Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.
An Investigation of Risk Factors for Fracture in Patients with Osteoporosis and Osteopaenia using Biomechanical, Biochemical and Radiological Assessments

A Thesis Presented in the University of Dublin for the Degree of Doctor of Medicine

By Joseph Browne

MB BCh BAO DME MRCPI

This work was carried out in the Mercer’s Institute for Research on Ageing, St James’s Hospital And Trinity Centre for Bioengineering Trinity College Dublin

April 2011
Declaration

I declare that the work contained in this thesis is my own, except where credit is given in the acknowledgements section to my colleagues in the Mercer’s Institute for Research on Ageing and the Department of Bioengineering, Trinity College Dublin. The project was approved by the St. James’s/AMNCH Ethics Committee and all participants gave full and informed consent. This thesis has not been submitted as an exercise for a degree to any other university. I agree that the library of the University of Dublin may lend or copy this thesis on request.

Joseph Browne
# Table of Contents

Declaration .......................................................................................................................... 2
List of Figures ........................................................................................................................ 8
List of Tables .......................................................................................................................... 11
Acknowledgements ............................................................................................................. 13
Abbreviations ...................................................................................................................... 15
Award, Publications and Presentations .................................................................................. 16
Summary ............................................................................................................................... 19
Chapter 1. Introduction and Literature Review ................................................................... 21
  1.1 Introduction .................................................................................................................. 21
  1.2 The Structure of Human Bone ...................................................................................... 24
  1.3 Types of Bone .............................................................................................................. 26
    1.3.1 Cortical Bone ........................................................................................................ 26
    1.3.2 Trabecular Bone .................................................................................................... 29
    1.3.3 Bone Cells ............................................................................................................ 30
  1.4 The Bone Modelling and Remodelling Process ............................................................ 32
    1.4.1 Bone Modelling .................................................................................................... 32
    1.4.2 Bone Remodelling ............................................................................................... 33
    1.4.3 Wolff's Law and Bone Remodelling .................................................................... 33
  1.5 Anatomical Structure of the Femoral Neck .................................................................. 34
  1.6 Osteoporosis ............................................................................................................... 35
    1.6.1 Epidemiology of Osteoporosis and Fractures ......................................................... 36
    1.6.2 Pathophysiology of Fractures .............................................................................. 40
    1.6.3 Peak Bone Mass .................................................................................................. 40
    1.6.4 Hip Fractures ....................................................................................................... 42
      1.6.4.1 Types of Hip Fractures .................................................................................. 43
      1.6.4.2 Morbidity ....................................................................................................... 44
      1.6.4.3 Mortality ........................................................................................................ 45
      1.6.4.4 Hip Fractures in Ireland ................................................................................ 45
      1.6.4.5 Time Trends and Future Projections in Hip Fractures ................................... 46
    1.6.5 Vertebral Fractures ............................................................................................... 47
    1.6.6 Distal Forearm (Wrist) Fracture .......................................................................... 48
  1.7 Bone Strength, Bone Quality and Skeletal Fragility ...................................................... 49
    1.7.2 Composition of Bone and Its Role on Bone Strength and Bone Quality ............... 52
    1.7.3 Role of Bone Geometry in Bone Strength ............................................................. 52
    1.7.4 The Role of Bone Microarchitecture in Bone Strength ........................................ 54
    1.7.5 The Role of Bone Matrix Properties in Bone Strength ......................................... 56
      1.7.5.1 Mineralisation ............................................................................................... 56
      1.7.5.2 Collagen Characteristics .............................................................................. 57
      1.7.5.3 Microdamage ............................................................................................... 57
    1.7.6 Factors affecting Bone Health in Older People .................................................... 58
  1.8 Vitamin D and Its Effects on Bone Health .................................................................... 60
    1.8.1 Physiology of Vitamin D ...................................................................................... 60
    1.8.2 Definition of Vitamin D Deficiency ....................................................................... 63
    1.8.3 Prevalence of Vitamin D Deficiency ..................................................................... 64

4
1.8.4 Optimal Vitamin D Level 65
1.8.5 Vitamin D and PTH Levels 65
1.8.6 Vitamin D and Bone Mineral Density 65
1.8.7 Vitamin D and Muscle Strength 66
1.8.8 Vitamin D and Falls 67
1.8.9 Vitamin D and Mortality 69
1.8.10 Treatment of Vitamin D Deficiency 69

1.9 Glucocorticoid Induced Osteoporosis (GIO) 71

1.10 Methods for Assessing of Bone Quality 73
1.10.1 Assessment of Bone Geometry and Microarchitecture 73
1.10.1.1 Dual X-ray Absorptiometry (DXA) 73
1.10.1.2 Limitations of DXA scanning 76
1.10.2 Lateral Vertebral Assessment 78
1.10.3 Calcaneal/Heel Quantitative Ultrasound 79
1.10.4 Micro-Computed Tomography (MicroCT) 81
1.10.2 Assessment of Bone Mechanical Properties 85
1.10.2.1 Nanoindentation 86
1.10.3 Assessment of Bone Turnover 90
1.10.3.2 Physiology of Bone Turnover 90
1.10.3.2 Bone Turnover as a Predictor of Bone Loss 92
1.10.3.3 Bone Turnover as a Predictor of Fractures 92
1.10.3.4 Bone markers in Men 93

1.11 Conclusion 94

Chapter 2. The Use of Dual-Emission X-Ray Absorptiometry and Clinical Risk Factors for the Diagnosis of Osteoporosis – Results from an Open Access DXA Service 95
2.1 Introduction 95
2.2 Materials and Methods 96
2.3 Results 98
2.4 Discussion 111

Chapter 3. The Prevalence of Vertebral Fractures in Irish patients attending for Dual X-Ray Absorptiometry (DXA) using Lateral Vertebral Assessment (LVA) 115
3.1 Introduction 115
3.2 Methods 116
3.3 Results 120
3.4 Discussion 137

Chapter 4. The Performance of Calcaneal Quantitative Ultrasound and Dual-energy X-ray Absorptiometry in the Discrimination of Prevalent Osteoporotic Fractures 142
4.2 Introduction 142
4.3 Materials and Methods 143
4.4 Results 146
4.5 Discussion 152

Chapter 5. The Prevalence of Vitamin D Deficiency in Irish Osteoporotic and Osteopaenic Patients and its Effects on Bone Metabolism 155
5.1 Introduction 155
5.2 Methodology 157
5.3 Results 159
5.4 Discussion 168
Chapter 6. An Audit of Hip Fracture Care in St James's Hospital compared to the UK National Hip Fracture Database (NHFD) 175
   6.1 Introduction 175
   6.2 Methods 176
   6.3 Results 178
   6.4 Discussion 182

Chapter 7. The Prevalence of Vitamin D Deficiency in Irish Patients with Hip Fractures 187
   7.1 Introduction 187
   7.2 Methods 189
   7.3 Results 190
   7.4 Discussion 195

Chapter 8. The Relationship of Bone Turnover Markers and Bone Mineral Density in Patients with Hip Fracture using MicroCT Analysis 200
   8.1 Introduction 200
   8.2 Materials and Methods 202
   8.3 Results 204
   8.4 Discussion 211

Chapter 9. The Influence of Bone Turnover and Serum Vitamin D Levels on Bone Elasticity Moduli and Hardness measured by Nanoindentation 214
   9.1 Introduction 214
   9.2 Materials and Methods 215
   9.3 Results 220
   9.4 Discussion 225

Chapter 10. Summary and General Discussion 229
   10.1 Introduction 229
   10.2 Risk Factors for Osteoporosis 230
   10.3 The Prevalence of Vertebral Fractures 231
   10.4 Quantitative Heel Ultrasound 232
   10.5 Vitamin D Deficiency, Secondary Hyperparathyroidism and Increased Bone Turnover 233
   10.6 Further Considerations 235
   10.7 Conclusion 237

Chapter 11. Appendices 238
   11.1 Bone Sample Analysis Flowchart 239
   11.2 Serum Biochemistry 240
   11.3 BMD Measurement 242
      Bone Densitometry 243
      Quality control procedures for bone densitometers 243
      Hip Scan Procedure 245
      Spine and LVA scan procedure 246
   11.4 Protocol for Theatre Specimen Collection 248
   11.5 Safety Protocol for Coring and Cutting of Human Femoral Heads 250
   11.6 Preparation of Bone Specimens 253
   11.7 Coring and Cutting 254
   11.8 Storage and Cleaning 256
   11.9 MicroCT Scanning 257
   11.10 Preparing & Embedding the Samples 260
   11.11 Polishing Protocol 263
      Polishing Procedure 264
   11.12 Nanoindentation Protocol 265
11.12.1 Theory 265
11.12.2 Testing Protocol 268
11.13 MicroCT Images 273
11.14 Nanoindentation Images 277

Chapter 12. References 286
List of Figures

Figure 1.1 The structure of cortical bone. 25
Figure 1.2. Microstructure of compact bone tissue. 27
Figure 1.3. Microscopic picture of the typical appearance of healthy cortical bone with several secondary osteons with central canals. 28
Figure 1.4. Trabecular bone tissue in the endosteal region of a long bone 29
Figure 1.5. The activity of osteoclasts, osteoblasts and osteocytes 31
Figure 1.6. The Bone Remodelling Cycle 32
Figure 1.7. Coronal section through a human femur showing compact bone at the external surfaces and trabecular bone in the internal aspect 34
Figure 1.8. Fracture Rate and the Number of Women with Fractures According to Peripheral Bone Mineral Density 35
Figure 1.9. Age-specific and sex-specific incidence of radiographic vertebral, hip, and distal forearm fractures 38
Figure 1.10. Demonstrating areas around the hip joint 43
Figure 1.11. The integration of Outline of Bone Quality, Bone Strength and skeletal fragility. 50
Figure 1.12. Fractures occur as a result of both trauma and decreased bone strength 51
Figure 1.13. Increasing the diameter results in increased both in the compressive and bending strengths 53
Figure 1.14. Position and extent of bone loss in men and women. 53
Figure 1.15. The importance bone microarchitecture. 55
Figure 1.16. Sunlight is important in the synthesis of vitamin D in the skin. 61
Figure 1.17. An example of a DXA scanner. 75
Figure 1.18. Genant semi-quantitative assessment of vertebral fractures 78
Figure 1.19. The above pictures are examples of MicroCT scans performed on femoral bone samples. 82
Figure 1.20. Diagram showing process of nanoindentation. 86
Figure 1.21. Schematic of load-displacement curve for an instrumented nanoindentation test. 84
Figure 1.22. The above pictures show interstitial bone of sheep where nanoindentation has been performed. 88
Figure 1.23 Bone turnover on the microscopic scale. 91
Figure 2.1 Number of DXA referrals according to Age Group 98
Figure 2.2 Source of referral for DXA scan 100
Figure 2.3 Overall DXA diagnosis according to age 102
Figure 2.4 Overall DXA diagnosis in female patients 103
Figure 2.5 Overall DXA diagnosis in male patients 104
Figure 2.6 Femoral Neck DXA Diagnosis in female patients according to age 105
Figure 2.7 Total Hip DXA Diagnosis in female patients according to age 106
Figure 2.8 Spinal DXA Diagnosis in male patients according to age 107
Figure 2.9 Femoral neck DXA diagnosis in male patients according to age ____________________________ 108
Figure 2.10 Total hip DXA diagnosis in male patients according to age ____________________________ 109
Figure 2.11 Spinal DXA diagnosis in male patients according to age ____________________________ 110
Figure 3.1 Prevalence of vertebral fractures based on LVA according to age group ________________ 124
Figure 3.2 Distribution of lateral vertebral assessments in male patients with evidence of at least one vertebral fracture according to age groups _____________________________________________ 125
Figure 3.3 Distribution of lateral vertebral assessments in female patients with evidence of at least one vertebral fracture according to age groups _____________________________________________ 126
Figure 3.4 Distribution of lateral vertebral assessments in male and female patients with evidence of at least one vertebral fracture according to age groups _____________________________________________ 127
Figure 3.5 Distribution of Vertebral Fractures in all patients attending for DXA. ________________ 130
Figure 3.6 Breakdown of underlying reasons for being on glucocorticoids at time of referral ____ 133
Figure 3.7 Prevalence of Vertebral fractures based on LVAs according to DXA diagnosis ______ 135
Figure 3.8 Distribution of Vertebral Fractures in Patients Using Glucocorticoids ________________ 136
Figure 3.9 Mean PTH Levels according to tertiles of serum 25(OH)D levels. Highest mean levels of PTH were seen in older patients with the lowest 25(OH)D levels ____________________________ 167
Figure 6.1 Distribution of Hip Fractures by Age _______________________________________________ 180
Figure 6.2 Fracture type by percentage according to gender ____________________________________ 180
Figure 6.3 Osteoporosis medications prescribed prior to admission and at discharge. ____________ 181
Figure 6.4 Outcomes of hip fracture patients at 30 days compared to NHFD UK 2009. ____________ 181
Figure 7.1 Relationship between 25(OH)D and parathyroid hormone. __________________________ 193
Figure 9.1 Correlation between serum PTH Levels and BV/TV in Wedge 1. ______________________ 205
Figure 9.2 Correlation of Trabecular Number and PTH Levels in Wedge 1. ______________________ 206
Figure 9.3 Correlation of Trabecular Thickness and PTH Levels in Wedge 1. ______________________ 206
Figure 9.4 Comparison of BV/TV and Serum PTH Levels in Wedge 1 and Wedge 4. _____________ 207
Figure 9.5 Comparison of Trabecular Number and PTH Levels in Wedge 1 and Wedge 4. __________ 207
Figure 9.6 Cortical bone dimensions according to PTH levels _________________________________ 208
Figure 9.1 Each indent produced a loading and unloading curve, as shown in the following diagram. ____________________________________________________ 216
Figure 9.2 and 9.3 Optical micrographs for the osteonal and interstitial cortical bone. ____________ 217
Figure 9.4 and 9.5 Displacement curves generated for 2 separate OP3 and OP4. _________________ 218
Figure 9.3 Vitamin D tertiles and elasticity measured in 3 types of bone tissue (trabecular, osteonal and interstitial). ________________________ 221
Figure 9.4 Vitamin D tertiles and hardness measured in 3 types of bone tissue (trabecular, osteonal and interstitial) ____________________________________________ 221
Figure 9.4 Correlation of serum 25(OH)D levels and mean elastic moduli. ______________________ 224
Figure 9.5 Correlation of serum 25(OH)D levels and mean hardness. ____________________________ 224
Figure 11.1. Images showing the coring procedure. __________________________________________ 254
Figure 11.2 Schematic diagram demonstrating the division of femoral head into cores and wedges. ________________________________________________________ 255
Figure 11.3: The inside of the scanner with the place for the container to slot into positioned in the centre of the picture 257
Figure 11.4 A finished embedded hip sample 262
Figure 11.6 Badly polished sample imaged under the microscope 264
Figure 11.8 Nano Indenter XP 268
Figure 11.9 The Berkovich diamond-indenting tip 269
Figure 11.10 Clear image of osteons with surrounding lamellae in cortical bone. 272
Figure 11.11 Trabecular Bone 277
Figure 11.12 Trabecular Bone 277
Figure 11.13 Cortical bone 278
Figure 11.14 Cortical Bone 278
Figure 11.15 Interstitial Cortical Bone 279
Figure 11.16 Interstitial bone 279
List of Tables

Table 1.1 Secondary Causes of Osteoporosis .................................................. 39
Table 1.2 Risks for Osteoporotic fractures .................................................... 41
Table 1.3. This table highlights the various ranges for Vitamin D and their respective definitions __64
Table 1.4 Sources of Error in the Diagnosis of Osteoporosis by DXA .................. 76
Table 2.1 Osteoporosis Risk factors referred to the DXA service. ...................... 101
Table 3.1 Demographics of all patients. ......................................................... 122
Table 3.2 Distribution of lateral vertebral assessments according to age-group with evidence of at least one vertebral fracture. .................................................. 123
Table 3.3 Vertebral fractures based on LVA according to DXA diagnosis at various sites and gender. .................................................. 128
Table 3.4 Percentage of vertebrae visualised and not visualised on lateral vertebral assessment __129
Table 3.5 Comparison of Baseline Characteristics in patients with hip fractures based on the presence of a vertebral fracture. .................................................. 132
Table 4.1 Characteristics of the population ..................................................... 146
Table 4.2 Comparison of all patients with and without prevalent fractures ............. 147
Table 4.3 Correlation between QUS parameters and DXA measurements ............. 148
Table 4.4 Relationship between sensitivity and specificity of bone mineral density (BMD) and heel ultrasound measurements in identifying patients with prevalent fractures in female patients using logistic regression and receiver operating characteristics (ROC). .............................................. 150
Table 4.5 Relationship between sensitivity and specificity of bone mineral density (BMD) and heel ultrasound measurements in identifying patients with prevalent fractures in male patients using logistic regression and receiver operating characteristics (ROC). .............................................. 150
Table 4.6 Relationship between sensitivity and specificity of bone mineral density (BMD) and heel ultrasound measurements in identifying patients with prevalent fractures in all patients using logistic regression and receiver operating characteristics (ROC). .............................................. 151
Table 4.7 Age-adjusted relative risk of fracture types using QUS and BMD Measurements. ______ 151
Table 5.1 Baseline Demographics of Patients referred to the Osteoporosis clinic. .................. 162
Table 5.2 Characteristics of population according to Vitamin D Supplementation. ................. 164
Table 5.4 Seasonal variability in all subgroups of patients including non-supplemented or supplemented patients.  

Table 6.1. Comparison of characteristics in patients admitted in 2008 and 2009 to St James's Hospital compared to the 2009 National Hip Fracture Database UK (NHFD).

Table 7.1 Baseline Characteristics of Study Group

Table 7.2 Distribution of Vitamin D Levels in subgroups of patients.

Table 8.1 Descriptive statistics of patients included in the study.

Table 9.1 Baseline Characteristics of Study Population.

Table 9.2 shows correlation co-efficients of bone turnover markers and elastic and hardness moduli.

Table 9.3 Correlation of Bone Turnover Markers and Nanoindentation Results
Acknowledgements

I would like to thank Professor James Bernard Walsh, my supervisor and friend, for his time, attention, patience, endurance, personal supervision and assistance for the last 5 years from the initiation to accomplishment of this thesis. I would like to Dr Miriam Casey for her advice, support, ingenuity and assistance throughout the project from the development of the concept of the project to its completion. I also wish to thank Dr Conal Cunningham, Dr Frances Horgan and Professor Davis Coakley for their unreserved advice and support throughout the project.

I am indebted to all the staff in the bone health and osteoporosis clinic in St James’s Hospital (Deirdre Cummins, Nessa Fallon, Kara Fitzgerald, Martha Gavin, Niamh Maher, Georgina Steen, Eilish Thornton and Zsofia Toth) for their assistance in recruiting and assessing patients. Their patience, organisation skills, and patient care are inspiring attributes that motivated me throughout this project. I want to also thank Caroline O Connor (St Camillus’s Hospital, Limerick) and Anne McCormack (Croom Orthopaedic Hospital, Limerick) for their support, assistance and time that were freely given to the project. I wish to thank all the staff in Theatre of St James’s Hospital and Colles ward for their assistance throughout the project. I want to thank Dr Cathal Walsh for advice and time on statistics, Gerry Cox and Dr Martin Healy in the biochemistry laboratory for their assistance in the analysis of blood results and their advice on the results.

I would like to thank Professor David Taylor and Peter O Reilly in the Trinity Centre for Bioengineering who always offered their help and advice in a patient, encouraging and constructive way. I also wish to acknowledge the help and assistance of Claire Picard, Tariq Mesallati, Claudine Murphy and Katie Reeve-Arnold in the preparation, analyzing and interpretation of the results. I would like to thank the medical colleagues and friends that have worked in the Bone Health Unit in the previous 4 years who have assisted in the collection and assessment of data.
The Mercer’s Institute for Research in Ageing (MIRA) and the Department of Bioengineering, Trinity College Dublin generously provided the equipment and facilities.

I wish to thank my parents, Mary and Gerard, who were so supportive of me in every way. Although, they may or may not understand what it is I actually do, I could not have done it without their help and encouragement.

Finally, this research would not have been possible without the interest, generosity of time and support of all of the participants, without whom the research would not be possible.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>Bone Mineral Density</td>
</tr>
<tr>
<td>vBMD</td>
<td>Volumetric Bone Mineral Density</td>
</tr>
<tr>
<td>aBMD</td>
<td>Areal Bone Mineral Density</td>
</tr>
<tr>
<td>BV</td>
<td>Bone Volume</td>
</tr>
<tr>
<td>TV</td>
<td>Total Volume</td>
</tr>
<tr>
<td>BV/TV</td>
<td>Bone Volume Fraction</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>Trabecular Thickness</td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>Trabecular Spacing</td>
</tr>
<tr>
<td>Tb.N</td>
<td>Trabecular Number</td>
</tr>
<tr>
<td>Conn.D</td>
<td>Connectivity density</td>
</tr>
<tr>
<td>SMI</td>
<td>Structural model index</td>
</tr>
<tr>
<td>MIL</td>
<td>Mean intercept length</td>
</tr>
<tr>
<td>DA</td>
<td>Degree of Anisotropy</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual energy X-ray absorptiometry</td>
</tr>
<tr>
<td>μCT</td>
<td>Micro Computed Tomography</td>
</tr>
<tr>
<td>St. Dev.</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>E</td>
<td>Elastic/Young’s Modulus</td>
</tr>
<tr>
<td>H</td>
<td>Hardness</td>
</tr>
<tr>
<td>RCSI</td>
<td>Royal College of Surgeons of Ireland</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
</tr>
</tbody>
</table>
Award, Publications and Presentations

Awards

President Prize (Best Poster): Irish Geriatric Society, Sept 2009

"Investigating bone quality in patients with hip fracture using newer bioengineering techniques"

Work arising from this thesis has been published as follows:

Publications

Seasonal variation of serum vitamin D in Irish community-dwelling older people.
R Romero-Ortuno, L Cogan, J Browne, M Healy, MC Casey, C Cunningham, JB Walsh, RA Kenny.
Age and Ageing 2010;0:1-7 doi: 10.1093/ageing/afq138

Abstracts

Hip Fracture Patients with Vertebral Fractures have more Severe Osteoporosis and are Candidates for more Active Treatment including PTH therapy. JG Browne, E Thornton, N Maher, MC Casey, JB Walsh. JBM R: Vol:23, September 2008 pp. S309:SU274.


Hip Fracture Patients with Vertebral Fractures have more Severe Osteoporosis and are Candidates for more Active Treatment including PTH therapy. JG Browne, E Thornton, N Maher, MC Casey, JB Walsh. Osteoporos Int 2009 20:Suppl 1 S75-76.


The Value of Lateral Vertebral Assessment in Patients Receiving Glucocorticoids Referred for Dual X-Ray Absorptiometry (DXA). Browne, J.G., Lim, Y., Casey, M.C., Walsh, J.B. Age and Ageing,102 (2), 2010

Vitamin D Deficiency is Highly Prevalent in Patients attending a Falls and Osteoporosis Clinic, Browne, J.G., Casey, M.C., Fallon, N., Healy, M., Ryan, D., Fitzgerald, K., Maher, N., Steen, G., Walsh, J.B., Bone,44 S2, 2009


17


**Vertebral Fracture Prevalence is High among osteopenic patients.** Walsh, J.B., Ryan, D., Browne, J.G., Thornton, E., Casey, M.C., Steen, G. *Irish Journal of Medical Science*, (Supp 8) 178, 2009


Summary

Osteoporosis is defined as a skeletal disorder characterised by compromised bone strength resulting in an increased risk for fracture. Hip fractures are the most common osteoporotic fracture in older adults and occur due to reduced bone strength and a propensity to falling. Dual X-ray absorptiometry (DXA) is the standard test to assess for osteoporosis in fracture patients, although there is a significant overlap between patients diagnosed with normal and osteoporotic bones who subsequently fracture.

