin deficiency, and others that result in resistance to insulin actions (insulin resistance/insulin insensitivity) (Amod *et al*., 2017).

Osteoporosis is defined as a combination of reduced bone mass and altered bone quality, with micro-architectural abnormalities, resulting in decreased bone strength with an increased risk of fractures. Based on the present definition, both bone density and quality, which encompass the structural and material properties of bone, are important factors in the determination of bone strength (Jackuliak & Payer, 2014). Classical criteria by the National Osteoporosis Foundation of South Africa (NOFSA, 2017) were used in this study to classify conditions of normal BMD, osteopenia and osteoporosis based on the T-score for subjects ≥50 years and Z-score for subjects <50 years.

The risk factors for the onset of osteoporosis are many and different from each other. Some of them cannot be modified, such as age, hereditary diseases and endocrine diseases. Others are modifiable, so that prevention is an advisable tool to reduce the incidence of osteoporosis. Among preventive tools, physical activity, dietary intake, obesity, etc. are certainly valid instruments of prevention (Castrogiovanni *et al.,* 2016). This highlight the importance of osteoporosis education and awareness programs. Taking the above mentioned into consideration there is numerous actions that can be taken to prevent or limit the incidence osteoporosis therefore highlighting the importance of education and awareness programs.

DM adversely affects the skeleton and is associated with an increased risk of osteoporosis and fragility fractures. Type 1 diabetes mellitus (T1DM) affects the skeleton more severely than type 2 diabetes mellitus (T2DM), probably because of the lack of the bone anabolic actions of insulin and other pancreatic hormones. Bone mass can remain high in patients with T2DM, but it does not protect against fractures, as bone quality is impaired. Increased awareness of osteoporosis is needed in view of the growing and aging population of patients with DM (Hamann *et al*., 2012).

One important way to describe bone quality is to assess its micro-architecture. Bone micro-architecture contributes to the mechanical strength of bone and, thus, to its ability to withstand fractures. Bone loss is often accompanied by deterioration in bone architecture, resulting from a decrease in the number of trabeculae of cancellous bone, increased intertrabecular distances, and a loss of trabecular connectivity. In addition, a reduction in the thickness of cortical bone and an increase in its porosity of trabecular bone can result in fragility of the femoral neck. Osteoporotic bone is, hence, called "porous" (Jackuliak & Payer, 2014). Quantitative computed tomography (QCT) and high-resolution peripheral QCT (HR-pQCT) are also commonly used when assessing bone mass and structure in patients with osteoporosis. Amstrup *et al.* (2016) stated that different information on bone quality is obtained, depending on the imaging technique and measuring site. They suggest that the various techniques measure different characteristics of the bone and may therefore be used in conjunction with dual-energy X-ray absorptiometry (DXA) for imaging in clinical practice.

Bone has trabecular and cortical components. Trabecular bone predominates in vertebrae and the proximal femur, whereas cortical bone is prominent in the long bone shafts. Trabecular remodelling occurs at a rate of approximately 25% per year, while the cortical rate is approximately 3% per year. Thus, changes in bone mineral density (BMD) occur more quickly and have greater clinical implications in trabecular bone, which is consistent with the prevalence of vertebral and femoral fractures in patients with osteoporosis (Lash *et al*., 2009).

Numerous studies have been done on BMD and metabolism in patients with DM and coexistence of this disease and osteoporosis, but controversies still exist. The most important aim of this study was to compare the BMD of T2DM patients with those of non-diabetic middle-aged Black South African women. The results of this study indicated that there was no significant change in the BMD of middle-aged Black South-African women diagnosed with T2DM compared to non-diabetic women.

There was little information to be found for this specific ethnic group. Studies were done on Black South African women and diabetes, and on Black South African women with osteoporosis. There was no information to be found on the effect of T2DM on BMD of Black South African women (Goedecke e*t al.,* 2017: Paruk *et al.,* 2017). The aim of their study was to ensure that existing treatments could be used in the best possible way should the study show a relationship between T2DM and BMD.

5.2 Demographic, anthropometric and bone density measurements

5.2.1 Demographic data

Black South African women are not only undiagnosed and unaware, but also unable to access the correct education and health services, since Africa lacks sufficient resources and infrastructure to effectively deliver such services. Consequently, most patients are diagnosed

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after presenting complications (Issaka e*t al*., 2018). The data collected for this study were data of Black South African women to address the lack of information available for this population.