Bone quality is a primary contributor to bone strength. A precise definition of bone quality remains elusive, due to characteristics other than bone mineral density (BMD) such as bone turnover, microarchitecture, and mineralisation of the bone matrix, which are important factors in the pathophysiology of osteoporosis and the mechanisms that underlie fracture. New techniques of assessment of bone quality and structure have been developed such as high-resolution computerized tomography (microCT) and nanoindentation assess the microarchitecture and mineralisation of bone respectively, which may account for the variance in patients who fracture.

In this study, we assessed the risk factors involved in patients being diagnosed with low bone mineral density on DXA. A high percentage of people being reviewed in our osteoporosis service had a fragility fracture Although, BMD should not be used in isolation to predict fractures, BMD has been shown to predict osteoporotic fractures independently of age, body weight and prevalent fractures. Advancing age and prior fragility fractures were markers of low bone mass in our patients. In addition, low body mass index, height loss, smoking, rheumatoid arthritis, steroid use and a history of thyroid disease were strong predictors for low bone mass in our study population.

Bone mineral density (BMD) alone underestimates the severity of osteoporosis in patients with previously undiagnosed vertebral fractures. Lateral vertebral assessment (LVA) was used as a diagnostic tool for the identification of vertebral fractures in patients attending for DXA and highlighted patients who required additional assessment and treatment for optimal bone health. There was a high prevalence of vertebral fractures in patients who attended our unit for a DXA examination,
particularlly in those with significant risk factors for osteoporosis such as a previous hip fracture or a history of glucocorticoid use.

Vitamin D is well recognised to be suboptimal in older patients and patients with fragility fractures are more likely to have lower serum vitamin D levels when compared to age-matched controls. We found that a large proportion of patients attending our hospital were vitamin D deficient, even though they were taking vitamin D supplementation. We found that patients with the lowest serum 25(OH)D levels had increased PTH and bone turnover marker (BTM) levels compared to those in the higher levels. Older patients appeared to have the highest PTH levels for a given vitamin D tertile level. Increased bone turnover, particularly bone resorption, can lead to bone loss and thus increased the likelihood of fracture. Thus, aggressive vitamin D replacement and anti-osteoporosis therapy should be promoted to normalise PTH levels and reduce bone turnover to protect those at high risk of fractures.

Bone quality encompasses micro-architecture of bone and the intrinsic properties of bone, along with bone mineral density. We noted that patients with increased PTH levels had reduced bone mass, trabeculae and cortical thinning, particularly in the superior portions of the femoral neck based on microCT scanning. Nanoindentation measured the elastic moduli and hardness of human bone samples and we found that patients with vitamin D deficiency had reduced hardness and stiffness of their bones. Vitamin D deficiency appeared to have a negative effect on the mechanical strength of bone with patients having the lowest vitamin D levels having reduced hardness and elasticity.

Comprehensive assessment of bone quality helps us to better understand the ability of bone to resist fracture. The assessment of all these parameters encompassed by bone quality would greatly add to future fracture predictions, evaluate new therapies and assess the influence of bone remodelling disorders on bone health.
Chapter 1. Introduction and Literature Review

1.1 Introduction

Fractures in older adults are an increasing medical problem all over the world with the vast majority of these fractures being associated with osteoporosis. Osteoporosis is the most common bone disease in humans, which affects 200 million people worldwide [1]. The lifetime risk of any osteoporotic fracture ranges between 40% and 50% in women and 13-22% in men [2]. The number of osteoporotic fractures in Europe in 2000 was estimated at €3.79 billion and is expected to increase to €76.7 billion in 2050 based on current demographic trends [3]. In the US, more than 2 million incident osteoporosis-related fractures occurred in 2005 in patients aged >50 years and these fractures were associated with healthcare costs of approximately $17 billion [4]. The most frequent fractures were vertebral (27%), wrist (19%) and hip (14%) fractures, with hip fractures accounting for 72% of the healthcare costs [4].

Osteoporosis is characterized by compromised bone integrity and an increased vulnerability to fractures that impair a person’s quality of life and increase the likelihood of mortality. Of greater importance is the fact that based on current population trends, the number of fractures is projected to double or even triple in the next few decades. The primary objective of osteoporosis treatment is to prevent fractures. This is dependent on accurate and effective methods for characterising bone integrity and the associated fracture risk in an individual. Early detection of a loss in bone integrity would enable the implementation of treatment regimens that may substantially improve outcomes and prevent the morbidity and costs that follow the occurrence of a fracture.
Hip fractures are a major public health issue, which will become more common with the ageing of the population. Hip fractures are a cause of significant morbidity and mortality of older individuals. In most populations, hip fracture incidence increases exponentially with age, and is the most common clinical fracture for adults over the age of 75 years. At the end of the first year after a hip fracture, up to 50% of individuals have permanent functional disability, 15 to 25% require long-term nursing home care and up to 20% will have died. In Ireland, it is estimated that 3500 to 4000 adults will sustain a hip fracture in a given year and this number is expected to increase in future years. Identifying any potential treatable factor for a further hip fracture is of paramount importance and should be diagnosed and treated appropriately.

Vitamin D deficiency is common among older adults and it is increasingly being recognised as a significant contributing risk factor for falls and fractures. Serum 25-hydroxyvitamin D (25(OH)D) is the most common biomarker used to assess vitamin D status and low 25(OH)D levels have been associated with an increased risk of fractures in older adults. The risk of falling may be in part related to muscle weakness and to changes in balance [7, 8]. The importance of vitamin D status for optimal bone health has received increased recognition in recent years, with higher intake levels being proposed by some investigators [9]. There is a lack of consensus on the serum 25(OH)D concentration that reflects optimal vitamin D status, but a serum 25(OH)D concentration > 75nmol/L has been suggested as an optimal level [10].

The most current view of osteoporosis defines bone strength as an integration of both quantity and quality. While bone quantity (mass) strongly correlates with bone
strength, substantial variability remains unexplained and growing evidence continues to demonstrate that measures or surrogates of bone quality enhance the evaluation of bone status. From an engineering perspective, bone strength depends on a combination of its structural and material properties, both of which can be modulated by bone turnover. Structural properties depend on the size and shape of the bone (i.e. cortical thickness, cross sectional area, moments of inertia), the microarchitecture of the bone (i.e. cortical porosity, trabecular morphology, degree of anisotropy) and the amount of accumulated damage (microcracks). Material properties depend on the degree of mineralization, the crystal size of minerals, the amount and type of collagen crosslinks, the interactions of mineral with the matrix, other proteins and fat.

The focus of this thesis is to assess the factors involved in fragility fractures, particular hip fractures, in older people using biomechanical, biochemical and radiological assessments. Chapter 3, 4 and 5 describes the osteoporosis patients attending the osteoporosis clinic including patients with a history of fracture. Chapter 6, 7 and 8 focus on assessment of bone quality in patients with hip fractures.
1.2 The Structure of Human Bone

Bone is a hierarchical structure that is mainly composed of organic and mineralised components, consisting of a matrix of cross-linked type 1 collagen mineralised with nanocrystalline, carbonated apatite. An increase in tissue mineral density increases the stiffness of the fabric but sacrifices flexibility [5]. Previous understanding based on studies performed more than 30 years ago, was that the elastic properties of bone were attributed to the mineral components of the tissue and that the plasticity of bone was due to the collagen matrix. This does not consider the interaction of the two phases or the roles that each plays in both the elastic and the plastic deformation of bone tissue. Like all composite materials each constituent of bone gives a varied, but intrinsically linked contribution to the overall quality of the tissue [6]. The strength of bone depends heavily on its material composition and structure. Bone must be stiff and able to resist deformation, thus making loading possible. But it must also be flexible being able to shorten and widen when compressed, and to lengthen and narrow in tension without cracking. If bone is too stiff and not able to deform, the energy imposed during loading will be released by structural failure. If bone is too flexible and deforms beyond its peak strain, it will crack and fracture. A combination and balance of these features gives bone its strength.

Depending on its porosity and microstructure, human bone can be classified into two main types:- cortical and trabecular. Cortical bone (or compact bone) is a stiff material with a relatively low porosity ranging between 5% and 10%. Trabecular bone (also known as cancellous or spongy bone) is a more porous structure consisting of small struts called trabeculae, with a porosity ranging from 50 to 90%.
Figure 1.1 The structure of cortical bone.
Within a cortical bone shaft, shown in the cross section (Panel A) are osteons surrounded by interstitial bone and many osteocytic lacunae distributed around the central Haversian canal (Panel B). Panel C shows a microcrack that is largely confined to interstitial bone. Panel D shows the Haversian canal system in cortical bone. In Panel E, alternating high-density and low-density concentric lamellae of an osteon produces a composite structure that is resistant to cracking, with an osteocytic lacuna at a higher resolution showing collagen fibres (Panel F) [6].
1.3 Types of Bone

1.3.1 Cortical Bone

In general, cortical bone can be found in the shaft of long bones and forms the outer shell around trabecular bone at the end of joints. It comes in the four main forms of woven, plexiform, primary and secondary bone. Woven bone has a poorly organised non-lamellar tissue structure with large vascular spaces and is relatively weak. Plexiform bone is an intermediate tissue type, which lies in between non-lamellar and lamellar bone. It is known as fibro-lamellar bone for this reason.

The osteon, first identified by Clopton Havers in 1691, is the fundamental functional unit of cortical bone. It consists of a central canal surrounded by concentric layers of well-organised bone tissue (lamellae). The central canal contains the bone's nerve and blood supplies. The typical diameter of an osteon is 150-200\(\mu\)m. Primary osteons are formed by mineralisation of cartilage, and do not contain as many lamellae as secondary osteons. The secondary osteons are created by the process of either replacing or remodelling the older tissue with new tissue (remodelling, described below).

In adults, most cortical bone consists of secondary bone, including whole osteons and also the remains of older partially resorbed osteons. These remnants of osteons are known as interstitial lamellae, and they occupy the space between the secondary osteons. Previous research on cortical bone has yielded much information about its properties and behaviour, however, a complete understanding of the fracture mechanics and general mechanical properties of the tissue at the nanoscale remain to
be obtained [7]. A more thorough understanding of these issues would allow for a more effective treatment of bone diseases such as osteoporosis.

The microstructural features of osteonal systems and secondary compact bone are illustrated below.

Figure 1.2. Microstructure of compact bone tissue [8].
Figure 1.3. Microscopic picture of the typical appearance of healthy cortical bone with several secondary osteons with central canals.
1.3.2 Trabecular Bone

This bone has a structure consisting of struts called trabeculae. Each strut is made of lamellar bone. Trabecular bone is found in the medullary cavity of flat and short bones, and also in the epiphysis and metaphysis of long bones [6]. The connections and orientation of the trabeculae in this bone were found to have precise patterns, which are related to the specific mechanical properties of the bone.

Trabecular bone is responsible for 20% of the total skeletal mass within the body while cortical bone makes up 80%. However, trabecular bone has a much greater surface area than cortical bone ($7 \times 10^6$ mm$^2$ vs. $3.5 \times 10^6$ mm$^2$). Although a lot of research has been carried out on trabecular bone in the past number of years, there is certain aspect of its behaviour that has yet to be fully understood.
1.3.3 Bone Cells

There are four main cell types in bone tissue; the osteoprogenitor cell, the osteoblast, the osteocyte and the osteoclast, each of which resides within the bone matrix.

**Osteoprogenitor cells**

These primitive cells, derived from the mesenchyme, line osteonal and Volkmann’s canals of compact bone. During periods of growth and remodelling, these cells are stimulated to differentiate into osteoblasts [9]. In mature bone that is not actively remodelling, these cells are called bone lining cells. Their processes extend through canaliculi to neighbouring cells suggesting that they are involved in cellular communication.

**Osteoblasts**

The purpose of these cells is to produce organic bone matrix. These cells synthesize and secrete small vesicles into the existing bone. Rupture of these vesicles initiates local mineralisation by releasing calcium and by negating local inhibiting mechanisms. This process makes the bone stiffer and more capable of bearing loads.

**Osteoclasts**

These cells break down bone tissue. When these cells are active they rest directly on the bone surface in a small cavity called a Howship’s lacuna. They are characterised by two easily identifiable features; the ‘ruffled border’ which is an infolded plasma membrane where the resorption takes place, and the ‘clear zone’ which is the point of attachment of the osteoclast to the underlying bone matrix.
Osteocytes

These cells are the most common in the bone matrix and are mature osteoblasts that have been trapped by the bone tissue laid down around them. They maintain healthy tissue by secreting enzymes and controlling the bone mineral content, and the calcium release from the bone tissue to the blood. Each osteocyte occupies a space (lacuna) within the matrix and communicates with other osteocytes via cell processes, which travel through canaliculi, by means of gap junctions.
1.4 The Bone Modelling and Remodelling Process

1.4.1 Bone Modelling

Bone is an amazingly active and dynamic tissue that is able to adapt its shape and size to mechanical loads and stresses. This adaptive process is known as modelling, in which bone are shaped and reshaped by the independent action of osteoblasts and osteoclasts. Modelling occurs not only during growth but also in the adult in response to a mechanical load. An example would be the adaptation of tennis players' upper limbs in which the playing arm has a thicker cortex and a larger external diameter than the contralateral upper limb [10, 11]. Conversely, rapid bone loss may occur by the unloading of the skeleton during bed rest or space-flight (between 2% and 9%) [12, 13].

Figure 1.6. The Bone Remodelling Cycle [14].
1.4.2 Bone Remodelling

Bone remodelling is a lifelong process in which old bone is removed from the skeleton and new bone is added. It consists of two distinct stages – resorption and formation – that involve the activity of osteoclasts and osteoblasts. Usually, the removal and formation of bone are in balance and maintain skeletal strength and integrity. Around midlife, remodelling becomes unbalanced so that every time bone matrix is remodelled, whether initiated for damage repair or adaptation to loading, more bone is removed than is replaced by cells of the basic multicellular unit, producing bone loss and structural decay. Although this negative balance can worsen as age advances, the driving force producing bone loss and structural decay is the remodelling intensity—the birth rate of the many new basic multicellular units arising on these surfaces after menopause in women and in both sexes late in life.

1.4.3 Wolff’s Law and Bone Remodelling

The relationship between the structure and function of bone is described by Wolff’s Law. This principle of functional adaptation states that mechanical stress is responsible for determining the internal architecture of bone [15]. The reason that bone is able to adapt to its mechanical environment is due to continuous bone resorption and formation. When these processes occur at different sites, the bone morphology is altered (modelling). Ideally bone resorption and formation are equal, with old bone continuously being replaced with new tissue. This is known as remodelling. With this process, no change in bone size or shape occurs. It is thought that once the bones have reached maturity, they form less new bone than they resorb during each remodelling cycle, causing an imbalance that contributes to net bone loss with ageing.
1.5 Anatomical Structure of the Femoral Neck

The femoral neck consists of a central trabecular network and a relatively thin cortical shell. Previous studies have shown that there is a decline in the bone mass in the hip with increasing age particularly in the infero-anterior and superoposterior regions [16]. As these regions bear the greatest strain during a fall, it is hypothesized that specific thinning of the cortex in these regions leads to an exaggerated propensity to fracture in those so affected, above that resulting from an equivalent general decrease in bone mass. The age-related changes at the femoral neck site are identical to those of the vertebral bodies i.e. a decline in trabecular density and connectivity and thinning of the cortex.

![Figure 1.7. Coronal section through a human femur showing compact bone at the external surfaces and trabecular bone in the internal aspect.](http://www.lab.anhb.uwa.edu.au/mb14/CorePages/Bone/Bone.htm#intramembranous)
1.6 Osteoporosis

Osteoporosis is a skeletal disease characterized by a loss of bone mass and micro-architectural deterioration of the skeleton leading to an increased risk of fracture [17]. As micro-architectural deterioration cannot be routinely measured by direct measurement, a panel of the World Health Organization (WHO) recommended that the diagnosis of osteoporosis be made when the T score on bone mineral density (BMD) measurement by DXA is -2.5 or lower [18]. It was also suggested that the term "osteopaenia" or "low bone mass" would be applied when T scores are between -1.0 to -2.5. There are more people with osteopaenia than persons with osteoporosis. As a result, approximately half of fragility fractures occur in the osteopenic group, although the relative risk of fracture is higher in the osteoporotic population, as can be seen in the figure below.

![Fracture Rate and Number of Women with Fractures According to Peripheral Bone Mineral Density](image)

Figure 1.8. Fracture Rate and the Number of Women with Fractures According to Peripheral Bone Mineral Density [19].
1.6.1 Epidemiology of Osteoporosis and Fractures

Osteoporosis is a significant cause of morbidity and mortality in older adults in the Western world, leading to large numbers of fractures of the hip, spine and wrist. As the population grows older, due to advances in healthcare and demographics change, so does the proportion of men and women suffering osteoporosis and fractures. Other trends have meant that the number of osteoporosis sufferers has increased at an even greater rate than that expected from demographic changes.

Osteoporotic fractures result from a combination of reduced bone strength and increased rate of falls. By the age of 80 years and over, 70% of people will have osteoporosis, with the hip being affected in 47% of people. In most populations, hip fracture incidence increases exponentially with increasing age. Above 50 year of age, there is a female to male incidence ratio of around two to one. Hip fracture mortality is higher in men than women, increases with age, and is greater for those with co-existing illnesses and poor pre-fracture functional status.

Fractures of the hip, vertebrae and distal forearm have long been regarded as the typical osteoporotic fractures. However, the effect of osteoporosis on the skeleton is systemic. Prospective studies have shown that there is an increased risk of almost all types of fracture in individuals with low bone density and, irrespective of fracture site, adults who sustain a fracture are at substantially greater risk of sustaining another fracture of a different type. Of all the fractures due to osteoporosis, hip fractures are the most serious and associated with the highest level of morbidity and mortality. Between 20-30% of patient who sustains a hip fracture will die within the first year, while half will suffer long-term pain and disability.
Fracture incidence in the community is bimodal, showing peaks in youth and in older adults. In young people, fractures of the long bone predominate, usually after substantial trauma. In older adults, fractures result from a combination of reduced bone strength and increased rate of falls. Although, bone mineral density remains the best available non-invasive assessment of bone strength in routine clinical practice, many other skeletal characteristics can contribute to bone strength. These include bone macro-architecture (shape and geometry), bone micro-architecture (both trabecular and cortical), matrix and mineral composition, as well as the degree of mineralization, microdamage accumulation, and the rate of bone turnover.
Figure 1.9. Age-specific and sex-specific incidence of radiographic vertebral, hip, and distal forearm fractures [20].
<table>
<thead>
<tr>
<th>Common</th>
<th>Less Common</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cushing’s syndrome or corticosteroid therapy</td>
<td>Oestrogen deficiency at a young age</td>
</tr>
<tr>
<td>Excessive alcohol use</td>
<td>Use of oral contraceptive</td>
</tr>
<tr>
<td>Primary or secondary hypogonadism</td>
<td>Low BMI</td>
</tr>
<tr>
<td>Low calcium intake and vitamin D deficiency</td>
<td>Lack of exercise or excessive exercise</td>
</tr>
<tr>
<td>Smoking</td>
<td>Thyrotoxicosis or thyroxine over-replacement</td>
</tr>
<tr>
<td>Family history of minimal trauma fracture</td>
<td>Primary hyperparathyroidism</td>
</tr>
<tr>
<td></td>
<td>Chronic liver or kidney disease</td>
</tr>
<tr>
<td></td>
<td>Malabsorption, including coeliac disease</td>
</tr>
<tr>
<td></td>
<td>Hypercalciuria</td>
</tr>
<tr>
<td></td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td></td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td></td>
<td>Multiple myeloma or other monoclonal gammopathies</td>
</tr>
<tr>
<td></td>
<td>HIV or its treatment with protease inhibitors</td>
</tr>
<tr>
<td></td>
<td>Organ transplantation or immunosuppressive agents</td>
</tr>
<tr>
<td></td>
<td>Mastocytosis</td>
</tr>
<tr>
<td></td>
<td>Osteogenesis imperfecta</td>
</tr>
</tbody>
</table>

39
1.6.2 Pathophysiology of Fractures

Osteoporosis-related fractures result from a combination of reduced bone strength and an increased tendency to falling. Bone mineral density remains the best available non-invasive assessment of bone strength in routine clinical practice, many other skeletal characteristics also contribute to bone strength. These include bone macro-architecture, bone micro-architecture, bone mineralization and the rate of bone turnover, which can affect the structural and material properties of bone.

1.6.3 Peak Bone Mass

The bone mass of an individual in later life is a result of the peak bone mass accrued during intrauterine life, childhood and puberty. The level of bone mass in adults is determined by the level of peak bone acquired during skeletal maturation and by the rate and duration of bone loss that occurs with ageing. Peak bone mass is attained by 20-25 years and is influenced by both genetic and environmental factors. The role of heredity in determining peak bone mass is dominant and accounts for 60-80% of the variance on peak bone mass.
### Table 1.2 Risks for Osteoporotic Fractures [21, 22]

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Premature menopause</td>
</tr>
<tr>
<td>Female gender</td>
<td>Primary or secondary amenorrhoea</td>
</tr>
<tr>
<td>Asian or Caucasian race</td>
<td>Primary or secondary hypogonadism in men</td>
</tr>
<tr>
<td>Low BMD</td>
<td>Previous fragility fracture¹</td>
</tr>
<tr>
<td>High bone turnover¹</td>
<td>Glucocorticoid therapy¹</td>
</tr>
<tr>
<td>Poor visual acuity¹</td>
<td>Family history of hip fracture¹</td>
</tr>
<tr>
<td>Neuromuscular disorders¹</td>
<td>Low Body weight¹</td>
</tr>
<tr>
<td></td>
<td>Smoking¹</td>
</tr>
<tr>
<td></td>
<td>Excessive alcohol consumption¹</td>
</tr>
<tr>
<td></td>
<td>Prolonged immobilisation</td>
</tr>
<tr>
<td></td>
<td>Low Dietary calcium intake</td>
</tr>
<tr>
<td></td>
<td>Vitamin D Deficiency</td>
</tr>
</tbody>
</table>

¹These characteristics capture aspects of fracture risk over and above that provided by BMD

Many risk factors for osteoporotic fracture have been identified (Table 2) [22]. In general, risk factor scores show relatively poor specificity and sensitivity in predicting either bone mineral density or fracture risk [23-28]. Some risk factors vary in importance according to age. For example, risk factors for falling such as visual impairment, reduced mobility and treatment with sedatives, are more strongly predictive of fracture in the elderly than in younger individuals [29].
1.6.4 Hip Fractures

Hip fractures are the most serious of all osteoporotic related fractures. They are associated with increased morbidity, functional decline, and death in older adults, as well as increased use of health care services. In most populations, hip fracture incidence increases exponentially with age. Mortality is increased in the year after hip fracture, with reported rates of 15 to 25% and an estimated 9 excess deaths per 100 patients among women 70 years of age or older.

Hip fractures are often a consequence of falls and underlying osteoporosis. Many potential risk factors for hip fracture, such as low body weight, cigarette smoking, caffeine intake, use of long-acting sedatives and inactivity have been identified in case–control [30, 31] and prospective studies [32-34]. The lifetime risk for sustaining a hip fracture is 6% in white men and 17.5% in white women.
1.6.4.1 Types of Hip Fractures

A hip fracture is generally a fracture of the proximal femur. Such injuries are divided into three categories, according to the anatomical area in which they occur.

![Diagram of hip joint areas](image)

Figure 1.10. Demonstrating areas around the hip joint.

Fractures of the proximal femur are classified on the basis of their location in the femoral neck, intertrochanteric region, or subtrochanteric region. Operative repair for these type of fracture include dynamic hip screw or total hip replacement, where the later is for fractures of the neck of femur when the blood supply has been disrupted.

Hip fractures occur as a result of a fall [31] and most are described as a fragility fracture. Any fracture in an adult (aside from a fracture of the digits) that occurs from a standing height or less, without major trauma such as a motor vehicle accident, can be considered a low-trauma or fragility fracture. A history of fragility fractures (unrelated to substantial trauma) strongly supports a diagnosis of osteoporosis, regardless of bone mineral density. Falls are a common problem in older populations with rates of 30 to 50% of community dwelling elderly falling each year. One in 20 falls is associated with hip fracture [31].
Patients with low-trauma hip fracture have one of the highest risks of subsequent fracture, with nearly one in five experiencing an event in the next 2 years [35, 36]. This extremely high subsequent fracture rate contributes significantly to loss in quality of life [37] and the cost of medical care [38, 39]. Many people who sustain a hip fracture are at high risk of sustaining a second hip fracture, with the frequency of a second hip fracture after an initial hip fracture being estimated being between 2% and 11% [40-49]. Most of these fractures occur in the first two years after the incident hip fracture [50, 51]. Several factors predispose people to a second hip fracture, which include low BMI, increasing age and female sex [28, 29, 52]. This represents a subgroup of patients where secondary prevention is of major importance. Identifying and treating factors for these repeat factors will reduce the incidence of recurrent fractures.

### 1.6.4.2 Morbidity

There is significant morbidity associated with hip fracture. In one study, 40% of patients could not walk alone, 60% have with difficulty at least one activity of daily living, 27% have been admitted to a nursing home for the first time and 20% have died within the first year after the hip fracture [53]. Osteoporosis in older men is a major public health problem, 25% to 30% of osteoporotic fractures occur in men[2]. Morbidity, loss of independence, institutionalisation and mortality are higher in men compared to women [54].

One source of the excess morbidity and cost incurred by patients with hip fractures is new osteoporotic fractures [55]. Such fractures occur at a rate of 10.4 per 100 patients per year, which is 2.5 times as high as the rate in age-matched persons without previous hip fracture. However, recent data suggests that few patients with hip
fractures actually receive pharmacologic therapy for osteoporosis [55, 56]. Recurrence of hip fracture occurs in approximately 10% of patients within 5 years of the original hip fracture.

1.6.4.3 Mortality

The increased mortality following hip fractures has been widely reported. The cumulative mortality 1 year following a hip fracture ranges from 20% to 40% [57]. Hip fracture mortality is higher in men than in women and increases with age [58]. This is greatest for people with co-existing illnesses and poor functional statues prior to the fracture [58, 59].

1.6.4.4 Hip Fractures in Ireland

Hip fractures are often the consequence of a fall [31]. Most hip fractures occur in the older person, and have devastating consequences for the patient. One review of hospitalisations secondary due to falls in older persons in the Eastern region of Ireland, showed that there were 2029 hospitalisations in 2002 [60]. Fractures accounted for 84% of the falls with nearly half of them sustaining a hip fracture [60].

In Ireland, there are few studies published on hip fractures. A typical hip fracture incident admission episode has been estimated to cost €14,300 [61]. There is also significant morbidity and mortality attached to hip fractures. A previous study at St Vincent’s University Hospital showed early mortality was 10.8% and this rose to 27% at three months [62]. It also has been demonstrated that at 2 years mortality associated with hip fracture was 23.6% compared to controls at 10.6%, and a further 26.6 % being institutionalised [63].
1.6.4.5 Time Trends and Future Projections in Hip Fractures

Life expectancy is increasing around the world and the number of older individuals is rising in every geographic region. The world population is expected to rise from the current 323 million individuals over 65 years of age to 1.555 billion by 2050. These demographic changes alone can be expected to increase the number of hip fractures occurring among people >35 years worldwide. The incidence is estimated to rise from 1.66 million in 1990 to 6.26 million in 2050. In the United Kingdom, the number of hip fractures is expected to increase from 46,000 in 1985 to 117,000 in 2016. However, in the developed world, recent studies from Switzerland and Finland suggest that the age-adjusted incidence of hip fracture has declined over the last decade. This may be related to an increase in obesity or improved screening and treatment of osteoporosis. These factors may potentially offset the impact of the projected increase in the older population.
1.6.5 Vertebral Fractures

Vertebral fractures are the hallmark of osteoporosis and they are associated with height loss, spinal deformity, chronic pain and reduced quality of life. Vertebral fractures are the most common type of osteoporotic fracture. Data from the European Vertebral Osteoporosis study (EVOS) has shown that the age-standardised population prevalence of vertebral fractures across Europe was 12.2% for men and 12.0% for women 50-79 yrs of age, with the prevalence of vertebral fractures increasing in those age over 80 years with age, with up to 25% of women over 75 years demonstrating vertebral fractures [64].

Vertebral fractures are critically important as a strong predictor of further fracture risk at any site, which is independent of BMD [51, 65]. The risk of sustaining a new vertebral fracture increases in those who have previously had a vertebral fracture compared to those without vertebral fractures. Vertebral fractures increase exponentially with the number and severity of prevalent vertebral fractures [66] and the high rate of subsequent vertebral fracture following an initial fracture is often referred to as the “vertebral fracture cascade” [67]. Up to 20% of women with a prevalent vertebral fracture will suffer a new fracture within one year [66].

Vertebral fractures are often asymptomatic with up to two thirds of fractures being asymptomatic. Another issue is that some physicians do not realise the clinical relevance of vertebral fractures with the result that vertebral fractures are under-reported and untreated. Vertebral fractures have been associated with increased premature mortality [68, 69]
1.6.6 Distal Forearm (Wrist) Fracture

Wrist fractures show a different pattern of occurrence to hip and vertebral fractures. There is an increase in incidence in white women between the ages of 45 and 60 years of age, followed by a plateau in most countries. The annual incidence of distal forearm fractures in male and female were estimated to be 1.7 and 7.3 per 1000 person-years, respectively [70].

This may relate to altered neuromuscular reflexes with ageing with a resultant tendency to fall sideways or backwards and thus not to break the fall with an outstretched arm. The increase in morbidity due to wrist fractures is less than that of hip or vertebral fractures. Mortality is similar to that of the general population.
1.7 Bone Strength, Bone Quality and Skeletal Fragility

Bone strength is a term used to describe the ability of bone to resist fractures [71]. Determining bone strength reflects the integration of three factors: quantity, quality and turnover [72]. Bone mineral density (BMD) measured by DXA reflects bone quantity. Measurements from DXA are an important tool in measuring BMD and assessing fracture risk, but less than 50% of variation in whole-bone strength is attributable to variations in BMD [73]. As outlined previously, the majority of patients who experience fragility fracture have BMD T-scores above -2.5 (figure 1.8). Although, it must be noted that age also has a significant effect on similar T scores. Clinical trials have shown that the risk of fracture in a 75 year old woman is 4-7 times that of a 45 year old woman with an identical bone mass [74]. Additionally, DXA has a limited ability to diagnose secondary causes of bone loss. Therefore, the other two determinants of bone strength (quality and turnover) should be included in assessing fracture risk in patients with osteoporosis. Since results of densitometry are a widely used endpoint in clinical practice and research, an appreciation of the limitations of this method is needed if the pathogenesis of bone fragility is to be understood [75, 76].

Bone quality is a primary contributor to bone strength and it is a function of the structural and material properties of bone. Bone quality encompasses additional aspects of bone structure other than BMD such as bone turnover, microarchitecture and mineralisation of the bone matrix which are important factors in the pathophysiology of osteoporosis and the mechanisms that underlie fracture [77].
Figure 1.1. The integration of Bone Quality, Bone Strength and Skeletal Fragility.

The strength of bone is determined by its material composition and structure. Bone must be stiff and able to resist deformation, thereby making load bearing possible. Bone must also be flexible: it must be able to absorb energy by deforming, to shorten and widen when compressed and to lengthen and narrow in tension without cracking. If bone is brittle (i.e., too stiff and unable to deform a little), the energy imposed during loading will be released by structural failure — initially by the development of microcracks and then by complete fracture. If bone is too flexible and deforms beyond its peak strain, it will also crack. Bone must also be light to facilitate movement. A unique feature of bone is that it can serve these contradictory needs of stiffness, flexibility, lightness and strength.

Bone trabecular and cortical macro- and micro-architecture strongly influence biomechanical strength, but there is not yet a consensus as to which parameters improve prediction of fracture risk in osteoporotic patients. Current knowledge is
limited, in part, by the predominant use of 2D techniques such as dual x-ray absorptiometry (DXA). Areal BMD (aBMD) has been shown to be only a partial predictor of fracture. DXA does not distinguish specific attributes of 3D geometry, cortical and trabecular density, trabecular architecture and intrinsic properties of the bone matrix.

Figure 1.12. Fractures occur as a result of both trauma and decreased bone strength.

Trauma depends on factors related to falling and to the force of the impact. Once the trauma force exceeds the bone strength, a fracture will occur [78].

In summary, a comprehensive evaluation of bone strength, including quantity, quality and turnover is critical in the identification of individuals with increased risk of fractures.
1.7.2 Composition of Bone and Its Role on Bone Strength and Bone Quality

Bone is composed of type I collagen stiffened by crystals of calcium hydroxyapatite. An increase in tissue mineral density increases the stiffness of the fabric but sacrifices flexibility. Variations in tissue mineral density affect function. Human bone is about 60 percent mineralized. The composition and degree of collagen cross-linking also influence function. The triple helix of type I collagen confers strength in tension. The cross-links in collagen keep its helixes fastened. If there are too few cross-links, the helixes may separate; if there are too many, the ability to absorb energy diminishes.

1.7.3 Role of Bone Geometry in Bone Strength

The loads applied to the skeleton generally are a combination of compression or tension (outward-pulling) forces with bending or torsional (twisting) moments. The resistance to bending and torsional loading is particularly important, as the highest stresses in the appendicular skeleton are due to these loading modes. (The highest stresses on the vertebral skeleton are due to compression loading).

The most efficient design for resisting bending and torsional loads involves distributing the bone material far from the neutral axis of bending or torsion (generally this axis is near the centre of the bone). The distribution of mass around the neutral bending axis is quantitatively described by a geometric property called the area moment of inertia. Importantly, the area moment of inertia of a solid circular bar is proportional to its diameter to the fourth power. Thus, small increases in the external diameter of a long bone can markedly improve its resistance to bending and torsional loading (Figure 1.13).
Effect of Cross-sectional Geometry on long Bone Strength

<table>
<thead>
<tr>
<th>Areal BMD (DXA)</th>
<th>No change</th>
<th>No change</th>
<th>Reduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compressive strength</td>
<td>No change</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Bending strength</td>
<td>No change</td>
<td>Greatly increased</td>
<td>Greatly increased</td>
</tr>
</tbody>
</table>

Figure 1.13. Increasing the diameter results in increased both in the compressive and bending strengths [78].

Figure 1.14. Position and extent of bone loss in men and women.

Absolute amount of bone resorbed on the inner bone surface and formed on the outer bone surface is more in men than in women during ageing [6, 79].
Considerable evidence indicates that age-related declines in the material properties of bone tissue are accompanied by a redistribution of cortical and trabecular bone. Specifically, in the appendicular skeleton, these changes involve endosteal resorption within bone combined with periosteal apposition on bone’s exterior. This leads to an age-related increase in the diameter of long bones but a decrease in cortical thickness (Figure 1.14).

1.7.4 The Role of Bone Microarchitecture in Bone Strength

Bone density is among the strongest predictors of the mechanical behaviour of trabecular bone, however, both empirical observations and theoretical analyses show that aspects of trabecular microarchitecture influence trabecular bone strength. Isolated trabeculae may fail by buckling, which describes the failure mode of a long slender column. Loss of horizontal trabecular elements leads to a marked increased in the unsupported length of a trabecular, markedly decreasing its buckling strength. Inversely, preservation of one or more horizontal struts can profoundly influence trabecular bone buckling strength with very little change in bone mass [80].

Another potential mechanism by which trabecular bone declines with increased bone resorption is the hypothesis that resorption cavities themselves serve as sites of local weakness where cracks in trabeculae may initiate. Using an analytical model of vertebral trabecular bone, a 20% decline in bone mass was induced either by thinning the entire trabecular structure or by randomly introducing resorption cavities [81].
Figure 1.15. The importance of bone microarchitecture.

The above compares normal bone to osteoporotic bone. Disruption of trabecular continuity by trabecular perforation results in reduced connectivity of the trabecular bone structure, increased bone fragility and increased fracture risk [14].

The importance of trabecular bone microarchitecture has since been supported by clinical studies showing altered trabecular microarchitecture in subjects with fragility fractures compared to age-matched controls with no fractures [81]. Studies have also shown altered trabecular microarchitecture in people with vertebral fracture and have related the extent of microarchitectural deterioration to vertebral fracture severity.
1.7.5 The Role of Bone Matrix Properties in Bone Strength

In addition to the macro- and microarchitecture, the features of bone matrix itself influence bone mechanical properties. Matrix characteristics that affect bone mechanical properties include:

- Mineralisation
- Collagen characteristics
- Microdamage

1.7.5.1 Mineralisation

It is well established that the degree of matrix mineralisation strongly influences bone mechanical properties. The stiffness and strength of bone are positively related to the degree of matrix mineralization. However, the ability of bone to absorb energy may either increase (if the bone is relatively under mineralized to begin with) or decrease (if the bone is already fully mineralized) with increasing mineral content.

Physiological changes or drug therapies that decrease bone turnover will eventually increase the degree of matrix mineralization by prolonging the period of secondary mineralization. In contrast, physiological processes or agents that increase bone turnover may lead to a transient decrease in the degree of matrix mineralization as new remodelling units are initiated and new bone laid down. Thus, iliac crest biopsies from postmenopausal women treated with antiresorptive therapy (calcium + vitamin D, raloxifene, risedronate and alendronate) show an increase in the degree of mineralisation that mirrors the suppression of bone turnover, whereas iliac crest biopsies from men treated with teriparatide show a slight temporary decrease in the degree of mineralisation. These effects on matrix mineralization will be reflected in
BMD measurements and likely contribute to the anti-fracture efficacy of these agents.

1.7.5.2 Collagen Characteristics

Bone is a composite material with two primary constituents: mineral and collagen. Mounting evidence indicates an important role for age- and disease-related changes in collagen content and structure. The majority of evidence suggests that in normal bone, the mineral provides stiffness and strength, whereas collagen affords bone its ductility and ability to absorb energy before fracturing. The extreme fragility seen in osteogenesis imperfecta underscores the potential for collagen abnormalities to influence bone strength. However, more subtle alterations in collagen, as noted by polymorphisms in the COL1A1 gene, have also been associated with fracture risk independent of BMD status [82]. Post-translational modifications of collagen have also been shown to influence bone mechanical properties [83], although their contribution to age-related skeletal fragility remains to be defined.

1.7.5.3 Microdamage

Throughout life, physiologic loading of the skeleton produces fatigue damage in bone. Although the optimal methods to quantify microdamage in bone are under debate, numerous studies show that the accumulation of damage weakens bone. Moreover, it appears that microdamage initiates activation of remodelling, presumably to repair the damaged tissue.

This observation suggests that one important role of bone remodelling is to repair fatigue-induced microdamage in bone. There is on-going debate regarding the optimal level of bone turnover to prevent architectural deterioration while preserving the ability of bone to maintain calcium homeostasis, respond to altered mechanical
loading, and to repair microdamage. The role of microdamage in age-related fragility fractures has yet to be established.

1.7.6 Factors affecting Bone Health in Older People

The risk for osteoporotic fracture is mainly determined by three factors: the risk of falling, force of impact in the event of a fall and the underlying bone strength. Age-related fractures are the most common manifestation of osteoporosis and these fractures are responsible for the greatest proportion of the morbidity and mortality from this disease. Over the next quarter century, as the population ages, fracture prevalence will also rise. Biochemical, biomechanical, and non-skeletal factors contribute to fragility fractures in the elderly.

Several studies have demonstrated a progressive reduction in bone mineral density at nearly every skeletal site with increasing age, however, fracture risk increases with age independent of BMD [21]. Other skeletal and non-skeletal factors must contribute to overall fracture risk. Recent advances in imaging technology and the availability of more longitudinal studies showed significant changes in trabecular microarchitecture that can be linked to bone strength and ultimately fracture risk in the older patients. Other qualitative factors that are influenced by age include the degree of mineralisation, microcrack number and frequency, anisotropy, skeletal geometry, matrix changes such as advanced glycation end-products.

Currently, it is difficult to clinically measure qualitative characteristics of bone. Newer techniques such as high-resolution CT are able to assess micro-architecture,
which may independently contribute to fracture risk. Also, risk factors such as age and previous fracture capture some of these qualitative determinants of fracture risk.

During ageing, bone resorption on the endocortical, intracortical, and trabecular surface reduces the amount of bone within the periosteal envelope as trabeculae thin and disappear, and as cortices thin and become porous. Simultaneously, periosteal bone formation partly offsets removal of bone on the inner surface. The net loss of bone of connectivity tends to dominate. However, those with osteoporosis and fractures have greater loss of connectivity than men with osteoporosis but without fractures [84].

Women and men with hip fractures have normal vertebral size and modest deficits in vertebral BMD [85, 86]. In women, femoral neck diameter can be reduced, normal, or increased [87-90]. BMD is reduced because of thinning of cortices, which contain large intracortical cavities [91]. Men have reduced femoral neck diameter with reduced BMD, probably due to cortical thinning [86].
1.8 Vitamin D and Its Effects on Bone Health

Vitamin D is necessary for a wide variety of essential biological functions such as bone and mineral metabolism, muscle function and immunity [92]. Vitamin D deficiency is extremely common [93-96] and can contribute to the development of osteoporosis and osteomalacia [97, 98]. In addition, vitamin D deficiency can increase the risk for falls, fractures [99, 100], and muscular weakness [101]. Low serum vitamin D levels appear to play a role in non-musculoskeletal diseases, including a variety of cancers [102-104], multiple sclerosis [105, 106], infection [107], hypertension [108], and diabetes mellitus [109, 110]. Recently, a meta-analysis of 18 randomised controlled trials found that oral supplementation of cholecalciferol (vitamin D₃) significantly reduced total mortality [111]. Despite its established benefits, vitamin D supplementation use is low and often inadequate [112].

The circulating level of serum 25(OH)D is the best indicator of vitamin D status in humans [113]. The cut-off value to define low vitamin D status and a definition for an optimal level of serum vitamin D remains controversial [114]. This is partly due to the variability of vitamin D concentrations by geographical location and differences in assay methodology [115-119].

1.8.1 Physiology of Vitamin D

Vitamin D status depends on adequate synthesis in the skin and a sufficient intake and absorption from the gastrointestinal tract [120]. The high prevalence of vitamin D deficiency is assumed to result from inadequate sun exposure, however, there is variability among individuals, causing some to have low vitamin D despite having a
high sun exposure [121], with some patients appearing to be more dependent on oral vitamin D intake to maintain adequate serum levels of 25(OH)D.

Vitamin D in the body is primarily produced in basal epidermis by ultraviolet B radiation [122]. Vitamin D normally enters the circulation after UVB radiation (290-315 nm) from sunlight that interacts with 7-dehydro-cholesterol in the skin, converting it to vitamin D₃ or cholecalciferol (Figure 1.16). When taken orally, the body metabolises vitamin D similarly to that generated in the skin. The liver hydroxylates vitamin D to 25(OH)D. Several organs and tissues in the body use 25(OH)D as a substrate to make the end-product, 1,25(OH)₂D, known as activated vitamin D, a pleiotropic seco-steroid.

The skins manufacture of vitamin D is rapid and robust. Production only after a few minutes of sunlight easily exceeds dietary sources. Incidental sun exposure, not dietary intake is the principal source of vitamin D stores.

![Figure 1.16. Sunlight is important in the synthesis of vitamin D in the skin.](image)

Vitamin D is further hydroxylated in the liver to form 25(OH)D. This is considered to be the storage form of vitamin D. 25(OH)D is further hydroxylated to 1,25(OH)₂D which has been implicated in the prevention and improvement of some disorders.
Cytochrome P450 enzymes are responsible for both the initial metabolism and subsequent catabolism of vitamin D. Drugs dependent on cytochrome P450 enzymes may affect vitamin D metabolism. Of the research done to date, corticosteroids [123], anticonvulsants [124-126], cimetidine [127, 128], antituberculosis agents [128, 129], theophylline [130], and orlistat [131] may alter serum 25(OH)D levels. Patients on these medications should have frequent testing of 25(OH)D levels and when identified as vitamin D deficient may require vitamin D3 doses above 2000 IU/day.

Insufficient intake of calcium and vitamin D leads to reduced calcium absorption and increased serum concentrations of parathyroid hormone (PTH). Elevated PTH levels lead to an increased bone turnover [132] and bone loss [133], particularly in cortical bone [134, 135]. Recent studies have shown a high incidence of secondary hyperparathyroidism (sHPT) contributes to bone fragility and even mild forms should be treated [136]. Long-term treatment with calcium and vitamin D supplementation is successful in reducing secondary hyperparathyroidism.
1.8.2 Definition of Vitamin D Deficiency

There is no consensus on optimal levels of serum 25(OH)D. However, most experts define vitamin D deficiency as a serum 25(OH)D level of less than 50 nmol/L [137-140]. 25(OH)D levels are inversely associated with parathyroid hormone levels until the former reach 75 to 100 nmol/L, at which point parathyroid hormone levels begin to level off [141]. Furthermore, intestinal absorption calcium transport increased by 45 to 65% in women when 25(OH)D levels were increased from an average of 50 to 80 nmol/L [142].

A consensus panel in 2005 concluded that an adequate serum 25(OH)D should be between 70 and 80 nmol/L [114, 143]. Serum 25(OH)D <25nmol/L is considered severe vitamin D deficiency and a level between 25 to 50 nmol/L is considered vitamin D deficiency. These serum vitamin D levels are based on the observations that serum PTH is still decreasing when serum 25(OH)D increases to 100 nmol/L. However these observations are from population-based studies, and the assessment of serum PTH is not helpful in the individual patient. When serum 25(OH)D is low, serum PTH is relatively high, but often still in the normal range, and the increase of serum PTH is blunted in many patients (i.e. functional hypoparathyroidism) [144].

Vitamin D toxicity does not typically occur until 25(OH)D concentrations are >350 nmol/L [142, 145, 146]. Vitamin D toxicity has not been reported from excessive sunlight exposure, and has only been associated with dietary intake when daily doses exceed 10,000 IU (250 µg) [50]. Doses of 4000 IU (100 µg) daily and 50000 IU (1.25 mg) weekly have been given without toxicity [139, 147]. Other studies using higher doses of vitamin D at longer intervals have also been conducted. For instance, the
results of a recent randomized, double-blind, placebo-controlled trial (n=2686) demonstrated that 100,000 IU (2.5 mg) vitamin D₃ every 4 months was safe and effective in decreasing the incidence of osteoporotic fractures [148].

<table>
<thead>
<tr>
<th>Vitamin D Level</th>
<th>Health Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 nmol/L</td>
<td>Severe Vitamin D Deficiency</td>
</tr>
<tr>
<td>25 – 50 nmol/L</td>
<td>Vitamin D Deficiency</td>
</tr>
<tr>
<td>50 – 75 nmol/L</td>
<td>Vitamin D Insufficiency</td>
</tr>
<tr>
<td>75 – 350 nmol/L</td>
<td>Vitamin D Adequacy</td>
</tr>
<tr>
<td>&gt;350 nmol/L</td>
<td>Vitamin D Toxicity</td>
</tr>
</tbody>
</table>

Table 1.3. This table highlights the various ranges for Vitamin D and their respective definitions [149].