Data received for this study were recorded for the parent study previously done: Genetic polymorphisms in Black South Africans with T2DM from the central Free State area (ECUFS No 162/2012). DXA was done on 140 participants. Sixty-five per cent of these participants were diagnosed with T2DM and acted as the test group, and thirty-five per cent were volunteers with no diagnoses of T2DM, who acted as the control group (see Figure 4.1). Women between the age ≥ 40 and ≤ 60 years were included in this study.

According to Goedecke e*t al.* (2017), due to the ageing population, increasing urbanisation and the associated lifestyle changes, Sub-Saharan Africa (SSA) has the highest projected rates of increase in T2DM over the next 25 years. The Black South African population has a strong believe in their culture. Cultural perceptions regarding weight loss and limited financial resources are the major limitations to the management of T2DM. Prevention is vital; therefore, this specific population group was included.

Sotunde e*t al.* (2015) examined the association between body composition (fat mass, lean mass and body mass index {BMI}) and bone health (BMD and fracture risk) in 189 healthy postmenopausal urban Black South African women aged ≥ 43 years. They conclude that lean mass and fat mass were positively associated with femoral neck, spine and hip BMDs, and negatively associated with fracture risk. Unfortunately, only 6% of the participants in the test group had a normal BMI, and 4% in the control group had a normal BMI, which was insufficient data to examine the association between BMI and BMD for this study (see Figure 4.2).

A study done in India by Kumar e*t al.* (2015), included a test group (n=41) with T2DM and mean age 51.9, and a healthy control group (n=41) without T2DM and mean age 51.4 years. All the participants were females between 40 and 60 years of age. The subjects in the test

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group received treatment with oral hypoglycaemic agents for a period of at least three consecutive years. The mean BMI were comparable between both groups. They found no significant difference between the two groups in the BMD and T-scores at the femoral neck and lumbar spine among T2DM patients receiving treatment and controls. In their study the presence of normal BMD (9/41 vs 8/41), osteopenia 16/41 vs 18/41) and osteoporosis (16/41 vs 15/41) was comparable between the test and control groups.

Thakur and Dash (2018) conducted a study in western Odisha which included 60 diabetic and 60 non-diabetic subjects between 40 and 65 years of age. They found no significant difference of the BMD in both groups. On further analysis, the incidence of osteoporosis was higher among the diabetic subjects, whereas incidence of osteopenia was higher among non-diabetic subjects. Our study supports the findings of Thakur and Dash (2018) for we have also demonstrated no significant difference in the BMD of both groups. However, osteopenia was more prevalent that osteoporosis in both groups.

The median age for the test group in this study was 52 years, with the youngest being 40 years and the oldest 60 years of age. The median age for the control group was 49 years, with the youngest being 40 years and the oldest 59 years of age. Although the normal distribution of the patients' age of both the test group and the control group was skewed according to the Shapiro-Wilk test, the Mann Whitney U-test showed no significant difference between the median values between the two groups. It is therefore appropriate to assume that the age variable between the two groups are statistically similar (see Table 4.3). As the distribution of age was skewed, the BMD on different age intervals could not be determined.

Compston (2017) performed a meta-analysis which only included postmenopausal women. She found strong evidence that T2DM is associated with an increased risk of hip fracture, and weaker evidence of an increased risk of wrist, spine and foot fractures. Although the risk for fracture was not investigated in our study there was evidence that the prevalence of

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osteopenia was higher in the AP Spine, lower in the femoral neck of both hips and the lowest in the total hip regions.

5.2.2 Anthropometric data

Due to the weight limit of the DXA (HOLOGIC W QDR, USA, model S/N70494) the participants weighed ≤ 130 kg. Statistical analysis shows no notable difference in weight between the two groups, although the maximum weight in the test group was slightly higher (see Table 4.6). The control group shows a slight increase in the minimum height as well as the maximum height when compared to the test group, but the mean between the two groups is similar (see Table 4.7). With similar weight and height, the BMI shows no significant difference between the two groups.

Thakur and Dash (2018) found no significant difference in the BMI of the diabetic group vs. the non-diabetic group and stated that the study did not signify BMI as a predictor for BMD.