1.8.3 Prevalence of Vitamin D Deficiency

Several studies demonstrate that vitamin D deficiency is widespread, especially among osteoporotic patients and the elderly. Extremely low serum 25(OH)D levels (<25 nmol/L) are common among institutionalized patients [150], although several studies have found a high prevalence among community-dwelling and healthy ambulatory older adults [151]. Most studies report that the prevalence of low vitamin D was higher in women than in men. In most studies of older adults, the prevalence of low serum vitamin D (< 20 ng/mL) was higher than 30%, regardless of season [152], gender [153], latitude [153] or race [154, 155].
1.8.4 Optimal Vitamin D Level

Currently, there is no standard definition of optimal vitamin D status. There are several skeletal and non-skeletal endpoints, which are defined for an optimal vitamin D and are outlined below. The circulating 25(OH)D level needed to suppress maximally the serum parathyroid hormone (PTH) concentration has been proposed and used by several investigators.

1.8.5 Vitamin D and PTH Levels

The effect of vitamin D on PTH is partly mediated by its effect in promoting calcium absorption. In a recent study, mean calcium absorption was 65% greater at serum 25(OH)D levels averaging 86.5 nmol/L than at levels averaging 50 nmol/L [156]. PTH suppression may also be mediated by a more direct mechanism involving metabolism of 25(OH)D to 1,25(OH)₂ within parathyroid tissue [157].

There is some disagreement as to whether PTH truly attains a lower plateau as serum 25(OH)D levels increase [158, 159]. The estimates of 25(OH)D levels needed for maximal suppression of PTH have been placed between 30 and 99 nmol/L [139, 160-164]. The studies yielding the highest estimates were cross-sectional studies [162-164].

1.8.6 Vitamin D and Bone Mineral Density

Vitamin D deficiency is a common in patients with hip fractures and can precipitate osteopaenia and osteoporosis, causing osteomalacia, muscle weakness and increased risk of fracture [165]. According to several studies, 40 to 100% of U.S. and European elderly men and women still living in the community (not in nursing homes) are deficient in vitamin D [137].
In older women, serum 25(OH)D and bone mineral density (BMD) of the hip were positively associated at 25(OH)D levels below 30 nmol/L, but not at higher 25(OH)D levels [166]. In the NHANES III study, the patients with the highest serum 25(OH)D had the highest BMDs [167]. The association was present in men and women and in Caucasians, Mexican-Americans and African-Americans, and is not dependent upon the level of physical activity. With regard to the effect of vitamin D on change in BMD, bone loss from the spine and hip was reduced during the wintertime with vitamin D supplementation that increased serum 25(OH)D levels from about 60 to 90 nmol/L [168, 169].

The mechanism by which vitamin D is thought to improve BMD is by suppressing secondary hyperparathyroidism and thus reducing bone resorption. There may be other direct effects on osteoblasts and osteoclasts that enhance bone health and bone formation.

1.8.7 Vitamin D and Muscle Strength

Muscle weakness below a certain threshold affects functional ability and mobility, which puts an older person at increased risk of a fall [170]. The evidence for improvement in muscular function comes from several studies. In NHANES III, women over the age of 60 years, with higher levels of serum 25(OH)D had improved lower limb function (faster walking and sit-to-stand speeds) [170]. More recently, a meta-analysis of 5 randomized controlled vitamin D intervention trials showed that supplementation lowered the risk of hip fracture by 26%. The trials in which the effect was most apparent used doses of 800IU per day [171]. However, not all studies have detected an effect of vitamin D on falls and fractures, which may be related to poor compliance or not achieving sufficient vitamin D levels.
There is conflicting evidence whether vitamin D contributes to proximal muscle weakness. Some studies have found an association [172-175], whereas other studies have found no relationship between vitamin levels and muscle weakness [176-178]. A recent study [179] investigated the relationship between quadriceps strength and vitamin D levels. Based on univariate analysis, there was a significant relationship between quadriceps strength and vitamin D levels, however, there were no statistically significant relationship once potential confounders such as physical activity, age, and comorbidities were controlled for. A systemic review conducted in 2003, concluded that vitamin D alone cannot be recommended for use in clinical where the primary aim is to improve muscle strength or physical function or reduce the risk of falling in frail older people [180]. This study did not review the methodological quality of the included studies.

In summary, the serum threshold of vitamin D at which at plateau effect on improvements in muscle strength has not been established and this is a potential area of further research.

1.8.8 Vitamin D and Falls

Falls are a major source of morbidity and mortality in older people with significant consequences including injury, fractures, hospital admissions and even death [181]. About one third of community-dwelling adults over the age of 65 and nearly one half of institutionalised persons or persons over the age of 80 will fall each year [181]. Almost half of those who fall in 1 year will experience a repeat fall within the next year. Although most falls result in no injury, 31% of falls result in an injury requiring medical attention or restriction of activities for at least 1 day. 10% to 15% of falls
result in fracture, and 5% of those result in more serious soft tissue injury or head trauma [182].

Increased bone fragility and increased number of falls caused by impaired muscle function are known risk factors for hip fractures in older patients [183]. Furthermore, postural sway has been identified as a risk factor for osteoporotic and Colles’ fractures [184, 185]. The percentage of older people who fall increases steeply in those older than 70 years of age, with over 90% of hip fractures in older people occurring as a result of a fall [186, 187]. Impaired balance and increased body sway are important causes of falls. Nguyen et al showed that besides bone density at the femoral neck, the main predictive factors for non-vertebral fractures were body sway and quadriceps strength [183]. It is thought that optimal serum vitamin D levels have an impact on body sway through an interaction on muscle cells mediated by a Vitamin D Receptor (VDR) [188]. Through this interaction, optimal vitamin D levels may enhance balance and reduce falls and fractures. Factors affecting VDRs include age [189], concurrent drug treatments and variation in receptor types [190].

Several studies have examined the effect of vitamin D supplementation on falls. It has been noted that vitamin D with calcium intake can be linked to higher bone density levels, vitamin D has also been found to help improve muscle mass. Older women given vitamin D showed increases in the number and size of type II muscle fibers within 8 to 12 weeks of initiating supplementation.

Falls may have other important consequences, even among older people without a fall-related injury. Falls are associated with greater functional decline, social
withdrawal, anxiety, and depression, and an increased use of medical services. Fear of falling is common among the elderly and has been associated with impaired mobility and decreased functional status. As a result, older adults who have fallen are at greater risk of becoming institutionalised regardless of whether they have experienced an injurious fall.

1.8.9 Vitamin D and Mortality

A recent meta-analysis of 18 randomised controlled trials found that cholecalciferol (vitamin D₃) significantly reduced total mortality [111]. This discovery is all the more remarkable because of the relatively low doses of vitamin D used (mean dose of 528 IU) with the finding persisting across a number of subgroup analyses.

1.8.10 Treatment of Vitamin D Deficiency

There are no definitive guidelines on appropriate dosages for vitamin D replacement. According to most studies, maximum benefit from vitamin D replacement is obtained when serum levels are at least 75 nmol/L. Most studies show that fall risk does not decrease unless the dose of vitamin D (cholecalciferol) is at least 700 IU/day and there is concurrent supplemented calcium intake [191].

The Institute of Medicine has suggested that up to 4000 IU cholecalciferol per day as the tolerable upper intake level for vitamin D [192], however, not all authorities have endorsed these guidelines [193]. Some studies suggest that 700 to 1000 IU of vitamin D₃ per day may be sufficient to bring 50% of younger and older adults up to 75–100 nmol/L. Thus, to bring most of the older adults to the desirable range of 75-100 nmol/L, vitamin D₃ doses higher than 700 – 1000 IU daily would be required. The
current intake recommendation for older adults (600 IU per day) may bring most individuals to 50 – 60 nmol/L, but not to the 75-100 nmol/L.

Recently, the NOF have published guidelines that 2000 IU daily is recommended in high-risk groups. These patients include patients with known vitamin D deficiency, malabsorption, chronic renal insufficiency, housebound patients, chronically ill patients, and others with limited sun exposure. Serum 25(OH)D levels should be measured in patients at risk of deficiency and vitamin D supplemented in amounts sufficient to bring serum 25(OH)D level to 30 ng/ml (75 nmol/L) or higher.
1.9 Glucocorticoid Induced Osteoporosis (GIO)

Glucocorticoid (steroid) excess is the third leading cause of osteoporosis after loss of sex steroids and advancing age. It is estimated that as many as 50% of patients requiring glucocorticoids for the control of pulmonary, rheumatological, autoimmune, haematopoietic, gastrointestinal disease or to prevent transplant rejection will ultimately suffer fractures. Fractures due to glucocorticoid excess are closely related to an increase in bone turnover and alterations in bone strength, which ultimately lead to bone loss and bone fragility. There is a biphasic pattern to bone loss due to glucocorticoids, with an initial rapid phase of approximately 12% in the first few months, followed by a slower of about 2-5% annually. Both cortical and trabecular bones are lost, but the bone loss tend to affect the axial skeleton predominantly.

The diagnostic thresholds in GIO have not been established. It is important to that the diagnostic guidelines for postmenopausal osteoporosis suggested by the WHO do not apply to GIO. Patients exposed to glucocorticoids are at increased risk of fractures. At similar BMD levels, patients who use glucocorticoids appear to have a higher risk of fractures than non-users. The Royal College of Physicians of London have proposed to consider a T score of -1.5 or lower to be indicative of a need of therapeutic intervention. The higher risk of fractures at comparable BMDs points to issues of bone quality that may be affected by glucocorticoids, but not measured by DXA. Despite these concerns, only a small proportion of patient on continuous glucocorticoid therapy receive any treatment to prevent bone loss. A study on practice attitudes in the United States revealed that only one-quarter of patients with rheumatic diseases receiving glucocorticoid therapy underwent diagnostic testing with BMD measurement and received supplementation calcium and vitamin D [194].
Most patients receiving glucocorticoids have an underlying disease, which frequently by itself carries a further risk for osteoporosis. These underlying disorders include rheumatological diseases, as well as chronic pulmonary disorders, inflammatory bowel disease and transplant patients. Patients with chronic obstructive pulmonary disease (COPD) are at increased risk of osteoporosis due to a variety of factors associated with the disease, such as poor health, poor nutrition, and smoking.
1.10 Methods for Assessing of Bone Quality

Bone mass or bone mineral density as measured by dual-energy x-ray absorptiometry (DXA), is one of the main predictors for fracture risk. However, multiple factors contribute to the structural integrity of bone. These factors include total bone mass, bone geometry and the properties of the bone tissue. Despite the complexity of these contributors to bone strength, bone mass is primarily used clinically to diagnose osteoporosis and assess fracture risk. Bone mass, characterized by bone mineral density (BMD) is assessed by dual-energy x-ray absorptiometry [195]. BMD is a limited predictor of fracture risk [196] and as a result clinical and scientific interest has increased to improve measures of bone quality that could improve fracture risk prediction [77].

1.10.1 Assessment of Bone Geometry and Microarchitecture

1.10.1.1 Dual X-ray Absorptiometry (DXA)

In 1991, a consensus panel defined osteoporosis as “a loss of bone mass and microarchitectural deterioration of the skeleton leading to an increased risk of fracture” [17]. Since microarchitectural deterioration cannot be directly measured, a panel of the World Health Organisation (WHO) recommended that the diagnosis of osteoporosis be made when T score on bone mineral density measurement by dual x-ray absorptiometry is -2.5 or lower [18]. They also suggested that the term osteopenia or low bone mass be applied when T scores are from -1.0 to -2.5. Due to more people having osteopenia than people with osteoporosis, approximately half of fragility fractures occur in the osteopenic group. Although the relative risk of fracture is higher in the osteoporotic population [197].
The current practice is to perform dual-energy x-ray absorptiometry (DXA) of the lumbar vertebrae (L1 to L4) and of the hip, including the femoral neck and total hip [198]. DXA quantifies bone mineral density per surface area (g/cm²). Measurement of mineral density in the forearm is not used routinely but is recommended for patients with primary hyperparathyroidism, since this site may show the greatest bone loss [199].

DXA uses the attenuation of x-rays through bone to measure bone mineral content at a skeletal site. This type of measurement is areal, providing a two-dimensional representation of bone. DXA does not yield a volumetric, or 3 dimensional picture of the bone. Bone strength is directly related to its 3-dimensional properties. DXA does not measure volumetric characteristics of bone and it does not provide an accurate picture of bone health in all people. Measurement of markers of bone turnover and risk factor assessment helps in modifying treatment in patients with osteoporosis.

Modern DXA scanners allow lateral vertebral assessment (LVA) which is recognised as being of increasing value for demonstrating vertebral fractures. Vertebral fractures are considered a hallmark of established osteoporosis and reduced bone quality. A problem with spinal measurements in older patients is that sclerotic changes that occur with age, largely owing to osteoarthritis, may result in an artificial increase in measured bone mineral density.
Figure 1.17. An example of a DXA scanner. A patient lies flat on the bed and X rays are directed at two sites, usually the lumbar spine and the hip.

DXA scanning is areal, providing a 2D representation of bone. It does not yield a volumetric, or 3D picture of the bone. Many other factors influence fracture risk and should be considered in making recommendations regarding bone densitometry and therapy.
1.10.1.2 Limitations of DXA scanning

As DXA does not measure the volumetric characteristics of bone, it does not provide an accurate picture of bone health in all people. The following table shows the limitations associated with DXA scanning.

Table 1.4 Sources of Error in the Diagnosis of Osteoporosis by DXA [200]

<table>
<thead>
<tr>
<th>Incorrect diagnosis of osteoporosis can be caused by:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Osteomalacia</td>
</tr>
<tr>
<td>• Osteoarthritis</td>
</tr>
<tr>
<td>• Soft tissue calcification</td>
</tr>
<tr>
<td>• Overlying metal objects</td>
</tr>
<tr>
<td>• Contrast media</td>
</tr>
<tr>
<td>• Previous fracture (spine, hip, and wrist)</td>
</tr>
<tr>
<td>• Severe scoliosis</td>
</tr>
<tr>
<td>• Extreme obesity or ascites</td>
</tr>
<tr>
<td>• Vertebral deformities due to osteoarthritis</td>
</tr>
<tr>
<td>• Inadequate reference ranges</td>
</tr>
<tr>
<td>• Inadequate operating procedures (e.g. calibration, region selection, acquisition mode positioning)</td>
</tr>
</tbody>
</table>
Current conventional thinking suggests that the most dominant factor related to skeletal fragility due to ageing or osteoporosis is reduced bone mass. Measures of bone mineral density (BMD) have been demonstrated to explain a substantial portion of the risk of osteoporotic fractures [201-203]. Despite its limitations, areal bone mineral density (aBMD) by dual-energy x-ray absorptiometry (DXA) is the most widely used assessment in clinical practice for the diagnosis and management of osteoporosis. aBMD can predict subsequent vertebral fracture with an increase in relative risk by 50% to 150% with each standard deviation decrease in bone mass, which is at least as good as the ability of blood pressure measurements to predict stroke and better than the predictive ability of serum cholesterol for cardiovascular disease.
1.10.2 Lateral Vertebral Assessment

Vertebral fractures are the commonest osteoporotic fracture, however, they can be clinically asymptomatic and under-reported in up two thirds of people. Vertebral fractures are often the hallmark of osteoporosis and are associated with considerable morbidity and mortality.

Lateral vertebral assessment provides practical and relevant clinical information to help predict future fracture risk. LVA seems to be a good alternative to conventional spinal radiographs, due to the possibility to perform BMD and vertebral morphometry at the same time and lower radiation exposure. The vertebrae T4 to L4 are considered for analysis, as T1 to T4 and L5 are better detected with conventional radiography than with LVA. Several studies have assessed the benefits of DXA-based techniques for the assessment of vertebral fractures, even in patients with normal BMD or osteopenia.

![Vertebral Fracture Grades](image)

Figure 1.18. Genant semi-quantitative assessment of vertebral fractures [204].
1.10.3 Calcaneal/ Heel Quantitative Ultrasound

Calcaneal quantitative ultrasound (QUS) or heel ultrasound is a diagnostic test that assesses the density and quality of bone in the heel using high frequency sound waves. It has been introduced as a "screening" tool for osteoporosis. The technology is relatively cheap, radiation-free and portable, but its accuracy in diagnosing osteoporosis is unclear.

Calcaneal QUS for bone assessment involves placing ultrasound inducers on either side of the calcaneus; one acts as a wave transmitter and the other acts as the receiver [205]. These devices assess 3 main types of parameters: broadband ultrasound attenuation (BUA), speed of sound (SOS) or velocity of sound (VOS) and quantitative ultrasound index stiffness (SI). Broadband ultrasound attenuation measures the frequency dependence of attenuation of the ultrasound signal that occurs as energy is removed from the wave, primarily by absorption and scattering in the bone and soft tissue [206]. Speed of sound measures the distance the ultrasound signal travels per unit time [207]. Quantitative ultrasound index and stiffness are composite parameters derived from BUA and SOS [207, 208].

Bone mineral density (BMD), measured by DXA, is the best predictor of fracture risk and is currently considered the "gold standard" for diagnosing osteoporosis. However, due to the relatively higher cost and a lack of availability of DXA, only a small percentage of women with osteoporosis are diagnosed by DXA [209]. Heel ultrasound has been suggested as a screening technique for people who are at risk of fracture. Several prospective studies have shown that quantitative ultrasound (QUS) predicts future fracture risk, including hip fractures, independently of BMD, and
nearly as well as DXA [210-218]. The use of clinical risk factors and calcaneal quantitative ultrasound can correctly identify more women at low risk fracture for hip fracture compared to either the stiffness index on QUS or the clinical risk factors alone [219]. In addition, QUS may be able to assess bone quality along with BMD [220-222].
1.10.4 Micro-Computed Tomography (MicroCT)

In recent years, the use of high-resolution micro-computed tomography (MicroCT) imaging has increased for the assessment of trabecular and cortical bone morphology in both animals and humans. MicroCT technologies are now emerging to better define the relationship between BMD and micro-architectural structure, as well as skeletal fragility, and to establish the clinical utility of bone structural measurements.

Excellent reproducibility and accuracy of microCT measurements of bone morphology has been shown in several studies. The accuracy of microCT measurements has been evaluated by comparing these results with traditional measurements from 2D histomorphometry. These studies have shown that 2D and 3D morphological measurements by microCT generally are highly correlated with those from 2D histomorphometry.

Areal bone mineral density estimates for the proximal femur using DXA are currently considered the gold standard for making a diagnosis of osteoporosis in an individual patient [223] and in predicting fracture risk [224]. However, structural information can be derived from microCT methods that are difficult to obtain using DXA methods. MicroCT studies of the proximal femur have shown a strong relationship between bone mass and its distribution and bone strength [225]. In particular, geometric measurements derived from microCT may represent in vivo strength better than DXA BMD, particularly cortical strength.

High-resolution assessment of trabecular and cortical architecture and volumetric BMD of the hip may demonstrate changes evident in hip fracture patients when
compared to age-matched osteoarthritic patients. Previous studies have shown at the radius that trabecular/tissue volume (BV/TV) declines with age similarly in men and women, but the structural basis for the decrease in trabecular bone volume is quite different between men and women. Some parameters evaluated through high-resolution microCT were different between osteopaenic women with and without a history of fracture.

Unlike DXA, microCT can differentiate between cortical and trabecular bone and can measure the vBMD of bone. Peripheral microCT is already a diagnostic method for BMD assessment. But it is only used at peripheral sites, like the tibia and distal radius, and generally it does not extend to the hip region [226].

Computed Tomography scanners use x-rays to produce detailed images of anatomical structures. These structures are usually organs or soft tissue. Digital geometry processing then produces a 2D image of the inside of the object from a large series of two-dimensional x-ray images taken around a single axis of rotation (Picard, 2008). These scanners can be peripheral Quantitative CT scanners (pQCT) usually used in clinical settings or micro CT scanners (µCT) usually used in research.

Figure 1.19. The above pictures are examples of MicroCT scans performed on femoral bone samples.
Micro CT scanning is used to examine the microarchitecture of bone in a qualitative and quantitative manner. Though not yet widely used clinically, it has been adapted for in vivo use in humans. A number of studies have been conducted to examine the microstructural indices of bone and the significance of bone quality in osteoporosis. One study examined microstructural indices in sheep trabecular bone using μCT [227]. The results showed that indices that are structural in nature (e.g. structural model index (SMI)) are as good as more density oriented indices (e.g. BV/TV) in predicting the ultimate strength of a region of trabecular bone. They also found that indices related to global changes in trabecular structure, such as degree of anisotropy, are less able to predict the mechanical properties of bone. The study also showed that a loss of bone primarily affects the connectivity and overall number of trabeculae, while an increase in strength results from an increase in the overall thickness of trabeculae, but does not necessarily improve the connectivity.

Studies investigating regional variations in the 3-D microstructure of trabecular bone with respect to ageing in the proximal femur, demonstrated that age-related changes in trabecular microstructure significantly vary in different sub-regions of the femur [228]. The researchers measured BV/TV, Tb.N, Tb.Th and Tb.Sp using μCT and found there was a significant decrease in BV/TV and Tb.Th associated with ageing at any region in male patients. They also found a significant difference in structural indices between the superior and inferior neck.

A study looking at trabecular bone in the proximal femur of Caucasian females found that with increasing age there is a significant reduction in BV/TV in all regions [228]. However is was observed they that the inferior neck retained a relatively high volume
of trabecular bone after 50 years of age, and also found a significant difference in the reduction in magnitude of BV/TV between the superior and inferior regions of the femoral neck. The superior part of the neck is believed to experience the greatest stress during a fall, as well as being the site of initial neck fracture.
1.10.2 Assessment of Bone Mechanical Properties

The biomechanical properties of bone are the basic parameters that reflect its structure and function and can be measured by testing either whole bone or specimens, which are prepared to isolate particular structural components. Bone is a viscoelastic, anisotropic and heterogeneous material and as such is complex to analyse mechanically. The inherent ability of bone to adapt continually to metabolic and environmental changes in vivo creates an even more complex system. The purpose of studying the biomechanical properties of bone is to characterize and better understand the relationship between its structural, material and mechanical behaviours.

Measuring the microscopic mechanical properties of bone tissue is important in support of understanding the aetiology and pathogenesis of many bone diseases [229]. The mechanical properties of bone are the basic parameters describing its function and structure. Bone is a complex structure due to it being viscoelastic, anisotropic, and heterogeneous. The mechanical behaviour of bone depends on various different factors including the stiffness, porosity, orientation of the microstructure and the degree of mineralisation [230].

Mechanical properties of the two bone types can vary under different stresses. Cortical bone is stiffer than trabecular bone, withstanding greater stress but less strain before fracture. The properties of trabecular bone are mostly determined by the structural properties of the tissue due to its architecture of trabeculae. Trabecular bone is more porous than cortical bone, and so it has a large capacity for energy storage. The physical difference between the two bone tissues is quantified in terms of their apparent density.
1.10.2.1 Nanoindentation

Nanoindentation has been developed over the last 15 years and is now used widely in the materials science community for probing the mechanical properties of thin films, small volumes and small micro-structural features. It can be used to derive values of the elastic modulus, hardness, and time-dependent deformation effects, which will indicate the material properties of the structure being investigated. Features less than 100 nm across and thin films less than 5 nm thick can be evaluated.

![Diagram of nanoindentation process](image)

**Figure 1.20. Diagram showing process of nanoindentation.**

Nanoindentation provides a means of examining the mechanical competence of bone at a micron scale, averaging the effect of osteonal lamellae but sensitive to variation in mineral content within the bone. Careful identification and selection of indentation sites by nanoindentation can obtain material characteristics separate from any effects of porosity. At the microscopic level, the mechanical behavior of bone is poorly understood although the bulk mechanical behavior of bone has been extensively studied in the past.

Human bone is composed of mixture of collagen, bone cells and hydroxyapatite crystals. Variations in the composition of these components can affect the hardness and elasticity, which is described as a Young’s’ modulus. Early nanoindentation
studies applied on bone examined the influence of microstructure, drying, anatomical location and age and compared the mechanical properties of compact and trabecular bone. It was reported that drying increases Young's modulus of compact bone by approximately 9-16%, but does not change the relative stiffness of the bone constituents.

In nanoindentation, small loads and tip sizes are used, so the indentation area may only be a few square micrometres or nanometres. During the course of the instrumented indentation process, a record of the depth of penetration is made, and then the area of the indent is determined using the known geometry of the indentation tip. While indenting, various parameters including load and depth of penetration can be measured. A record of these values can be plotted on a graph to create a load-displacement curve (such as the one in Figure 21). These curves can be used to extract the mechanical properties of the material.

Figure 1.21. Schematic of load-displacement curve for an instrumented nanoindentation test.

A load is applied to a sample causing displacement of the material. As the load increases the displacement increases. Release of the load will result in the material returning to the original state.
Figure 1.22. The above pictures show interstitial bone of sheep where nanoindentation has been performed.

At the microscopic level, the mechanical behaviour of bone is poorly understood [231], although the bulk mechanical behaviour of bone has been extensively studied in the past [232, 233]. Recent research has shown that significant variations in Young’s modulus may exist within a given microstructural component [234], and has suggested that the modulus of trabecular bone is substantially lower than that of cortical bone. Townsend et al [235] reported values of elastic moduli for trabecular bone to be between 11.4 GPa and 14.1 GPa. The values of cortical bone were between 12 GPa and 22 GPa. Zysset et al [236] found that elastic modulus and hardness values were significantly influenced by the type of lamella, showing that the structure of bone at the nanoscale must differ among lamellar types and anatomical sites.

The difficulty in preparing and testing specimens consisting of trabecular bone has contributed to the variation in measured elastic properties [237]. Some nanoindentation studies have examined the various influences of microstructure, drying, anatomical location and age, and have compared the mechanical properties of cortical and trabecular bone [238].

The technique used in these studies is critical. The storage, cleaning, embedding, polishing and testing parameters are all essential considerations. However, the results
from the majority of studies cannot be compared without the adoption of a universal indent methodology. Hengsberger et al [238] tested BSU's (bone structural units) from the femoral neck of an 86 year old. The modulus and hardness were investigated as a function of lamella type and indentation depth. For low depth indents, thick lamellae showed a higher indentation modulus than thin lamellae. But with increased indentation depth, thick lamellae experienced a significant decrease in indentation modulus and hardness, whereas thin lamellae experienced an insignificant decrease in these properties. Hoffler et al [229] looked at the effects of experimental testing parameters on nanoindentation measures of cortical bone. They found that consistent modulus values were obtained from a 500 nm deep indent. They also found that the moduli obtained at a 5nm/s loading rate was significantly lower than the values obtained at 10 and 20 nm/s loading rates, but the latter two were not significantly different from one another. The literature available is lacking in terms of a standardised procedure for preparing and testing specimens using nanoindentation. Many issues can cause different degrees of variability in the results.
1.10.3 Assessment of Bone Turnover

Markers of bone turnover are increasingly being used to improve fracture risk prediction and monitor treatment efficacy in osteoporosis. Bone turnover is characterized by the formation of new bone by osteoblasts followed by resorption of older bone matrix by osteoclasts. In osteoporosis, bone turnover is altered, with predominantly more osteoclastic activity compared to osteoblastic activity, with a resultant loss of bone and an increase in bone fragility. The development of serum assays for biochemical markers with improved specificity and sensitivity reflecting either enzymatic activities of osteoblasts and osteoclasts or breakdown products of bone tissue has been of great importance in understanding the complex pathways of bone turnover and their alterations in osteoporosis.

1.10.3.2 Physiology of Bone Turnover

Bone metabolism is characterized by two-opposite activities coupled at a basic multicellular unit (BMU). During bone resorption, dissolution of bone mineral and catabolism of the bone matrix by osteoclasts results in the formation of resorption cavities and the release of bone matrix components. During bone formation, osteoblasts synthesize bone matrix that fills in the resorption cavities and which then undergoes mineralization. There are two groups of biochemical bone turnover markers (BTMs). Bone formation is assessed by osteocalcin (OC), bone specific alkaline phosphatase (BALP) and N-terminal propetides of type 1 procollagen (P1NP). Bone resorption is assessed by osteoclasts by C-terminal cross-linking telopeptides of type 1 collagen.
In young adults, the quantity of bone formed at every BMU is equal to the quantity of bone removed by resorption. After menopause, BTMs increase rapidly [239]. Bone formation increases to fill in the higher number of resorption cavities, which increases the serum levels of bone formation markers. The quantity of bone formed is lower than the quantity of bone resorbed and as a result, there is a net bone loss at the BMU. The increased number of BMUs is the principal determinant of the postmenopausal BTM levels and BMDs [239, 240].

![Figure 1.23 Bone turnover on the microscopic scale.](image)

A microcrack severs canaliculi, which causes osteocytic apoptosis, with the location and extent of the damage defined by signals to lining cells. Lining cells and osteocytes release local factors that attract cells from blood and marrow into the remodelling compartment in which osteoclastogenesis occurs. Osteoclasts resorb the matrix and the microcrack, and then successive teams of osteoblasts deposit new lamellar bone. Osteoblasts that are trapped in the matrix become osteocytes; others die or form new, flattened osteoblast lining cells.

It is thought that increased bone turnover affects bone quality in a number of ways:

1. Highly mineralized bone is removed and replaced with younger bone, which contains fewer minerals and results in a reduction of the material stiffness.

2. The presence of more resorption sites creates more potential stress concentrations that predispose bone to micro-damage.
3. Increased remodelling impairs isomerization and maturation of collagen, which increases the fragility of bone.

1.10.3.2 Bone Turnover as a Predictor of Bone Loss

In some studies, baseline BTM levels correlated well with subsequent bone loss [241], which suggests that the bone turnover rate determines the subsequent bone loss. In the OFELY study, BMD was measured yearly for 4 years [242]. The BTM levels correlated negatively with the rate of bone loss at the distal radius where the precision error was comparable to the yearly bone loss. However, in the SOF study [243], BMD was measured twice and the coefficient of variation of DXA was higher than the average yearly bone loss.

1.10.3.3 Bone Turnover as a Predictor of Fractures

Increased bone turnover markers (BTM) levels predict fragility fractures independently of age, BMD and prior fracture. This association has been assessed in several prospective and case control studies [242, 244-246]. This association has been found in postmenopausal women and older women, but not in the frail older persons, where incident falls were the strongest of fracture [242, 247-251]. BTM levels are predictive of all fractures including vertebral fractures, hip fracture and multiple fractures. This association was found, though not consistently, for bone resorption markers [242, 245, 249].

However, for a given BTM level, there is a large scatter of individual values of bone loss [252], and BTM cannot be used for prediction of the accelerated bone loss at the individual level. High bone turnover is associated with lower BMD, faster bone loss
and poor bone micro-architecture both in the trabecular compartment (trabecular perforations, loss of trabeculae and poor trabecular connectivity) and in the cortical compartment (cortical thinning and increased porosity) [5, 253]. Vitamin D deficiency is a common finding in adults with osteoporosis who may have a subsequent rise in parathyroid hormone (PTH) and bone turnover [254].

1.10.3.4 Bone markers in Men

Serum levels of bone formation decrease in men decrease until the age of 50 to 60 years, then remain stable or increase slightly in cohorts including older men [255]. However, bone resorption increases after the age of 60. Men with high bone turnover have lower BMD and thus age-related bone loss in men results in part from increased bone resorption. Recent prospective studies have shown that BTMs may not be helpful in predicting osteoporotic fracture in older men [256].
1.11 Conclusion

Fragility fractures are a main contributor to mortality, morbidity and diminished quality of life in older people. Comprehensive assessment of all patients who are at risk of fracture may prevent these fractures in the first instance and also improve the outcome of those patients who have already fractured. The focus of this thesis is to evaluate bone health with patients who have osteoporosis and at risk of fractures with novel clinical, biochemical and bioengineering techniques.
Chapter 2. The Use of Dual-Emission X-Ray Absorptiometry and Clinical Risk Factors for the Diagnosis of Osteoporosis – Results from an Open Access DXA Service

2.1 Introduction

The internationally agreed description of osteoporosis is “a systemic skeletal disorder characterised by low bone mass and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture” [257]. This definition indicates that low bone mass is but one of the important components in predicting the risk of fracture, and that other changes occur in the skeleton which contribute to bone fragility. Thus, any assessment for future fracture risk should capture all these aspects of fracture risk. At present, the assessment of bone mineral density is the only aspect that can be routinely measured in clinical practice.

Bone mineral density (BMD) measurements form the cornerstone of general osteoporosis management and treatment. The gold standard for the diagnosis of osteoporosis and low bone mass in adults is dual emission X-ray absorptiometry (DXA). DXA measures the bone mineral density, which is the amount of bone mass per unit volume (volumetric density, g/cm^3) or per unit area (areal density g/cm^2). The absorption of X-rays is very sensitive to the calcium content within the tissue being studied. Thus, any disorder or process that alters the mineral content of bone can also affect the DXA scan result.
The exact number of patients affected by osteoporosis in Ireland is unknown, however, the World Health Organisation (WHO) estimates applied to the Irish population would suggest that there is in the region of 300,000 Irish people affected by osteoporosis which accounts for >25% of the people over the age of 50 [258]. Osteoporosis affects both men and women with its prevalence increasing with age, but women appear more susceptible to fracture compared to men. Several factors are associated with an increased risk of fractures and the development of the 10-year fracture risk algorithm FRAX allows for the identification of patients at high risk of osteoporotic fractures, with or without assessment by dual-emission X-ray absorptiometry (DXA) scanning.

The aim of this study was to determine which risk factors were more commonly referred for DXA and more likely to predict a positive DXA scan for osteoporosis.

2.2 Materials and Methods

Subjects

Our service is an open access service for both GPs and hospital consultants. This was a retrospective review of all DXA referrals to the Bone Health Clinic in St James’s Hospital from 2003 to 2009. Baseline demographics were recorded in all patients including reasons for the referral and the referral source. Baseline DXA scans were only included in the analysis. These patients included those who had been referred after a fracture, from their general practitioner or from a hospital consultant. Routine practice within our service is to perform a lateral vertebral assessment on all patients unless they are unable to lie on their left side. We recorded
all scans performed between January 2003 and April 2009 (n=8119). A further subgroup of patients (n=4122) completed a questionnaire, which included their osteoporosis risk profile (see appendices).

**Bone Densitometry**

Patients had their bone mineral density measures using a Lunar DXA system. This provided measurements of BMD in g/cm² together with the T-score and Z-score. Bone mineral density (BMD) was measured at three sites primarily, the hip, the forearm and the L1-L4 spine. In cases where one site was not available (e.g. patients with bilateral hip replacements), the distal one third of the radius was assessed. The hip measurements included the total hip and the femoral neck.

Daily quality control was performed out by measurement of the Lunar phantom. The patient’s BMD was measured at the lumbar spine (antero-posterior projection at L1-L4) and at the femurs (i.e. femoral neck and total hip). The World Health Organisation (WHO) classification system was applied, defining osteoporosis as a T score ≤−2.5 and osteopenia as −2.5 to −1. Study participants were categorized by the lowest T-score of the L1–4 lumbar spines, femoral neck, or total femur. We acknowledged patient DXA results should be represented by Z-score if they are below 40 years of age. However, for clarity we have presented those patient’s DXA results as T-scores.
2.3 Results

8119 patients had a DXA scan performed in St James's Hospital. 4960 (61.1%) patients were between 65 and 79 years and 1603 (19.7%) patients were 80 years old and over (Figure 1). Female patients were predominantly referred for a DXA scan, with 6537 (80.5%) patients being female patients and a further 1582 (19.5%) being male. The mean age of the overall cohort was 64.2 (+/-16.7; age range 18 - 98), with the mean age of female patients was 64.7 (+/-16.4) and the mean age of male patients was 62.1 (+/-17.9).

![Figure 2.1 Number of DXA referrals according to Age Group](image-url)
Referral Source

The main referral sources are listed in figure 1. The most frequent referral source were from Medicine for the Elderly (2160, 26.6%), general practitioner (1920, 23.6%) and rheumatology services (1379, 17.0%).

DXA Results

The prevalence of osteoporosis increased which each decade of age. Figure 2 demonstrates that the prevalence of patients according to age group. Patients over 80 years had an osteoporosis prevalence of 75%. Figures 3 – 11 demonstrates the prevalence of osteoporosis according to age at various sites (total hip, femoral neck and spine) for both male and female patients.

Risk Factors

Table 1 demonstrates the reasons for referral to the DXA service. Low BMI (OR 2.64; 95% CI 2.53 – 2.76; p=0.001), history of fracture (OR 2.04; 95% CI 2.00 – 2.08; p=0.001), rheumatoid arthritis (OR 1.36; 95% CI 0.98 – 0.98; p=0.001), steroid use (OR 1.33; 95% CI 1.29 – 1.36) and smoking (OR 1.35; 95% CI 1.31 – 1.39) were the most predictive risk factors for osteoporosis on DXA. Female sex was a strong predictor of osteoporosis (OR 1.23; 95% CI 1.18 – 1.29).
Figure 2.2 Numbers of Patients Referred for DXA scan by Referral Source
<table>
<thead>
<tr>
<th>Reason for Referral</th>
<th>Number of Patients N (%)</th>
<th>Mean Age (Std Dev, Yrs)</th>
<th>% Osteoporosis (T score &lt; -2.5)</th>
<th>Unadjusted Odds Ratio (95% CI)</th>
<th>P-value</th>
<th>Age-Adjusted Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Group</td>
<td>8119 (100)</td>
<td>64.2 (16.7)</td>
<td>46.7%</td>
<td></td>
<td></td>
<td>1.23 (1.18 – 1.29)</td>
<td>0.001</td>
</tr>
<tr>
<td>Female</td>
<td>6537 (80.5)</td>
<td>64.7 (16.4)</td>
<td>48.2%</td>
<td>1.02 (0.99 - 1.05)</td>
<td>-</td>
<td>0.64 (0.63 - 0.66)</td>
<td>0.001</td>
</tr>
<tr>
<td>Male</td>
<td>1581 (19.5)</td>
<td>62.1 (17.9)</td>
<td>40.7%</td>
<td>0.68 (0.57 - 0.66)</td>
<td>0.001</td>
<td>0.64 (0.63 - 0.66)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Subgroup Analysis (n=4122)**

<table>
<thead>
<tr>
<th>Reason for Referral</th>
<th>Number of Patients N (%)</th>
<th>Mean Age (Std Dev, Yrs)</th>
<th>% Osteoporosis (T score &lt; -2.5)</th>
<th>Unadjusted Odds Ratio (95% CI)</th>
<th>P-value</th>
<th>Age-Adjusted Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low BMI</td>
<td>162 (3.9)</td>
<td>69.7 (16.8)</td>
<td>71.6%</td>
<td>2.74 (1.93 – 3.87)</td>
<td>0.001</td>
<td>2.64 (2.53 – 2.76)</td>
<td>0.001</td>
</tr>
<tr>
<td>History of Fracture</td>
<td>1179 (28.6)</td>
<td>61.8 (18.3)</td>
<td>41.0%</td>
<td>2.11 (1.82 - 2.46)</td>
<td>0.001</td>
<td>2.04 (2.00 – 2.08)</td>
<td>0.001</td>
</tr>
<tr>
<td>X-ray evidence of OP</td>
<td>493 (12.0)</td>
<td>66.9 (15.5)</td>
<td>51.2%</td>
<td>1.11 (0.92 – 1.34)</td>
<td>0.269</td>
<td>1.05 (1.03 – 1.07)</td>
<td>0.001</td>
</tr>
<tr>
<td>Steroid Use &gt; 3 months</td>
<td>196 (4.7)</td>
<td>63.1 (16.9)</td>
<td>42.9%</td>
<td>0.77 (0.58 - 1.04)</td>
<td>0.084</td>
<td>1.33 (1.28 – 1.36)</td>
<td>0.001</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>495 (12.0)</td>
<td>64.4 (14.0)</td>
<td>44.8%</td>
<td>0.83 (0.69 - 1.00)</td>
<td>0.050</td>
<td>1.36 (0.98 – 1.92)</td>
<td>0.001</td>
</tr>
<tr>
<td>GI Disorder</td>
<td>128 (3.1)</td>
<td>52.7 (16.2)</td>
<td>36.7%</td>
<td>0.60 (0.41 – 0.86)</td>
<td>0.005</td>
<td>0.65 (0.63 – 0.69)</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoker</td>
<td>259 (6.3)</td>
<td>66.0 (14.4)</td>
<td>56.8%</td>
<td>1.40 (1.09 – 1.81)</td>
<td>0.009</td>
<td>1.35 (1.31 – 1.39)</td>
<td>0.001</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>33 (0.8)</td>
<td>67.7 (14.2)</td>
<td>54.5%</td>
<td>1.26 (0.63 – 2.50)</td>
<td>0.514</td>
<td>1.27 (1.17 – 1.39)</td>
<td>0.001</td>
</tr>
<tr>
<td>Height loss</td>
<td>146 (3.6)</td>
<td>77.4 (12.1)</td>
<td>58.9%</td>
<td>1.52 (1.09 – 2.13)</td>
<td>0.014</td>
<td>1.43 (1.37 – 1.48)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>101 (2.5)</td>
<td>72.7 (13.6)</td>
<td>45.5%</td>
<td>0.87 (0.59 – 1.29)</td>
<td>0.497</td>
<td>1.12 (1.06 – 1.19)</td>
<td>0.030</td>
</tr>
<tr>
<td>Alcohol</td>
<td>43 (1.0)</td>
<td>68.6 (12.2)</td>
<td>41.9%</td>
<td>0.75 (0.41 – 1.38)</td>
<td>0.354</td>
<td>0.60 (0.58 – 0.65)</td>
<td>0.001</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>31 (0.8)</td>
<td>62.5 (16.6)</td>
<td>29.0%</td>
<td>0.43 (0.20 – 0.93)</td>
<td>0.027</td>
<td>0.41 (0.37 – 0.45)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Table 2.1 Osteoporosis Risk factors referred to the DXA service.*
Figure 2.3 Overall DXA diagnosis according to age (n=8119, percentages demonstrated in table).
Figure 2.4 Overall DXA diagnosis in female patients (n=6509).
Figure 2.5 Overall DXA diagnosis in male patients (n=1566).
Figure 2.6 Femoral Neck DXA Diagnosis in female patients according to age (n=6509).
Figure 2.7 Total Hip DXA Diagnosis in female patients according to age (n=6509).
Figure 2.8 Spinal DXA Diagnosis in male patients according to age (n=1566).
Figure 2.9 Femoral neck DXA diagnosis in male patients according to age (n=1566).

<table>
<thead>
<tr>
<th>Osteoporosis</th>
<th>Osteopaenia</th>
<th>Normal</th>
<th>Unavailable</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30</td>
<td>23</td>
<td>62</td>
<td>4</td>
</tr>
<tr>
<td>30-40</td>
<td>43</td>
<td>62</td>
<td>2</td>
</tr>
<tr>
<td>40-50</td>
<td>84</td>
<td>73</td>
<td>5</td>
</tr>
<tr>
<td>50-60</td>
<td>122</td>
<td>83</td>
<td>4</td>
</tr>
<tr>
<td>60-70</td>
<td>169</td>
<td>75</td>
<td>9</td>
</tr>
<tr>
<td>70-80</td>
<td>153</td>
<td>69</td>
<td>24</td>
</tr>
<tr>
<td>80-90</td>
<td>94</td>
<td>47</td>
<td>14</td>
</tr>
<tr>
<td>&gt;90</td>
<td>11</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 2.10 Total hip DXA diagnosis in male patients according to age (n=1566).
Figure 2.11 Spinal DXA diagnosis in male patients according to age (n=1566).
2.4 Discussion

Many techniques are available to assess bone mineral at multiple sites including those where osteoporotic fractures predominate. Dual emission x-ray absorptiometry (DXA) is the most widely validated technique in assessing patients for osteoporosis. The foremost requirement for the use of bone mineral testing in the diagnosis and the assessment of osteoporosis is its predictive performance for fracture. Previous population based studies have shown that osteoporosis has a prevalence of approximately 16-30% [20, 259, 260]. In our study, 48.9% of the overall patients had osteoporosis and a further 35.6% had osteopaenia. The prevalence of osteoporosis increased with age, with the prevalence in patients over 70 years being over 60%. This higher prevalence in osteoporosis based on DXA diagnosis may be due several factors including a heightened awareness of amongst physicians within the locality, appropriate screening of DXA referrals and a possibility that there is a genuine higher prevalence of osteoporosis within the local catchment area.

There has been increased interest in the combined use of risk factors with BMD to predict fractures. Osteoporosis and bone fragility are asymptomatic and the morbidity of osteoporosis is secondary to the fractures that can occur if not treated. Most fractures occur in men and women with normal bone mineral density or osteopaenia according to DXA [19, 261]. Population screening can be restricted by access to DXA and the cost of the examination. Our service is open access service for both general practitioners and hospital consultants. The service is widely utilised by several services, however, medicine for the elderly, general practitioners and the
rheumatology services were the most frequent services to refer for DXA scans. This may reflect the high-risk population being seen by these services. Previous studies have shown that access to DXA can be linked to access to osteoporosis care. Thus, a reduced availability of DXA can lead to reduced osteoporosis treatment. Even in Ireland, the availability and access to DXA can vary geographically. Therefore, highlighting people at risk of fracture should be the priority in osteoporosis management. In recent years, there have been several fracture prediction algorithms developed, FRAX [262] and Qfracture [263], for the assessment of fracture risk. Both these assessment techniques use various risk factors for the assessment of fracture risk, although Qfracture excludes BMD measurements. To date in Ireland, there has been no development of fracture prediction algorithms for osteoporotic patients and this would be an opportunity for longitudinal ageing studies in Ireland.

Table 1 demonstrates the significant risk factors for referral to the DXA service. Age, history of fragility fracture, x-ray evidence of osteopaenia, steroid use and rheumatoid arthritis were significant risk factors in predicting osteoporosis and should be prioritised in an open access DXA service. Increasing age is an important risk factor for osteoporosis, with the prevalence increasing with each decade. Interestingly, early menopause was not a strong predictor of osteoporosis (age-adjusted OR 0.61, 95% CI 0.59 – 0.63). This may be accounted for patients being on HRT or having their bone health optimised prior to being referred for DXA. A prior history of osteoporosis treatment in this group may account for this finding. For example, patients have been treated with hormone replacement therapy but this has not been recorded. Any future studies within out department should clarify prior medications and treatments, which may influence bone health.
Osteoporosis is generally considered to be a disease of postmenopausal women. This misconception has led to a comparatively low number of referrals of men for DXA scans. In our study, there were substantially more females referred for DXA as compared to men. This continuing lack of awareness of the real magnitude of reduced bone mass among men results in under-referral for DXA and, consequently, under-diagnosis of osteoporosis in this group. Several studies have shown up to 6% of men above 50 years have osteoporosis and up to 47% have osteopenia [264].

There are a number of limitations in the general application of DXA for the diagnosis of osteoporosis, which should be acknowledged [200]. The presence of osteomalacia will underestimate total bone mass due to the decreased mineralization of bone. Osteoarthritis at the spine or hip is common in older patients and contributes to the density measurement, but not necessarily to the bone strength. Heterogeneity of density due to osteoarthritis, previous fracture or scoliosis can often be detected on the scan and in some cases excluded from the analysis. As can be seen in table 1, osteoarthritis was a negative predictor of osteoporosis (OR 0.41; 95% CI 0.37 – 0.45). These issues can be overcome by having highly trained staff and rigorous quality control. As mentioned previously, DXA provides an areal BMD measurement rather than true volumetric BMD. As a consequence, the computation of BMD is sensitive to changes in bone size. This may not be a negative issue, as increasing bone size can increase to bone strength.