A study done by Adeniyi *et al.* (2015) investigated the prevalence and the determinants of overall obesity among patients with T2DM in rural and semi-urban areas surrounding the town of Mthatha, South Africa. They found that 60.2% of their sample population were defined as obese. After calculations of the BMI for this study group, it seems that obesity is a problem in the middle-aged Black South African women population. According to Figure 4.2, this study indicates that 77% of the test group was obese, and 16% was overweight. The control group consists of 69% obese participants and 27% overweight participants.

A study done by Micklesfield *et al.* (2018) highlight the need for more tailored intervention to slow down the obesity epidemic in middle-aged Black South African women. Dickie *et al.*

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(2016) conclude that both physical activity and cardiorespiratory fitness were associated with reduced total and central fat mass and reduced T2DM.

Johnson and Mincey (2016) stated that obesity continues to be a public health concern across the globe. They mentioned in their article that obesity has increased worldwide over the past 2 decades. Chooi e*t al.* (2019) found that the worldwide prevalence of overweight and obesity has doubled since 1980. They stated that obesity rates have increased in all ages and both sexes irrespective of geographical locality, ethnicity or socio-economic status, although the prevalence of obesity is generally greater in older persons and women.

5.2.3 Bone density measurements

5.2.3.1 Bone mineral density (BMD)

A cross-sectional study in South Karnataka, done by Asokan *et al*. (2017) was conducted on 150 patients between 40 and 70 years of age which included 75 T2DM subjects and 75 nondiabetic subjects. No significant difference was observed in BMD of both the groups. This study shows similar results to the study conducted by Asokan *et al.* (2017) regarding the BMD between T2DM subjects and non-diabetic subjects.

Investigation shows that the left femoral neck (LFN) of the test group (p = 0.0342) and the total group (p = 0.005) did not follow a normal distribution (see Table 4.8), and therefore the mean differences and 95% confidence interval (CI) were investigated (see Table 4.10). None of the mean BMD values of the different sites between the two groups showed a significant difference.

Bayani e*t al.* (2016) investigated the relationship between osteoporosis and T2DM in elderly people. They examined 1 151 elderly people in Amirkola, northern Iran, of which 31.45%

had T2DM. Their results demonstrated that the mean lumbar and femur BMD in older people with T2DM were higher than in people without DM.

In an article compiled by Walsh and Vilaca (2017), they articulate that T2DM is associated with higher BMD, but increased overall and hip fracture risk. They postulate that it is possible that, even if BMD increases in response to obesity, the capacity for increase is limited and eventually the load-to-strength ratio rises far enough to cause fracture in low-trauma injuries. Unfortunately, no patient history of possible fractures was recorded in this study.

5.2.3.2 T-score

Osteopenia in the anteroposterior (AP) spine was more prevalent than in any other region. The overall prevalence of osteopenia is higher than the prevalence of osteoporosis. It seems that the femoral neck and AP spine are more prone to low bone mass than the total hip region (see Figure 4.5). Other than the study done by Asokan *et al.* (2017), this study indicates that the incidence of osteopenia was higher compared to the incidence of osteoporosis among middle-aged Black South African women regardless of their T2DM status. Asokan *et al.* (2017) found that the incidence of osteoporosis was higher among diabetic subjects, whereas incidence of osteopenia was higher among non-diabetic subjects. The subjects were from South Karnataka. These findings were supported by the study carried out by Thakur and Dash (2018) in western Odisha.

The results of this study are in accordance with a study done by Raska *et al.* (2017). They studied the BMD of 68 postmenopausal women with T2DM and 71 controls. In their cohort of 68 postmenopausal women with T2DM, 32.4 % of women had normal BMD, 48.5 % had

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osteopenia and 19.1 % osteoporosis, whilst in the non-diabetic control group it was 28.2 %, 50.7 % and 21.1 % of subjects, respectively.

When investigating the T-score between the test group (n=91) and the control group (n=49), there was no significant difference between the two groups (see Table 4.13). The results for this study indicated that T2DM did not influence BMD for this group.

On further analysis when the control group and the test group were divided according to age, the test group consisted of 54 subjects ≥50 years, whilst the control group consisted of 23 subjects ≥50 years. They were compared according to the T-score as seen in Table 4.14. There was no significant difference between the proportions of the two groups, except at the right femoral neck (RFN), where $p < 0.05$.