In conclusion, our open access service shows that there is a high prevalence of osteoporosis being diagnosed within our service. Some risk factors, such as age, prior
fragility fracture, steroid usage and low BMI should be prioritised as they have the highest prevalence of osteoporosis.
Chapter 3. The Prevalence of Vertebral Fractures in Irish patients attending for Dual X-Ray Absorptiometry (DXA) using Lateral Vertebral Assessment (LVA)

3.1 Introduction

Vertebral fractures are the most common osteoporotic fractures [265] and are strongly associated with low bone mineral density [266, 267]. Vertebral fractures cause significant morbidity, mortality and reduced quality of life [268-270] and they are highly prevalent in older populations [271]. The prevalence of vertebral fractures increases with age and for women aged over 50 years the overall prevalence of vertebral fractures is 20-25% [272]. Several studies have found that these fractures are associated with an increased risk of further fracture of the spine or hip [65, 66, 273, 274], which can be independent of bone mineral density [275, 276].

Despite the high prevalence of vertebral fractures, it has been demonstrated that between two thirds to three quarters of people with vertebral fractures are unaware of their presence and do not come to clinical attention [277-279]. Several studies have suggested that vertebral fractures which present on x-rays are not being reported routinely or if they are reported their significance is not always recognised [280]. Lateral vertebral assessment can be used to identify vertebral fractures and a number of methods have been developed for interpretation of spinal assessments, including the Genant semi-quantitative method, which has been used as a surrogate gold standard in a number of key osteoporosis studies [281].
The aim of this study was to assess the overall prevalence of vertebral fractures as demonstrated on lateral vertebral assessment and to focus on two high risk groups – patients with a history of hip fracture and patients with a history of glucocorticoid use.

3.2 Methods

Subjects

3164 patients were reviewed between January 2007 and January 2011. Two subgroups were identified: One group consisted of 413 patients with hip fractures and a second group of 657 patients with a history of glucocorticoid use of 5.0 mg/day for >3 months.

Overall Population

Patients were reviewed who had been referred after a history of fracture, a referral from their GP or from a hospital consultant. Routine practice within the service is to perform a lateral vertebral assessment on all patients unless they are unable to lie on their left side. Patients with hip fractures completed a questionnaire at their baseline visit. This included a medical history, medication use such as hormone replacement therapy (HRT), calcium and vitamin D supplementation and prior bisphosphonate therapy.

Patients with Hip Fractures

A total of 413 patients with hip fractures (age range: 39 –100 years) were identified in the overall group who had been admitted with a low trauma hip fracture. A fracture was considered osteoporotic when the patient presented after a low impact trauma,
such as a fall from a standing height. The BMD of the lumbar spine and of the un-
fractured proximal femur of these patients were used where possible.

*Patients with a History of Glucocorticoid Use*

Patients referred to the osteoporosis unit for a DXA were reviewed for a prior history
of glucocorticoid use >5.0 mg daily for more than 3 months. Prior treatments for
osteoporosis and the underlying conditions for glucocorticoid use were reviewed.

*Bone Densitometry*

Bone mineral density (BMD) was measured primarily at 2 sites, the hip and the L1-L4
spine. In cases where one of these sites was not available (e.g. patients with bilateral
hip replacements), the distal one third of the radius was assessed. The hip
measurements included the total hip and the femoral neck.

Daily quality control was performed by measurement of a Lunar phantom. Patient
BMD was measured at the lumbar spine (antero-posterior projection at L1-L4) and at
the femurs (i.e. femoral neck and total hip). The World Health Organisation (WHO)
classification system was applied, defining osteoporosis as a T score ≤−2.5 and
osteopenia as −2.5 to −1. Study participants were categorized by the lowest T-score of
the L1–4 lumbar spine, femoral neck, or total femur.

Lateral vertebral assessments were classified using a combination of Genant semi-
quantitative approach and morphometry in the following manner: each LVA image
was inspected visually to decide whether it contained a fracture in any of the
visualised vertebrae. Each vertebra that was judged as fractured by visual inspection
was subsequently measured using built-in morphometry and assigned a grade based on Genant SQ scale, where grade 1 (mild) fracture is a reduction in vertebral height of 20–25%, grade 2 (moderate) a reduction of 26–40%, and grade 3 (severe) a reduction of over 40%. Subjects with no fractures or a grade 1 vertebral fracture were included in the non-fracture group, whereas those with grade 2 or higher fractures were included in the fracture group.

Lateral vertebral assessment images were inspected by Dr Joseph Browne (Specialist Registrar in Geriatric Medicine/Clinical Fellow in Bone Health) and Dr Davinia Ryan (Specialist Registrar in Radiology). Dr Browne has been trained in DXA imaging and has been certified International Osteoporosis Foundation Advanced Training Course in Osteoporosis in 2010.

Initially, all LVA images and excluded non-evaluable vertebrae from the study. Subsequently, all adequately visualized vertebrae were evaluated for deformity using an established semi quantitative visual scoring system (Genant scoring). Using this system, a grade 1 (mild) fracture is defined as an approximate 20–25% reduction in either anterior or middle or posterior height relative to the adjacent vertebral bodies; a grade 2 (moderate) fracture is an estimated 25–40% reduction in any height and a grade 3 (severe) fracture is a reduction of greater than approximately 40% in any height. Two physicians visually evaluated the LVA images independently then mutually agreed upon a consensus interpretation.

In all equivocal and abnormal scans, further analysis was performed by placing six points on each of the assessable vertebrae. These six points marked the anterior,
3.3 Results

Overall Patients

**Patient demographics**

3412 consecutive patients were reviewed initially. 249 (7.3%) patients were excluded as they did not have a LVA performed or the LVA was of too inferior quality to be analysed. 3163 patients who attended for DXA and who had a lateral vertebral assessment performed were included in the subsequent analysis. There were 2513 (79.4%) females and 652 (20.6%) male patients assessed. The mean age was 65.9 (+/-17.1) years, with 2080 (65.8%) patients over the age of 60 years. Mean weight and height were 160.0 (+/-16.3) cm and 65.9 (+/-15.2) kg, respectively. Baseline demographics are outlined in Table 1.

**Vertebral visualisation and fracture identification on LVA**

536 (16.9%) patients had evidence of at least one vertebral fracture evident on LVA and a total of 972 vertebral fractures were identified. Table 4 shows the percentage of vertebrae visualised and not visualised. The vertebral levels of T4, T5 and T6 levels that were not visualised were 41.2%, 26.9% and 13.0%, respectively.

**Prevalence of Vertebral Fractures**

536 (16.9%) patients were identified with vertebral fractures in the overall group. In the female group, 425 (16.9%) patients were identified with at least one vertebral fracture. In the male group, 111 (17.0%) patients were identified with at least one vertebral fracture. Table 2 demonstrates the distribution of vertebral fractures according to age. The highest number of vertebral fractures was observed in the 80-89 and >90 age groups i.e. 31.4% and 44.9%, respectively. Men in the 50-59 year old
group had a significantly higher prevalence of vertebral fracture compared to females (10.5% vs. 4.0%, p<0.05). The converse was observed in women in the age group 80-89 and >90 years compared to men (27.0% vs. 32.4%, and 23.5% vs. 48.5%, p<0.05).

Vertebral Fractures Identified in Patients with Normal and Osteopaenic DXA Results

Vertebral fractures were identified in 124 (10.8%) patients with osteopaenia and a further 20 (3.3%) patients with normal BMD’s in the overall group (Table 3). In women, there were 96 (10.9%) patients with osteopaenia and 13 (2.8%) patients had normal DXA scans.
### Table 3.1 Demographics of all patients.

<table>
<thead>
<tr>
<th></th>
<th>Overall Group (N=3163)</th>
<th>Patients with Hip Fractures (N=378)</th>
<th>Glucocorticoids (N=657)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Age (+/-SD) yrs</strong></td>
<td>65.9 (+/-17.1)</td>
<td>77.6 (+/-10.2)</td>
<td>62.5 (+/-16.2)</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>66.8 (+/-16.3)</td>
<td>79.7 (+/-9.8)</td>
<td>62.7 (+/-15.9)</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>62.2 (+/-19.2)</td>
<td>74.1 (+/-10.8)</td>
<td>61.9 (+/-16.9)</td>
</tr>
<tr>
<td><strong>Gender (n, % Female)</strong></td>
<td>2511 (79.4%)</td>
<td>288 (76.2%)</td>
<td>475 (72.3%)</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>160.0 (+/-9.3)</td>
<td>158.7 (+/-9.3)</td>
<td>161.3 (+/-8.6)</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>65.9 (+/-15.2)</td>
<td>59.6 (+/-12.9)</td>
<td>68.0 (+/-14.4)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.7 (+/-5.3)</td>
<td>23.6 (+/-4.5)</td>
<td>26.1 (+/-5.1)</td>
</tr>
<tr>
<td><strong>Age Group (n, % of total)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>124 (3.9%)</td>
<td>0</td>
<td>23 (3.5%)</td>
</tr>
<tr>
<td>30-39</td>
<td>155 (4.9%)</td>
<td>1 (0.3%)</td>
<td>51 (7.8%)</td>
</tr>
<tr>
<td>40-49</td>
<td>293 (9.3%)</td>
<td>5 (1.3%)</td>
<td>72 (11.0%)</td>
</tr>
<tr>
<td>50-59</td>
<td>511 (16.2%)</td>
<td>18 (4.8%)</td>
<td>112 (17.1%)</td>
</tr>
<tr>
<td>60-69</td>
<td>588 (18.6%)</td>
<td>54 (14.2%)</td>
<td>161 (24.5%)</td>
</tr>
<tr>
<td>70-79</td>
<td>722 (22.8%)</td>
<td>125 (33.1%)</td>
<td>141 (21.5%)</td>
</tr>
<tr>
<td>80-89</td>
<td>652 (20.6%)</td>
<td>152 (40.2%)</td>
<td>88 (13.4%)</td>
</tr>
<tr>
<td>&gt;90</td>
<td>118 (3.7%)</td>
<td>23 (6.1%)</td>
<td>9 (1.4%)</td>
</tr>
</tbody>
</table>

**DXA Results**

<table>
<thead>
<tr>
<th></th>
<th>Overall Group (N=3163)</th>
<th>Patients with Hip Fractures (N=378)</th>
<th>Glucocorticoids (N=657)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lumbar Spine BMD</strong></td>
<td>1.023 (+/-0.205)</td>
<td>0.903 (+/-0.216)</td>
<td>0.944 (+/-0.191)</td>
</tr>
<tr>
<td><strong>Lumbar Spine T Score</strong></td>
<td>-1.6 (+/-1.7)</td>
<td>-2.4 (+/-1.7)</td>
<td>-1.9 (+/-1.5)</td>
</tr>
<tr>
<td><strong>Total Hip BMD</strong></td>
<td>0.834 (+/-0.180)</td>
<td>0.708 (+/-0.161)</td>
<td>0.862 (+/-0.171)</td>
</tr>
<tr>
<td><strong>Total Hip T Score</strong></td>
<td>-1.4 (+/-1.5)</td>
<td>-2.6 (+/-1.2)</td>
<td>-1.2 (+/-1.4)</td>
</tr>
<tr>
<td><strong>Femoral Neck BMD</strong></td>
<td>0.806 (+/-1.52)</td>
<td>0.580 (+/-0.155)</td>
<td>0.824 (+/-0.149)</td>
</tr>
<tr>
<td><strong>Femoral Neck T Score</strong></td>
<td>-1.5 (+/-1.3)</td>
<td>-2.3 (+/-1.3)</td>
<td>-1.3 (+/-1.3)</td>
</tr>
<tr>
<td>Age Group</td>
<td>&lt;30</td>
<td>30-39</td>
<td>40-49</td>
</tr>
<tr>
<td>-----------</td>
<td>-----</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Total Cohort (N=3164)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total DXA with a LVA performed</td>
<td>125</td>
<td>155</td>
<td>293</td>
</tr>
<tr>
<td>No of LVAs with fracture</td>
<td>3</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>% Fractures evident</td>
<td>2.4</td>
<td>3.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Female (N=2512)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total DXA/LVAs performed</td>
<td>73</td>
<td>96</td>
<td>225</td>
</tr>
<tr>
<td>No of LVAs with fracture</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>% Fractures evident</td>
<td>2.7</td>
<td>2.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Male (N=652)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total DXA/LVAs performed</td>
<td>52</td>
<td>59</td>
<td>68</td>
</tr>
<tr>
<td>No of LVAs with fracture</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>% Fractures evident</td>
<td>1.9</td>
<td>5.1</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Table 3.2 Distribution of lateral vertebral assessments according to age group with evidence of at least one vertebral fracture.
Figure 3.1 Prevalence of vertebral fractures based on LVA according to age group (N=3164).
(Percentage demonstrated above each column.)
Figure 3.2 Distribution of lateral vertebral assessments in male patients with evidence of at least one vertebral fracture according to age groups (N=652). (Percentage demonstrated above each column.)
Figure 3.3 Distribution of lateral vertebral assessments in female patients with evidence of at least one vertebral fracture according to age groups (N=2512). (Percentage demonstrated above each column.)
Figure 3.4 Distribution of lateral vertebral assessments in male and female patients with evidence of at least one vertebral fracture according to age groups (*Pearson Chi-Square).
<table>
<thead>
<tr>
<th></th>
<th>Osteoporosis (T Score &lt;-2.5)</th>
<th>Osteopaenia (T Score -1 to -2.5)</th>
<th>Normal (T Score &gt;-1.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall DXA Result</td>
<td>373 (28.2%)</td>
<td>124 (10.8%)</td>
<td>20 (3.3%)</td>
</tr>
<tr>
<td>(N=3164)</td>
<td>(N, % of total DXA result)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP Spine Result</td>
<td>298 (28.3%)</td>
<td>106 (11.3%)</td>
<td>64 (7.0%)</td>
</tr>
<tr>
<td>(N=2909)</td>
<td>(N, % of total DXA result)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip Result</td>
<td>267 (32.3%)</td>
<td>191 (14.8%)</td>
<td>36 (4.1%)</td>
</tr>
<tr>
<td>(N=2989)</td>
<td>(N, % of total DXA result)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (N=2512)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall DXA Result</td>
<td>303 (27.7%)</td>
<td>96 (10.9%)</td>
<td>13 (2.8%)</td>
</tr>
<tr>
<td>(N=2512)</td>
<td>(N, % of total DXA result)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP Spine Result</td>
<td>244 (27.5%)</td>
<td>84 (11.3%)</td>
<td>40 (6.1%)</td>
</tr>
<tr>
<td>(N=2289)</td>
<td>(N, % of total DXA result)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip Result</td>
<td>213 (32.2%)</td>
<td>152 (15.1%)</td>
<td>26 (3.7%)</td>
</tr>
<tr>
<td>(N=2367)</td>
<td>(N, % of total DXA result)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (N=652)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall DXA Result</td>
<td>70 (30.6%)</td>
<td>28 (10.5%)</td>
<td>7 (5.0%)</td>
</tr>
<tr>
<td>(N=652)</td>
<td>(N, % of total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP Spine Result</td>
<td>54 (32.5%)</td>
<td>22 (11.5%)</td>
<td>24 (9.2%)</td>
</tr>
<tr>
<td>(N=620)</td>
<td>(N, % of total DXA result)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip Result</td>
<td>54 (32.3%)</td>
<td>39 (13.7%)</td>
<td>10 (5.9%)</td>
</tr>
<tr>
<td>(N=632)</td>
<td>(N, % of total DXA result)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3 Vertebral fractures based on LVA according to DXA diagnosis at various sites and gender.
Table 3.4 Percentage of vertebrae visualised and not visualised on lateral vertebral assessment.

<table>
<thead>
<tr>
<th>Vertebral Levels</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>T8</th>
<th>T9</th>
<th>T10</th>
<th>T11</th>
<th>T12</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage Vertebrae Visualised</td>
<td>58.2</td>
<td>73.1</td>
<td>87</td>
<td>94</td>
<td>98</td>
<td>98.6</td>
<td>99.5</td>
<td>98.7</td>
<td>98.7</td>
<td>98.6</td>
<td>98.3</td>
<td>96.2</td>
<td>91.2</td>
</tr>
<tr>
<td>Percentage Vertebrae Not Visualised</td>
<td>41.8</td>
<td>26.9</td>
<td>13</td>
<td>6</td>
<td>2</td>
<td>1.4</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>1.7</td>
<td>3.8</td>
<td>8.8</td>
</tr>
</tbody>
</table>
Figure 3.5 Distribution of Vertebral Fractures in all patients attending for DXA.
Patients with Hip Fracture

Patient demographics

413 patients with an osteoporotic hip fracture were assessed over the 4-year period. 35 patients were not included in the analysis (28 patients did not have a LVA performed and a further 7 patients had a LVA which was not suitable for analysis). 378 patients with hip fractures who had a DXA and lateral vertebral assessment (LVA) performed were analysed for this study. The mean age of this group was 77.5 (+/-10.5) years (range 39 to 100) - 287 (75.7%) patients were female and 92 (24.3%) were male.

Overall, 195 (52.7%) patients with hip fractures had osteoporosis, 103 (27.8%) had osteopaenia and a further 72 (19.5%) had a normal DXA scan. All study participants were Caucasian. 9.7% had a previous hip fracture on the opposite side.

141 (38.1%) patients with hip fractures had evidence of at least one vertebral fracture on LVA, with a total of 240 vertebral fractures identified. More vertebral fractures were identified in the thoracic region compared to the lumbar region (55% vs. 45% respectively). The T12 vertebra was the most common vertebra to have a fracture (Figure 5). Most vertebral fractures (15.7%) occurred at T12 (Figure 5).
<table>
<thead>
<tr>
<th></th>
<th>No Vertebral Fractures</th>
<th>Vertebral Fractures Present</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>77.0 (+/-10.7)</td>
<td>78.3 (+/-10.2)</td>
<td>0.23</td>
</tr>
<tr>
<td>Weight</td>
<td>61.3 (+/-13.3)</td>
<td>57.1 (+/-11.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Height</td>
<td>160.2 (+/-9.0)</td>
<td>156.5 (+/-9.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI</td>
<td>23.9 (+/-4.8)</td>
<td>23.2 (+/-4.0)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

**DXA Results**

<table>
<thead>
<tr>
<th></th>
<th>No Vertebral Fractures</th>
<th>Vertebral Fractures Present</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck BMD (g/cm²)</td>
<td>0.727 (+/-0.124)</td>
<td>0.660 (+/-0.107)</td>
<td>0.01</td>
</tr>
<tr>
<td>Neck T Score</td>
<td>-2.2 (+/-1.0)</td>
<td>-2.8 (+/-0.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Hip BMD (g/cm²)</td>
<td>0.741 (+/-0.165)</td>
<td>0.652 (+/-0.139)</td>
<td>0.01</td>
</tr>
<tr>
<td>Hip T Score</td>
<td>-2.3 (+/-1.2)</td>
<td>-3.0 (+/-1.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Spine BMD (g/cm²)</td>
<td>0.929 (+/-0.210)</td>
<td>0.861 (+/-0.220)</td>
<td>0.01</td>
</tr>
<tr>
<td>Spine T Score</td>
<td>-2.1 (+/-1.7)</td>
<td>-2.8 (+/-1.7)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Table 3.5** Comparison of Baseline Characteristics in patients with hip fractures based on the presence of a vertebral fracture.
Patients Receiving Glucocorticoids

Patient demographics

657 patients were identified as having received glucocorticoids for over 3 months. Mean age was 59.8 (+/-17.9) years (Range: 21.8 – 93.6 years). There were predominantly female patients referred to the Osteoporosis clinic. Mean BMI was 26.4 (+/- 5.4) kg/m². Demographics are outlined in Table 1.

Rheumatoid arthritis was the most common reason to be on glucocorticoids at the time of referral. Figure 6 gives the percentages of underlying reasons for being on glucocorticoids.

![Figure 3.6 Breakdown of underlying reasons for being on glucocorticoids at time of referral (n=657).](image)

642 of the 657 patients had an LVA performed. 15 (2.2%) patients either did not have a LVA performed or their LVA that was not suitable for analysis. 124 (19.3%) patients in the glucocorticoid group were identified as having at least one vertebral fracture.

Figure 7 demonstrates the prevalence of vertebral fractures in the glucocorticoid
group based on DXA result. 47 (21.3%) patients with osteopenia demonstrated at least one vertebral fracture with 9 (6.7%) patients having a normal DXA.

Figure 8 shows the distribution of vertebral fractures in the patients treated with glucocorticoids. There was a bimodal distribution of vertebral fractures observed on LVA. T8 and T12 were the most common vertebrae to be fractured on analysis.
Figure 3.7 Prevalence of Vertebral fractures based on LVAs according to DXA diagnosis (N=642)
Figure 3.8 Distribution of Vertebral Fractures in Patients Using Glucocorticoids

| No of Fract | 1 | 5 | 8 | 22 | 25 | 17 | 15 | 24 | 27 | 22 | 16 | 10 | 9 |
3.4 Discussion

The aim of this study was to determine the value of LVA added to BMD measurement. This study confirms that there is a high prevalence of vertebral fractures in patients attending for DXA, particularly in patients who have had a previous hip fracture or those using glucocorticoids. The gold standard to identify vertebral fractures is spine x-rays but the LVA technique has been validated for reproducibility, sensitivity and specificity as compared to spine X-rays [282, 283]. LVA can be performed at the time of BMD measurement, with little time added to the overall procedure. Of additional note, the radiation from LVA is about 10 uSv which is far less than the radiation used for antero-posterior and lateral spine x-rays (800 uSv) [284].

The prevalence of vertebral fractures observed in this study is in general agreement with other studies, although the epidemiology of vertebral fractures is less well documented as compared to hip fractures. In European-based studies prevalence figures in the order of 13%, 20% and 30% are found in age groups 50-59, 60-69 and 70-79, respectively [285, 286]. In our study, the overall percentage of patients with vertebral fractures was 16.1%. The prevalence of vertebral fractures in the age groups 50-59, 60-69 and 70-79 were 5.1%, 11.6% and 22.7%, as can be seen in table 2.

Historically, it was believed that vertebral fractures were more common in men than women but the EVOS data [287] suggests that this is not the case at younger ages. In our study, the prevalence of deformities in 50-59 year olds is higher in men than in females, possibility due to a higher incidence of trauma. In the EVOS study, the
prevalence is greater among middle-aged men (10%) than women (5%), but the converse is true after the age of 80 years, such that the prevalence is the eighth decade is 20-25% in women and 15-20% in men. A similar finding was found in our study.

Several studies have assessed the benefits of DXA-based techniques for assessing vertebral fractures, even in patients with normal BMD or osteopaenia [288, 289]. The detection of a vertebral fracture may lead to medical treatment in patients that would have otherwise not have been treated. The presence of vertebral fractures were identified in 3.3% in patients with normal BMDs in the overall population, and a further 10.8% of osteopenic patients had at least one vertebral fracture. In the glucocorticoid group, the percentage of vertebral fractures detected was higher in patients with normal and osteopenic BMDs (6.7% and 21.4%, respectively) as compared to the overall group (3.3% and 10.8%, respectively). Glucocorticoids appear to double the prevalence of vertebral fractures as compared to baseline. This underlies the importance of LVA in highlighting patients with vertebral fractures who may have been under-treated if a lateral vertebral assessment had not been performed. The use of glucocorticoids doubles the risk of subsequent vertebral fractures irrespective of the BMD score result. The presence of vertebral fractures is a powerful additional risk factor, which weights in the direction of the use of an anabolic, such as teriparatide, in the therapy of such a patient.

An interesting observation is that despite osteoporosis and low bone mass being thought of as a systemic disorder, vertebral fractures did not uniformly occur along the vertebral column in our population. Previous studies have shown a similar non-uniform distribution of vertebral fractures, which more often occurs at the mid-
thoracic (T7-T8) and thoracolumbar (T11-L1) regions than elsewhere in the spine [290, 291]. The reasons for this bimodal distribution are not completely understood but it has been suggested that biomechanical factors as a consequence of variations in the curvature of the spine contribute to the increased vertebral fractures in these regions. For example, maximum thoracic kyphosis occurs around T7-T8, which may result in greater anterior bending moments and increase the risk of anterior wedge fractures in this region. At the thoracolumbar junction, the spinal curvature transitions from kyphotic to lordotic, and the rigid thoracic cage gives way to a more mobile lumbar spine. The higher prevalence of vertebral fractures at T12/L1 may be related to increased load-bearing by the vertebral bodies as the rib cage no longer helps support loads at these spinal levels.

In patients with hip fractures, there was a higher prevalence of vertebral fractures with over one-third of patients (38.1%) having at least one concurrent vertebral fracture evident on LVA scanning. This group had an older mean age compared to the general group, which may account for the higher prevalence of vertebral fractures. Those patients who had a vertebral fracture had significantly lower weight, height, BMI and BMD results compared to those without vertebral fractures (table 4), despite no statistical difference between the groups mean ages. Other factors, such as co-morbidities and nutritional status may account for the difference in these subgroups of patients with hip fractures.

The prevalence of vertebral fractures was higher in patients treated with glucocorticoids than that seen in the general population. In our study group, 19.3% of patients receiving glucocorticoid had at least one vertebral fracture. Almost 1 in every
6 referrals to the osteoporosis clinic was due to a history of glucocorticoid use, indicating a high level awareness of the association of osteoporosis and glucocorticoid use. There may be several reasons for patients to be receiving glucocorticoids. In our population, rheumatoid arthritis and chronic obstructive pulmonary diseases were the most common reasons to be on glucocorticoids. Rheumatoid arthritis in itself results in an increased risk of fractures [292-294]. It has been reported that there can be at least a 2-fold increase of vertebral fractures in patients with rheumatoid arthritis [294, 295] and a higher risk, up to 6-fold, has been reported in patients with long-standing disease [296]. In this study, it must be noted that 47/220 (21.4%) patients who had an osteopaenic scan had at least one vertebral fracture. LVA identified a further group of patients that would require active treatment of their osteoporosis risk. Currently, there are no Irish recommendations or guidelines for the assessment and treatment of steroid-induced osteoporosis. However, patients with a prior history of glucocorticoid use should be prioritized for osteoporosis assessment and treatment.

There are some limitations in this study. Lateral vertebral analysis was not performed on all patients due to patients not being able to lie on their left-hand or the vertebrae were not always visualised as a result of artefacts. This may lead to a selection bias for suspected vertebral fractures and thus under-report the true prevalence of vertebral fractures. Reasons for vertebrae being not visualised include severe scoliosis, osteoarthritis and multiple vertebral fractures. These patients would require further assessment using standard spinal radiographs. For patients were either the vertebrae are not visualised or that the patient cannot lie on their side, an x-ray of the lateral spine should be performed to reflect a true prevalence of vertebral fractures.
This study demonstrates that DXA-based lateral vertebral assessment is an invaluable technique to detect vertebral compression fractures. Although, spinal x-rays remain the gold standard for vertebral fracture detection and differentiation, LVA is a more practical screening tool for the detection of patients with grades 2 and 3 vertebral fractures. LVA has only one tenth the radiation exposure of a set of spinal x-rays. The presence of vertebral fractures here on LVA is important as it identifies a very vulnerable group of patients with low bone mass. This study demonstrates that a previous history of a hip fracture or glucocorticoid use is strong risk factors for vertebral fractures. Previous studies have suggested that patients with vertebral fractures appear to be at higher risk of subsequent fractures requiring active osteoporosis treatment. Lateral vertebral assessment serves a very valuable function by quickly establishing the presence or absence of unappreciated vertebral fractures and allows a physician to prescribe optimal therapy for that patients’ osteoporosis. We recommend that LVA should be a routine part of a DXA study in all osteoporosis treatment units. LVA may be an efficient screening tool for osteoporosis clinical studies.
Chapter 4. The Performance of Calcaneal Quantitative Ultrasound and Dual-energy X-ray Absorptiometry in the Discrimination of Prevalent Osteoporotic Fractures

4.2 Introduction

Bone mineral density (BMD), measured by dual x-ray absorptiometry (DXA) is the best predictor of fracture risk in clinical practice and is considered the “gold standard” for diagnosis osteoporosis [297]. However, due to relatively high costs, poor awareness for osteoporosis and a lack of availability of DXA, only a small number of women with osteoporosis are diagnosed by DXA [209]. Due to its low cost, portability and non-ionizing radiation, quantitative ultrasound (QUS) of the heel is an alternative tool in the assessment of bone health in addition to dual X-ray absorptiometry (DXA).

Quantitative ultrasound (QUS) of the heel has been used as an alternative to the assessment of bone health and to predict future risk of fracture in several studies [298-304]. Calcaneal quantitative ultrasound (QUS) is a diagnostic test that assesses the density and quality of bone in the heel. Several prospective studies have shown QUS predicts future fracture risk, including hip fractures, independently of BMD, and nearly as well as DXA [210-218]. The use of clinical risk factors and calcaneal quantitative ultrasound can correctly identify more women at low risk fracture for hip fracture compared to either the stiffness index on QUS or the clinical risk factors alone [219]. More recently, the EPISEM study demonstrated that the combination of the predictive power derived from clinical risk factors (CRFs) and QUS measurements can be used to predict 10-year probability of hip fracture [305, 306]. To date, there are no published studies from Ireland looking at heel ultrasound in the
diagnosis of osteoporosis and its discriminating ability in patients with prevalent fractures.

We aim in this chapter to compare the ability of heel ultrasound and DXA in discriminating patients with hip, wrist and vertebral fractures who were attending the osteoporosis clinic in St James’s Hospital and derive a QUS T-score that would provide an optimal threshold for the diagnosis of fracture risk.

4.3 Materials and Methods

Subjects

Four hundred and ninety three patients, both male and female, were assessed who had been referred to the bone health and osteoporosis clinic in St James’s Hospital, Dublin. Patients with a history of fracture were included in the study, along with DXA and QUS measurements. A fracture was considered osteoporotic if the fracture occurred with minimal trauma (i.e. a fall from a standing height). Four groups of patients with fractures were identified:

(1) Hip fracture,
(2) Wrist fracture,
(3) Known vertebral fracture and
(4) LVA identified vertebral fractures.

Patients referred with known vertebral fractures (i.e. symptomatic or incidental findings on radiology) were included in the known vertebral fracture group. The LVA identified vertebral fracture group included patients from the known vertebral group
and also those identified as having an asymptomatic Genant grade 2 or higher vertebral fracture on lateral vertebral assessments.

**Densitometry measurements**

BMD was determined by a Lunar Prodigy DXA system (Lunar Corp., Madison, WI) and QUS (Achilles Insight). A full description of DXA and QUS measurements are described in appendices 11.3.

**BMD Measurement and Vertebral Fractures**

The variables used for analysis were BMD (g/cm²) and T score, expressed in SDs (difference between the patients BMD and young normal BMD). We used the total hip T-score to categorize subjects as normal ($T > -1$), osteopaenic ($-2.5 < T < -1$) or osteoporotic ($T < -2.5$). The DXA scans were obtained by standard procedures supplied by the manufacturer for scanning and analysis. For the DXA scan, daily quality control was carried out by measurement of a Lunar phantom.

Lateral vertebral assessments (LVA) using DXA were performed at the same time of BMD measurement. LVA was classified using a combination of visual and Genant semi quantitative (SQ) approach in the following manner: each VFA image was inspected visually by one clinician to decide whether it contained a fracture in any of the visualized vertebrae. Each vertebra that was judged as fractured by visual inspection was measured using built-in morphometry and assigned a grade based on Genant SQ scale, where grade 1 (mild) fracture is a reduction in vertebral height of 20–25%, grade 2 (moderate) a reduction of 26–40%, and grade 3 (severe) a reduction of over 40%. Subjects with no fractures were included in the non-vertebral fracture
group, whereas those with grade 2 or higher fractures were included in the LVA identified vertebral fracture group.

**Heel Ultrasound (QUS)**

We used the Achilles Insight (General Electric) for QUS. This system consists of two transducers, one being the transmitter and the other the receiver; ultrasound passes through the centre of the heel. The acoustic coupling between the two transducers is obtained by placing gel between the two membranes, at lateral sites, containing water at a temperature of 35°C.

The primary QUS variables are speed of sound (SOS) and broadband ultrasound attenuation (BUA), but the Achilles uses these to calculate a stiffness index based on normalized BUA and SOS, where \( n\text{BUA} = 0.67 \times (\text{BUA} -50) \) and \( n\text{SOS} = 0.28 \times (\text{SOS} - 1,380) \). Stiffness is the addition of \( n\text{BUA} \) and \( n\text{SOS} \). Stiffness corresponds to \((0.67 \times \text{BUA} + 0.28 \times \text{SOS} - 420)\).
4.4 Results

493 patients were assessed over a 3-year period between January 2008 and December 2010. The mean age of all patients was 67.2 (+/-14.0) years. They were predominantly female (n=401, 80.8%). Table 1 shows their basic demographic data. Of all patients in the study, 288 (58.4%) patients were osteoporotic at the spine and 152 (30.8%) were osteoporotic at the hip. Overall, 312 (63.3%) patients had osteoporosis at any site. 371 (74.8%) of patients had a history of any fracture.

<table>
<thead>
<tr>
<th>Table 4.1 Characteristics of the population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Group (n=493)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>History of Fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Fracture</td>
</tr>
<tr>
<td>Hip Fracture</td>
</tr>
<tr>
<td>Vertebral Fracture</td>
</tr>
<tr>
<td>LVA Vertebral Fracture</td>
</tr>
<tr>
<td>Wrist Fracture</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heel Ultrasound</th>
</tr>
</thead>
<tbody>
<tr>
<td>T Score</td>
</tr>
<tr>
<td>BUA</td>
</tr>
<tr>
<td>SOS Stiffness</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DXA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD Total Hip</td>
</tr>
<tr>
<td>T Score Hip</td>
</tr>
<tr>
<td>BMD Neck Hip</td>
</tr>
<tr>
<td>T Score Neck</td>
</tr>
<tr>
<td>BMD Spine</td>
</tr>
<tr>
<td>T Score Spine</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
</tr>
<tr>
<td><strong>Female (n, %)</strong></td>
</tr>
<tr>
<td><strong>BMD LS</strong></td>
</tr>
<tr>
<td><strong>T Score LS</strong></td>
</tr>
<tr>
<td><strong>BMD Hip</strong></td>
</tr>
<tr>
<td><strong>T Score Hip</strong></td>
</tr>
<tr>
<td><strong>% Osteoporosis</strong></td>
</tr>
<tr>
<td><strong>QUS BUA</strong></td>
</tr>
<tr>
<td><strong>QUS T Score</strong></td>
</tr>
<tr>
<td><strong>QUS SOS</strong></td>
</tr>
<tr>
<td><strong>QUS T Score &lt; -2</strong></td>
</tr>
</tbody>
</table>
As can be seen in table 2, patients with hip and vertebral fractures were older than those with no history of fracture or wrist fracture. There were predominantly female patients in all fracture groups and those with a history of wrist fracture had the number of female patients.

The analysis of ROC values in tables 4-6 showed that there were variations in discriminating thresholds of fractures when DXA or QUS measurement techniques were compared. DXA measurements had higher ROC values compared to QUS measurements for vertebral fractures. QUS was better at discriminating wrist and hip fractures when compared to DXA measurements, particularly in female patients.

The optimal T score (the T score highest sensitivity and specificity) for heel ultrasound varied between -2.2 and -3.0 depending on the fracture type as seen in table 4. For women, this variation was between -2.5 for LVA identified vertebral fractures and -3 for patients with known vertebral fracture.

Table 7 shows the age-adjusted relative risk for predicting fractures at various heel ultrasound and DXA thresholds. Heel ultrasound thresholds of <-2 and <-3 had higher

---

**Table 4.3 Correlation between QUS parameters and DXA measurements**

<table>
<thead>
<tr>
<th></th>
<th>BMD Total Hip</th>
<th>Total Hip T Score</th>
<th>BMD Neck T Score</th>
<th>BMD Spine Spine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heel U/S</td>
<td>0.60*</td>
<td>0.60*</td>
<td>0.59*</td>
<td>0.48*</td>
</tr>
<tr>
<td>Heel BUA</td>
<td>0.60*</td>
<td>0.59*</td>
<td>0.61*</td>
<td>0.47*</td>
</tr>
<tr>
<td>Heel SOS</td>
<td>0.47*</td>
<td>0.49*</td>
<td>0.44*</td>
<td>0.43*</td>
</tr>
<tr>
<td>Heel Stiff</td>
<td>0.47*</td>
<td>0.49*</td>
<td>0.44*</td>
<td>0.43*</td>
</tr>
</tbody>
</table>

*P<0.01
relative risks compared to DXA measurements for fractures for hip and wrist fractures in all patients.
**Table 4.4** Relationship between sensitivity and specificity of bone mineral density (BMD) and heel ultrasound measurements in identifying patients with prevalent fractures in female patients using logistic regression and receiver operating characteristics (ROC).

<table>
<thead>
<tr>
<th></th>
<th>Hip Fracture</th>
<th>Vertebral Fracture (Known)</th>
<th>LVA identified Vertebral Fracture</th>
<th>Wrist Fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heel T Score</td>
<td>0.67</td>
<td>0.59</td>
<td>0.63</td>
<td>0.58</td>
</tr>
<tr>
<td>Heel BUA</td>
<td>0.67</td>
<td>0.57</td>
<td>0.60</td>
<td>0.58</td>
</tr>
<tr>
<td>Hip T Score</td>
<td>0.67</td>
<td>0.63</td>
<td>0.68</td>
<td>0.50</td>
</tr>
<tr>
<td>Spine T Score</td>
<td>0.52</td>
<td>0.58</td>
<td>0.61</td>
<td>0.51</td>
</tr>
<tr>
<td>Heel U/S T Score Optimum</td>
<td>-2.2</td>
<td>-3.0</td>
<td>-2.5</td>
<td>-2.9</td>
</tr>
</tbody>
</table>

**Table 4.5** Relationship between sensitivity and specificity of bone mineral density (BMD) and heel ultrasound measurements in identifying patients with prevalent fractures in male patients using logistic regression and receiver operating characteristics (ROC).

<table>
<thead>
<tr>
<th></th>
<th>Hip Fracture</th>
<th>Vertebral Fracture (Known)</th>
<th>LVA identified Vertebral Fracture</th>
<th>Wrist Fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heel T Score</td>
<td>0.72</td>
<td>0.60</td>
<td>0.65</td>
<td>0.57</td>
</tr>
<tr>
<td>Heel BUA</td>
<td>0.72</td>
<td>0.58</td>
<td>0.62</td>
<td>0.57</td>
</tr>
<tr>
<td>Hip T Score</td>
<td>0.67</td>
<td>0.63</td>
<td>0.69</td>
<td>0.51</td>
</tr>
<tr>
<td>Spine T Score</td>
<td>0.54</td>
<td>0.60</td>
<td>0.63</td>
<td>0.51</td>
</tr>
<tr>
<td>Heel U/S T Score Optimum</td>
<td>-2.7</td>
<td>-3.1</td>
<td>-2.5</td>
<td>-2.9</td>
</tr>
</tbody>
</table>
Table 4.6 Relationship between sensitivity and specificity of bone mineral density (BMD) and heel ultrasound measurements in identifying patients with prevalent fractures in all patients using logistic regression and receiver operating characteristics (ROC).

<table>
<thead>
<tr>
<th></th>
<th>Hip Fracture</th>
<th>Vertebral Fracture (known)</th>
<th>LVA identified Vertebral Fracture</th>
<th>Wrist Fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heel T Score</td>
<td>0.61</td>
<td>0.61</td>
<td>0.61</td>
<td>0.55</td>
</tr>
<tr>
<td>Heel BUA</td>
<td>0.65</td>
<td>0.59</td>
<td>0.58</td>
<td>0.49</td>
</tr>
<tr>
<td>Hip T Score</td>
<td>0.72</td>
<td>0.63</td>
<td>0.66</td>
<td>0.47</td>
</tr>
<tr>
<td>Spine T Score</td>
<td>0.49</td>
<td>0.55</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>Heel U/S T Score Optimum</td>
<td>-2.3</td>
<td>-0.1</td>
<td>-3.0</td>
<td>-1.0</td>
</tr>
</tbody>
</table>

Table 4.7 Age-adjusted relative risk of fracture types using QUS and BMD Measurements.

<table>
<thead>
<tr>
<th>Site Measured</th>
<th>Hip Fracture</th>
<th>Vertebral Fracture (Known)</th>
<th>Vertebral Fracture (LVA)</th>
<th>Wrist Fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall DXA T score &lt;-2.5</td>
<td>1.02 (0.97 – 1.64)</td>
<td>1.67 (1.59 – 1.74)¹</td>
<td>1.51 (1.45 – 1.57)¹</td>
<td>1.00 (0.97 – 1.03)</td>
</tr>
<tr>
<td>Lumbar Spine T Score &lt;-2.5</td>
<td>0.84 (0.80 – 0.87)</td>
<td>1.67 (1.60 – 1.75)¹</td>
<td>1.52 (1.46 – 1.57)¹</td>
<td>1.00 (0.96 – 1.03)</td>
</tr>
<tr>
<td>Total Hip T Score &lt;-2.5</td>
<td>1.74 (1.66 – 1.82)¹</td>
<td>1.61 (1.55 – 1.67)¹</td>
<td>2.00 (1.93 – 2.06)¹</td>
<td>1.07 (1.03 – 1.10)¹</td>
</tr>
<tr>
<td>QUS T Score &lt;-2</td>
<td>2.00 (1.89 – 2.11)¹</td>
<td>1.44 (1.37 – 1.51)¹</td>
<td>1.73 (1.67 – 1.80)¹</td>
<td>1.37 (1.32 – 1.42)¹</td>
</tr>
<tr>
<td>QUS T Score &lt;-3</td>
<td>2.31 (2.21 – 2.42)¹</td>
<td>1.63 (1.57 – 1.70)¹</td>
<td>1.69 (1.63 – 1.75)¹</td>
<td>1.34 (1.30 – 1.39)¹</td>
</tr>
</tbody>
</table>

¹p<0.001
4.5 Discussion

Heel ultrasound and DXA have been used to identify subjects at risk of hip and vertebral fractures in patients referred to the osteoporosis clinic. One of the important reasons for assessing bone health status is to identify people who are at risk of sustaining fragility fractures. It is expected that as the number of people living longer increases in the coming decades, there will also be an increase in the number of people at risk of fragility fractures. Thus, in our population, heel ultrasound may be a useful alternative for predicting and screening those people at risk of fragility fractures. Many cross-sectional and prospective studies have demonstrated that calcaneus ultrasound indicates risk of osteoporotic fractures even after correction for BMD [298-304]. This study assesses the ability of two separate imaging techniques to discriminate between patients with and without prevalent fractures. Both DXA and heel ultrasound performed similarly in discriminating most fractures, however, heel ultrasound was better at discriminating both hip and wrist fractures in female patients when compared to DXA.

In this study, we found that QUS and BMD by DXA had differences in discriminating patients with prevalent fractures. This is demonstrated in tables 4-6, with higher ROC values indicating a higher sensitivity and specificity in the testing method. In all patients (table 4), QUS was a better discriminator of wrist fractures compared to DXA measurements. For female patients, QUS was able to discriminate hip and wrist fractures better than DXA. However, in male patients BMD measurements were better at discriminating hip fractures compared the heel ultrasound measurements. We found that heel ultrasound was better at discriminating osteoporotic fractures compared to DXA measurements, as indicated by the relative risk and ROC curves in
table 4-6. Other cross-sectional studies in healthy patients with no prior history of osteoporosis have shown that DXA was better at discriminating other fractures, such as vertebral fractures [307].

Among the aims of our study was to find an optimum T score for QUS. Although the literature supports the clinical application of QUS, there is no general agreement about where the T-score threshold should be. In our study, as can be seen in tables 4-6, the QUS T-score giving the best diagnostic accuracy varied between the fracture types. Our study confirms that different T-scores should be applied to different fracture types in order to discriminate prevalent fractures. This may reflect the age and gender differences for particular fracture types and should be recognised in assessing osteoporosis patients at risk of fracture.

There are some strengths and points noted in our study. Similar to DXA, heel ultrasound results can be influenced by local anatomical variations or operator training. This may be overcome by having highly trained operating staff as in our osteoporosis clinic. We have used a cross-sectional rather than longitudinal design to examine the relationship between densitometry and fracture. One commercial QUS imaging device has been assessed; and therefore, this one device may not be representative of other imaging devices or QUS imaging technology. However, the subjects in our sample were recruited from patients attending for fracture risk assessment in our bone health clinic and may be more representative of the population at risk for osteoporosis and fractures.
In summary, heel ultrasound appears to perform similarly to DXA in discriminating prevalent fractures however there are differences in gender and fracture type. As this is a cross-sectional study in patients with risk factors for osteoporosis and fractures, it would be necessary to assess the technique in healthy older Irish adults in a longitudinal setting to assess its ability in predicting fractures. Similarly this has been performed in several longitudinal studies, such as the EPIDOS and SEMOF studies [210, 308], but not in an Irish population to date.
Chapter 5. The Prevalence of Vitamin D Deficiency in Irish Osteoporotic and Osteopaenic Patients and its Effects on Bone Metabolism

5.1 Introduction

Vitamin D deficiency is being recognised as a major health problem for adults over the age of 50 years [95] and higher levels have been associated with optimal bone health, regulating bone turnover and reduced falls [93, 309, 310]. Vitamin D is required for the efficient absorption of calcium and for the normal mineralisation of bone [139]. Serum 25-hydroxyvitamin D (25(OH)D) is the most common biomarker used to assess vitamin D status. Low 25(OH)D levels have been associated with an increased risk of falls and fractures in older adults. This is thought to be mediated by its effects on bone metabolism and by contributing to an increase risk of falling. The risk of falling may be in part related to muscle weakness and to changes in balance [137, 311]. The importance of vitamin D status for optimal bone health has received increased recognition in recent years, with higher recommended intake levels being proposed by some investigators [143]. Despite this, there is a lack of consensus on the serum 25(OH)D concentration that reflects optimal vitamin D status, but a serum 25(OH)D concentrations > 75nmol/L has been suggested as an optimal level [145].

Several studies have observed an increased serum PTH concentration in elderly people with or without hip fracture associated with vitamin D deficiency [141, 312, 313], which have been documented as having a negative impact on functional recovery of patients who have sustained a hip fracture [314]. Patients with hypovitaminosis D and secondary hyperparathyroidism compared to those without elevated PTH levels have increased bone turnover [315-318], increased fracture risk
and a higher mortality rate, especially cardiovascular deaths [316, 319]. Previous cross-sectional studies have shown that low levels of serum 25(OH)D were associated with reduced muscle strength [320, 321], reduced ability to perform activities of daily living [322] and an increase in body sway and falls [322-325]. Vitamin D receptors for 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) have been identified in human skeletal muscle [317], with higher levels of serum 1,25(OH)₂D₃ being associated with a reduced fall rate in older community-dwelling women [326]. Another study has shown that patients with vitamin D deficiency at baseline assessment were associated with a significantly increased risk of sarcopenia [319, 327].