Data recorded according to the Z-score for women < 50 years in the test group consisted of 37 subjects, and the control group consisted of 26 subjects. There was no significant difference between the proportions of the two groups, as indicated in Table 4.15.

CHAPTER 6

CONCLUSION

6.1 Introduction

There is limited data available on the association between type 2 diabetes mellitus (T2DM) and osteoporosis for middle-aged Black South African women. T2DM is a chronic disease that affects several target organs (Martinez-Laguna *et al*., 2015). Data on the association between T2DM and osteoporosis are controversial. There was no information to be found for studies done to determine the effect of T2DM on bone mineral density (BMD) in Black South African women. Therefore, the aim of this study was to determine the effect of T2DM on this specific population group.

Given the current data, comparison of T2DM patients and non-diabetic controls showed no significant differences in BMD and T-score in the femoral neck, total hip and anteroposterior (AP) lumbar spine region. Osteopenia was also more prevalent than osteoporosis in both groups.

This study confirms the results of similar studies that there is no significant change in the BMD of women diagnosed with T2DM. Although the difference is not statistically significant, there is evidence of low bone mass (osteopenia) in general for this population. It has been observed that T2DM negatively affects bone strength regardless of BMD. According to Sanches *et al.*

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(2017) although T2DM patients have normal BMD, they have a greater risk for fractures. They claimed that this increased risk is probably due to abnormalities in bone material strength and biomechanical quality. Ahmad *et al*. (2016) gathered data from a large meta-analysis with a linkage disequilibrium using Utah residents with Northern and Western European ancestry. They detected a weak association between T2DM and femoral neck BMD, and no statistical significance between T2DM and AP spine BMD. They suspect that the effect of T2DM on BMD is site-specific, and that it could relate to the known disparate effects of T2DM on cortical and trabecular bone and the significant regional variation in bone microstructure throughout the skeleton. The results obtained from this study to determine the effect of T2DM on BMD in middle-aged Black South African women, confirm the possibility that BMD is site-specific. It seems that bone fragility in T2DM, which is not reflected by BMD, depends on bone quality deterioration rather than bone mass reduction.

It is important to keep an open mind for the probability of future fracture risks for patients with T2DM. The current osteoporosis guidelines for screening can be used for patients with T1DM and T2DM, but it is important to bear in mind that diabetes mellitus (DM) is a risk factor for osteoporosis and fracture, and that fractures can occur at higher BMD levels in patients with DM (Jackuliak & Payer, 2014).

According to Silva *et al*. (2015) BMD explains only 60% - 80% of bone strength, and several skeletal features other than BMD contribute to bone strength and fracture risk. BMD is not sensitive enough to assess the risk of osteoporotic fractures. The aetiology of DM-related bone fragility and diagnostic markers other than BMD need to be explored.

6.2 Limitations

This was a retrospective study and provides limited insight to unravel underlying pathophysiological mechanisms of the observed relationships.

It is well known that DXA techniques have its own limitations. Further techniques, such as peripheral quantitative computed tomography which allows for separate assessment of the trabecular and cortical compartments of the bone, may provide better insight into the trabecular-cortical bone relationships.

Shortcomings in this study:

1. There was no information available regarding medical history, e.g., insulin use, timeframe since diagnosis, possible previous fractures, glucocorticoid treatment, bone-active drugs, calcium intake, etc.
2. There was no information available with regards to smoking history, nutrition, physical activity, etc.
3. There was no information with regards to contraception use (e.g. oral or injectable).
4. The population group was very small. With a larger number of subjects more accurate results would represent this ethnic group.
5. Although the subjects were all middle-aged, they should also be divided into different age groups to account for the natural aging process of bone.
6. BMD does not reflect bone quality. Bone quality would be a better indication for future fracture risks.
7. Current osteoporosis screening guidelines do not account for human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) status, and clinical risk assessment tools are not sensitive in persons living with HIV/AIDS. HIV/AIDS status was not a considering factor in our study.

6.3 Recommendations

The following recommendations can be made from this research study:

- The study population could be increased and T1DM included.
- Advanced scientific investigations are required to assess the bone quality in T2DM, which is not reflected by BMD.
- Incorporating more than one assessment tool to evaluate the risk of fracture assessment, i.e., WHO fracture risk assessment tool (FRAX) algorithm and questionnaires.

Additional studies are required to determine whether osteoporosis is aggravated by T2DM, and whether it should be considered as one of the long-term complications of diabetes.

Healthcare in South Africa varies from the most basic primary healthcare, which is largely accessible free of charge in state hospitals and clinics. Public healthcare is generally over-burdened and poorly resourced, which makes it difficult to serve most of the population. Many times, state patients reach advanced care settings when their conditions have progressed or complicated.

Some risk factors for T2DM such as genetics, ethnicity and age, are not modifiable. Others, such as being overweight or obese, unhealthy diet, insufficient physical activity and smoking are modifiable through behavioural and environmental changes. Several effective policy options are available to facilitate these behavioural changes, and to create supportive environments for healthy lifestyles, e.g., improved diet and physical activity.

Diabetes prevalence, deaths attributable to diabetes and healthcare expenditure due to diabetes present a large social, financial and health system burden across the world. Bommer e*t al.* (2018) highlight the importance to take urgent action to prepare health and social security systems to mitigate the effects of diabetes.

CHAPTER 7

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APPENDIX A

ETHICS COMMITTEE APPROVAL

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

IRB nr 00006240
REC Reference nr 230408-011
IORG0005187
FWA00012784

01 February 2017

MS PHILNA PIENAAR
CLINICAL TECHNOLOGY
CUT

Dear Ms Philna Pienaar

ECUFS162/2012E [UFS-HSD2016/1513]
PROJECT TITLE: AFFECT OF TYPE 2 DIABETES MELLITUS ON BONE MINERAL DENSITY (BMD) IN MIDDLE-AGED
BLACK SOUTH AFRICAN WOMEN

1. You are hereby kindly informed that, at the meeting held on 31 January 2017, the Health Sciences Research
Ethics Committee (HSREC) approved this protocol after all conditions were met.

2. The Committee must be informed of any serious adverse event and/or termination of the study.

3. Any amendment, extension or other modifications to the protocol must be submitted to the HSREC for
approval.

4. A progress report should be submitted within one year of approval and annually for long term studies.

5. A final report should be submitted at the completion of the study.

6. Kindly use the **HSREC NR** as reference in correspondence to the HSREC Secretariat.

7. The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The
SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes
(2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research
Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by
the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6
Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration
of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well
as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty
of Health Sciences.

Yours faithfully

DR SM LE GRANGE
CHAIR: HEALTH SCIENCES RESEARCH ETHICS COMMITTEE
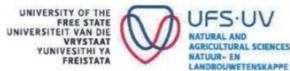
Cc Prof E van der Heever-Kriek

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | F: +27 (0)51 444 4359 | E: ethicsfhs@ufs.ac.za
Block D, Dean's Division, Room 0104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa
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APPENDIX B:

PERMISSION GRANTED

UNIVERSITY OF THE
FREE STATE
UNIVERSITEIT VAN DIE
VRYSTAAT
YUNIVESITHI YA
FREISTATA

UFS·UV
NATURAL AND
AGRICULTURAL SCIENCES
NATUUR- EN
LANDBOUWETENSKAPPE

19 Sept 2016

To Dr SM Le Grange
The Chair: Health Sciences Research Ethics Committee

For Attention: Mrs M Marais
Block D, Room 104
Francois Retief Building
PO Box 339 (G40)
Nelson Mandel Drive
Faculty of Health Sciences
University of the Free State
Bloemfontein, 9300

Regarding application to the Health Sciences Research Ethics Committee of the University of the Free, for the research proposal with the title:

Affect of Type 2 Diabetes Mellitus on Bone Mineral Density (BMD) in middle-aged black South African women.

I, Dr Gerda Marx, the principle investigator of research study with ethics approval number **ECUFS162/2012**, hereby give permission to Mrs P Pienaar to use the Bone Mineral Density (BMD) DXA data obtained from participants during this study. The data may only be used for the purpose of research study titled: Affect of Type 2 Diabetes Mellitus on Bone Mineral Density (BMD) in middle-aged black South African women. Mrs Pienaar will receive a blinded data set delinked from all personal information from the participants.

Regards,

Dr Gerda Marx

205 Nelson Mandela Drive/Rylaan, Park West/Parkwes, Bloemfontein 9301, South Africa/Suid-Afrika
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