The focus of this chapter is to assess the prevalence of vitamin D deficiency in patients attending a specialised osteoporosis clinic and the effects on parathyroid hormone levels.
5.2 Methodology

Osteoporosis Clinic Referrals

This group consisted of consecutive patients who had been referred to the Bone Health and Osteoporosis clinic for assessment of osteoporosis risk factors and biochemistry. Patients are referred to the clinic in three ways: (1) Referral from general practitioner/hospital consultant, (2) Referral from the fracture liaison service and (3) identification of severe osteoporosis from DXA reports. Patients were consented and a questionnaire was completed including risk factors for osteoporosis (See Appendix),

Serum Biochemistry

Serum Bone Markers

Fasting early morning venous blood was collected from patients for the measurement of 25(OH)D, calcium, creatinine and parathyroid hormone (PTH) levels. Samples were stored at -20°C. Serum 25(OH)D was assayed with a radioimmunoassay (RIA) kit (Diasorin, Stillwater, MN, USA) at the Department of Biochemistry, St James's Hospital, Dublin 8. Serum vitamin D status was classified using the cut off criteria outlined by International Osteoporosis Foundation [328]. A serum 25(OH)D less than 50 nmol/L was considered to be vitamin D deficient, levels between 50-75 nmol/L were classified as vitamin D insufficient, and levels >75 nmol/L were considered optimal. The mean 25(OH)D levels are presented for type of supplementation and season for each of the defined groups.
Statistical analysis

Descriptive statistics (means and frequencies) were used to assess the extent of vitamin D deficiency. The unpaired Student’s t test was used to compare means of 25(OH)D between gender and supplementation groups. The analysis of variance (ANOVA) was used to compare between age groups, supplementation types and season. To test for statistical significance between categorical variables, the chi-square test was used. P values of less than 0.05 were considered statistically significant for all tests.
5.3 Results

780 patients were assessed attending the bone health and osteoporosis clinic between January 2008 and December 2010. Baseline characteristics of the combined group are described in Table 1. All patients were Caucasians in their ethnicity. There were predominantly female patients referred to the osteoporosis clinic compared to male patients (649 and 124, respectively). Mean age of the group was 67.5 (+/-15.3) years. Table 2 compares the baseline characteristics between male and female patients.

Vitamin D Supplementation and Bone Treatment

278 (35.6%) patients in overall group were not on vitamin D supplements. Among the 502 (64.4%) supplemented patients, the most common of supplementation dosage was cholecalciferol (Vitamin D₃) 800IU/day (n=385), cholecalciferol 400 IU/day (n=60), 4 patients were on cholecalciferol 1000IU/day, 13 patients were on cholecalciferol 1200IU/day and a further 3 on cholecalciferol 1600IU/day. For the purposes of the analysis, the patients on 1000, 1200, and 1600 IU cholecalciferol per day have been combined into a single group (>1000IU/day). Tables 1 and 2 summarise the baseline characteristics of the study group.

337 (43.2%) patients had been on prior bone protection (bosphosphonates, strontium ranelate and hormone replacement therapy) at the time of referral. Bisphosphonates were the most prescribed medication (n=313), with strontium ranelate (n=24) and HRT (n=54). As can be seen in table 2, female patients compared to male patients at assessment were statistically more likely to be on
osteoporosis treatment on baseline assessment, either secondary bone protection or calcium and vitamin D (p=0.0001 and p=0.03, respectively).

**Serum 25(OH)D Levels**

Table 2 demonstrates the mean serum 25(OH)D for the study group. The mean 25(OH)D level for entire group was 57.6 (+/-29.9). Overall, 301 (38.6%) patients were not on vitamin D supplementation at baseline. There were more females patients on vitamin D supplementation compared to males. 65 (50.8%) of men compared to 236 (36.2%) females were not on vitamin D supplementation at baseline (p=0.04).

Overall, 25(OH)D levels <25 and <50 nmol/L were found in 16.2% and 42.6% of patients who attended the clinic. Levels >75nmol/L were detected in only 29.0% of participants. The mean serum 25(OH)D level seen in female patients was 58.5 (+/-30.3) and for male patients the mean serum 25(OH)D level was 53.0 (+/-27.9), although more female patients were on vitamin D supplementation at the time of referral (73.8% vs 49.2%, p=0.04).

Table 3 demonstrates mean serum 25(OH)D levels of patients according to type of vitamin D supplementation. The non-supplemented patients had a mean serum 25(OH)D level of 35.9 (+/-21.0) nmol/L. With increasing vitamin D supplementation doses, there was a significant increase in mean serum 25(OH)D levels (p<0.01) and a reciprocal decrease in mean serum PTH levels (p<0.01). There was no statistical significant change in mean serum calcium levels.
Figure 1 demonstrates the changes in mean PTH levels according to age and vitamin D levels. Patients with the highest PTH levels tended to be older and in the lowest tertile of vitamin D levels (10-40 nmol/L) as compared to the highest vitamin D tertile (70-169 nmol).

**Seasonal Variation and 25(OH)D Levels**

Table 4 demonstrates the seasonal variation of serum 25(OH)D levels according to season. There was a statistical variation of serum 25(OH)D levels seen in all groups studied, with highest levels of 25(OH)D being seen in Autumn months compared to Spring months. PTH levels tended to be higher in spring compared to autumn months, however, this was not statistically significant (p<0.05) in any of the groups studied.

**Bone Turnover Markers and 25(OH)D Levels**

Table 5 demonstrates bone turnover (Ctx, osteocalcin and P1NP) and its relationship to serum 25(OH)D levels. Increased bone turnover was significantly increased in all groups, particularly those who were not on anti—osteoporosis medication at baseline (p<0.01).
<table>
<thead>
<tr>
<th>Characteristic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), Mean (SD)</td>
<td>67.5 (+/- 15.3)</td>
</tr>
<tr>
<td>- Male (n=124)</td>
<td>63.2 (+/- 15.3)</td>
</tr>
<tr>
<td>- Female (n=619)</td>
<td>67.6 (+/- 14.1)</td>
</tr>
<tr>
<td>Age (years), n (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>272 (36.6)</td>
</tr>
<tr>
<td>65-74</td>
<td>212 (28.5)</td>
</tr>
<tr>
<td>75-84</td>
<td>197 (26.5)</td>
</tr>
<tr>
<td>&gt;85</td>
<td>62 (8.3)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>619 (84.5)</td>
</tr>
<tr>
<td>Fracture History, n (%)</td>
<td></td>
</tr>
<tr>
<td>- Any fracture</td>
<td>563 (75.7)</td>
</tr>
<tr>
<td>- Hip fracture</td>
<td>154 (20.7)</td>
</tr>
<tr>
<td>- Wrist fracture*</td>
<td>218 (30.2)</td>
</tr>
<tr>
<td>- Vertebral fracture</td>
<td>169 (22.7)</td>
</tr>
<tr>
<td>Vitamin D Supplementation, n (%)</td>
<td></td>
</tr>
<tr>
<td>- None</td>
<td>278 (37.4)</td>
</tr>
<tr>
<td>- Vitamin D3 400IU/day</td>
<td>60 (8.1)</td>
</tr>
<tr>
<td>- Vitamin D3 800IU/day</td>
<td>385 (51.8)</td>
</tr>
<tr>
<td>- Vitamin D3 1000IU/day</td>
<td>4 (0.5)</td>
</tr>
<tr>
<td>- Vitamin D3 1200IU/day</td>
<td>13 (1.8)</td>
</tr>
<tr>
<td>- Vitamin D3 1600IU/day</td>
<td>3 (0.4)</td>
</tr>
<tr>
<td>Prior osteoporosis treatment, n (%)</td>
<td></td>
</tr>
<tr>
<td>- None</td>
<td>406 (54.6)</td>
</tr>
<tr>
<td>- Bisphosphonate</td>
<td>313 (42.1)</td>
</tr>
<tr>
<td>- Strontium</td>
<td>24 (3.2)</td>
</tr>
<tr>
<td>- HRT**</td>
<td>54 (8.7)</td>
</tr>
<tr>
<td>Height (cm), Mean (SD)</td>
<td>158.9 (+/- 9.5)</td>
</tr>
<tr>
<td>Weight (kg), Mean (SD)</td>
<td>63.4 (+/- 15.2)</td>
</tr>
</tbody>
</table>

**Table 5.1 Baseline Demographics of Patients referred to the Osteoporosis clinic.**

*incomplete data in 22 patients

** Calculated for percentage females, n=619
<table>
<thead>
<tr>
<th></th>
<th>Overall (n=780)</th>
<th>Female (n=652)</th>
<th>Male (n=128)</th>
<th>Comparison female and male p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>67.4 (+/-15.0)</td>
<td>68.2 (+/-16.4)</td>
<td>63.4 (+/-14.9)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Gender (n, % Female)</td>
<td>652 (83.6%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Previous Fracture</td>
<td>592 (75.9%)</td>
<td>485 (74.4%)</td>
<td>107 (83.6%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hip Fracture</td>
<td>155 (19.9%)</td>
<td>119 (18.3%)</td>
<td>36 (28.1%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Vertebral Fracture</td>
<td>170 (21.8%)</td>
<td>138 (21.2%)</td>
<td>32 (25.0%)</td>
<td>0.34**</td>
</tr>
<tr>
<td>Colles Fracture</td>
<td>232 (29.7%)</td>
<td>209 (32.1%)</td>
<td>23 (17.9%)</td>
<td>0.01**</td>
</tr>
<tr>
<td>Osteoporosis Treatments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>389 (49.9%)</td>
<td>348 (53.4%)</td>
<td>95 (74.2%)</td>
<td>0.01**</td>
</tr>
<tr>
<td>Bisphosphonate</td>
<td>313 (40.1%)</td>
<td>283 (43.4%)</td>
<td>30 (23.4%)</td>
<td></td>
</tr>
<tr>
<td>Strontium Ranelate</td>
<td>24 (3.1%)</td>
<td>21 (3.2%)</td>
<td>3 (2.3%)</td>
<td></td>
</tr>
<tr>
<td>HRT</td>
<td>54 (8.7%)</td>
<td>54 (8.7%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Number of Patients on Vitamin D Supplementation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>301 (38.6%)</td>
<td>236 (36.2%)</td>
<td>65 (50.8%)</td>
<td>0.03**</td>
</tr>
<tr>
<td>400 IU/day</td>
<td>63 (8.1%)</td>
<td>57 (8.7%)</td>
<td>6 (4.6%)</td>
<td></td>
</tr>
<tr>
<td>800 IU/day</td>
<td>396 (50.8%)</td>
<td>342 (52.6%)</td>
<td>34 (22.8%)</td>
<td></td>
</tr>
<tr>
<td>&gt;1000 IU/day</td>
<td>20 (2.5%)</td>
<td>17 (2.6%)</td>
<td>3 (2.3%)</td>
<td></td>
</tr>
<tr>
<td>Ultrasound Heel T Score</td>
<td>-2.1 (+/-1.6)</td>
<td>-2.2 (+/-1.5)</td>
<td>-1.3 (+/-1.8)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Mean 25(OH)D (nmol/L)</td>
<td>57.6 (+/-29.9)</td>
<td>58.5 (+/-30.3)</td>
<td>53.0 (+/-27.9)</td>
<td>0.06*</td>
</tr>
<tr>
<td>&lt;25 nmol/L</td>
<td>128 (16.4%)</td>
<td>104 (16.0%)</td>
<td>104 (16.0%)</td>
<td>0.43**</td>
</tr>
<tr>
<td>25-50 nmol/L</td>
<td>204 (26.2%)</td>
<td>165 (25.3%)</td>
<td>24 (18.8%)</td>
<td></td>
</tr>
<tr>
<td>50-75 nmol/L</td>
<td>222 (28.3%)</td>
<td>190 (29.1%)</td>
<td>32 (25.0%)</td>
<td></td>
</tr>
<tr>
<td>&gt;75 nmol/L</td>
<td>226 (29.0%)</td>
<td>193 (29.6%)</td>
<td>33 (25.8%)</td>
<td></td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>44.8 (+/-28.8)</td>
<td>45.4 (+/-30.1)</td>
<td>41.7 (+/-20.5)</td>
<td>0.19*</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.3 (+/-0.1)</td>
<td>2.3 (+/-0.1)</td>
<td>2.3 (+/-0.1)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.0 (+/-0.2)</td>
<td>1.0 (+/-0.2)</td>
<td>0.9 (+/-0.2)</td>
<td>0.10*</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.8 (+/-0.1)</td>
<td>0.8 (+/-0.1)</td>
<td>0.8 (+/-0.1)</td>
<td>0.83*</td>
</tr>
</tbody>
</table>

*Table 5.2 Baseline characteristics of sample population according to gender

*Independent samples t test (ANOVA) **Pearson Chi-Sq test
<table>
<thead>
<tr>
<th></th>
<th>Non-supplemented (n=301)</th>
<th>Vitamin D 400IU/day (n=63)</th>
<th>Vitamin D 800IU/day (n=396)</th>
<th>Vitamin D &gt;1000IU/day (n=20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>67.3 (+/-14.7)</td>
<td>65.4 (+/-16.4)</td>
<td>68.0 (+/-14.9)</td>
<td>64.0 (+/-18.0)</td>
<td>0.43**</td>
</tr>
<tr>
<td>Gender (% Female)</td>
<td>80.8%</td>
<td>97.6%</td>
<td>88.0%</td>
<td>100%</td>
<td>0.02**</td>
</tr>
<tr>
<td>Previous Fracture</td>
<td>80.4%</td>
<td>73.0%</td>
<td>73.2%</td>
<td>70.0%</td>
<td>0.14**</td>
</tr>
<tr>
<td>Hip Fracture</td>
<td>21.6%</td>
<td>19.1%</td>
<td>18.7%</td>
<td>20.0%</td>
<td>0.82**</td>
</tr>
<tr>
<td>Vertebral Fracture</td>
<td>22.3%</td>
<td>28.6%</td>
<td>19.9%</td>
<td>30.0%</td>
<td>0.35**</td>
</tr>
<tr>
<td>Colles Fracture</td>
<td>29.9%</td>
<td>27.0%</td>
<td>30.3%</td>
<td>25.0%</td>
<td>0.27**</td>
</tr>
<tr>
<td>Ultrasound Heel T Score</td>
<td>-2.0 (+/-1.6)</td>
<td>-2.4 (+/-1.5)</td>
<td>-2.1 (+/-1.6)</td>
<td>-2.1 (+/-1.6)</td>
<td>0.48*</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>35.9 (+/-21.0)</td>
<td>47.9 (+/-23.0)</td>
<td>73.2 (+/-23.2)</td>
<td>108.6 (+/-35.0)</td>
<td>0.01*</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>51.1 (+/-31.0)</td>
<td>48.8 (+/-40.8)</td>
<td>39.6 (+/-23.9)</td>
<td>38.6 (+/-14.0)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.3 (+/-0.1)</td>
<td>2.3 (+/-0.1)</td>
<td>2.3 (+/-0.1)</td>
<td>2.3 (+/-0.1)</td>
<td>0.16*</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.0 (+/-0.2)</td>
<td>1.0 (+/-0.2)</td>
<td>1.0 (+/-0.2)</td>
<td>1.1 (+/-0.2)</td>
<td>0.63*</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.9 (+/-0.1)</td>
<td>0.9 (+/-0.1)</td>
<td>0.8 (+/-0.1)</td>
<td>0.9 (+/-0.1)</td>
<td>0.23*</td>
</tr>
</tbody>
</table>

**Table 5.3 Characteristics of population according to Vitamin D Supplementation.**

*Independent samples t test (ANOVA). (Standard deviation in brackets)

**Pearson Chi-Sq test
<table>
<thead>
<tr>
<th></th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean 25(OH)D (nmol/L)</td>
<td>48.1 (+/-29.2)</td>
<td>60.6 (+/-29.8)</td>
<td>62.7 (+/-29.2)</td>
<td>59.2 (+/-29.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean PTH (pg/mL)</td>
<td>48.1 (+/-38.1)</td>
<td>44.6 (+/-23.6)</td>
<td>42.5 (+/-21.2)</td>
<td>44.1 (+/-29.1)</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Non-supplemented</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean 25(OH)D (nmol/L)</td>
<td>30.9 (+/-19.8)</td>
<td>40.0 (+/-20.2)</td>
<td>41.9 (+/-23.7)</td>
<td>32.4 (+/-18.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean PTH (pg/mL)</td>
<td>53.7 (+/-39.2)</td>
<td>50.6 (+/-24.7)</td>
<td>48.5 (+/-22.6)</td>
<td>51.8 (+/-31.7)</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Vitamin D 400 IU/day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean 25(OH)D (nmol/L)</td>
<td>40.5 (+/-22.6)</td>
<td>38.8 (+/-17.3)</td>
<td>53.4 (+/-21.5)</td>
<td>65.9 (+/-23.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean PTH (pg/mL)</td>
<td>69.5 (+/-71.3)</td>
<td>42.8 (+/-21.1)</td>
<td>41.3 (+/-24.4)</td>
<td>40.5 (+/-9.9)</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Vitamin D 800IU/day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean 25(OH)D (nmol/L)</td>
<td>66.8 (+/-24.5)</td>
<td>75.0 (+/-25.3)</td>
<td>75.8 (+/-19.4)</td>
<td>74.0 (+/-23.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean PTH (pg/mL)</td>
<td>38.7 (+/-23.7)</td>
<td>41.7 (+/-23.5)</td>
<td>38.0 (+/-18.9)</td>
<td>40.1 (+/-28.5)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*Table 5.4* Seasonal variability in all subgroups of patients including non-supplemented or supplemented patients.
<table>
<thead>
<tr>
<th>Overall Group</th>
<th>Lowest Tertile Vitamin D (10 - 40 nmol/L)</th>
<th>Middle Tertile Vitamin D (40 - 70 nmol/L)</th>
<th>Highest Tertile Vitamin D (70 - 169 nmol/L)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid Hormone (pg/mL)</td>
<td>57.0 (+/-36.9)</td>
<td>41.4 (+/-21.8)</td>
<td>36.6 (+/-21.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>CTx (ng/mL)</td>
<td>0.42 (+/-0.28)</td>
<td>0.31 (+/-0.22)</td>
<td>0.23 (+/-0.16)</td>
<td>0.01</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>28.0 (+/-18.9)</td>
<td>22.5 (+/-13.6)</td>
<td>19.4 (+/-11.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>P1NP (ng/mL)</td>
<td>58.3 (+/-40.6)</td>
<td>49.2 (+/-36.0)</td>
<td>39.7 (+/-29.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>No Prior Anti-Osteoporosis Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parathyroid hormone (pg/mL)</td>
<td>55.9 (+/-34.4)</td>
<td>40.0 (+/-21.2)</td>
<td>32.8 (+/-15.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>CTx (ng/mL)</td>
<td>0.45 (+/-0.29)</td>
<td>0.37 (+/-0.21)</td>
<td>0.29 (+/-0.16)</td>
<td>0.01</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>28.9 (+/-19.0)</td>
<td>25.5 (+/-12.6)</td>
<td>21.9 (+/-11.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>P1NP (ng/mL)</td>
<td>64.1 (+/-42.0)</td>
<td>54.8 (+/-29.3)</td>
<td>45.7 (+/-28.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Prior Anti-Osteoporosis Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parathyroid hormone (pg/mL)</td>
<td>62.8 (+/-48.0)</td>
<td>43.2 (+/-22.9)</td>
<td>39.8 (+/-24.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>CTx (ng/mL)</td>
<td>0.27 (+/-0.19)</td>
<td>0.24 (+/-0.2)</td>
<td>0.18 (+/-0.13)</td>
<td>0.01</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>24.9 (+/-19.1)</td>
<td>18.9 (+/-14.1)</td>
<td>16.8 (+/-9.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>P1NP (ng/mL)</td>
<td>39.5 (+/-26.5)</td>
<td>42.3 (+/-42.1)</td>
<td>34.3 (+/-27.9)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**Table 5.5** The relationship of serum vitamin D levels and bone turnover markers
Figure 5.1. Mean PTH Levels according to tertiles of serum 25(OH)D levels. Highest mean levels of PTH were seen in older patients with the lowest 25(OH)D levels (n=780).
5.4 Discussion

In this sample of Irish patients, there was a high prevalence of vitamin D deficiency, particularly in those who were not supplemented with oral vitamin D$_3$. The patients taking vitamin D supplementation had a higher mean serum 25(OH)D than those not taking any vitamin D supplementation, however, this was only statistical significant in patients taking 800IU or higher daily doses of vitamin D$_3$ supplementation. Vitamin D supplementation was associated with a mean 25(OH)D increase of 37.34 nmol/L when comparing non supplemented patients with those taking 800IU/day vitamin D$_3$ (cholecalciferol). This effect of vitamin D$_3$ supplementation has been previously seen in recent Irish studies [329-331], which have demonstrated that higher doses of vitamin D$_3$ intakes are needed to increase mean serum 25(OH)D levels.

There is much debate on the optimal level of serum vitamin D (25(OH)D). Recently, the International Osteoporosis Foundation (IOF) have published recommendations for the optimal serum vitamin D level to be above 75nmol/L [143]. This level is based on maximal PTH suppression, reduced bone loss and a reduced rate of falls. The dose of vitamin D supplementation required by a patient would depend on several factors including the baseline level of serum 25(OH)D, body mass index, sun exposure and vitamin D metabolism. For individuals with effective sunlight exposure, a dose of 800 IU/day vitamin D$_3$ may be sufficient. However, patients with obesity, a history of falls, known osteoporosis and limited sun exposure may require higher doses of vitamin D$_3$ up to 2000 IU/daily [143]. In our study, less than a half of the study patients achieved an optimal level of vitamin D on 800 IU vitamin D$_3$/day and would
require a higher vitamin D supplemental dose to achieve an optimal serum level of 25(OH)D. The rate of replacement, mode of administration and the total dose required for reaching optimal levels still remains to be fully established.

A number of factors affect vitamin D levels including lifestyle, sunscreen usage, age and concomitant drug use. Endogenous production of vitamin D relies on 2 factors: solar UVB radiation penetrating the skin and the amount of 7-dehydrocholesterol in the skin. The amount of 7-dehydrocholesterol in skin is relatively constant until later in life, when it begins to decline. A 70 year old person exposed to the same amount of sunlight as a 20-year old person makes approximately 25% of the Vitamin D₃ that the 20-year old person can make [327, 332]. Prolonged sun exposure does not result in the production of excess quantities of vitamin D₃ and may increase the incidence of skin carcinoma [48]. As a result, older patients are more reliant on diet and supplements for adequate maintenance of serum vitamin D. Based on intervention studies, mean serum 25(OH)D levels in a vitamin D supplemented older population would be expected to be at least 50-60 nmol/L with a daily dose of 400 IU to 800 IU vitamin D₃ [138]. The levels in our study were consistent with published literature with a mean serum 25(OH)D levels being 47.9 (+/-23.0) nmol/L in the patients supplemented with 400IU/day vitamin D and a mean serum 25(OH)D of 73.2 (+/-23.2) nmol/L in the patients supplemented with 800IU/day.

Seasonality and latitude are well documented causes of vitamin D deficiency and can influence levels [333]. Our study was performed in Irish Caucasian patients living in a northern latitude (53° North), where sufficient sunshine for vitamin D₃ synthesis is limited to the summer and autumn months. In the non-
supplemented and supplemented groups, seasonality was detected, although, the mean 25(OH)D levels were much higher in the supplemented patients compared to the non-supplemented patients throughout the year. This seasonal variation has also been observed in previous studies of healthy older adults [334, 335]. This observation should be noted when prescribing vitamin D supplementation and is another area for further study in older patients.

Vitamin D deficiency can lead to secondary hyperparathyroidism which has an adverse affect on bone health by increasing bone turnover. Several studies have observed an increased serum PTH concentration in older people with or without fractures were associated with vitamin D deficiency [141, 312, 313]. In our study, serum PTH correlated with levels of serum 25(OH)D, with higher levels of PTH being observed in those with lower vitamin D levels. In our study, mean PTH levels increased with decreasing serum vitamin D levels. Some studies have suggested that patients with the highest levels of PTH in older patients have poorer outcomes post fracture [314]. Patients with an elevated PTH compared to those with serum PTH in the normal range have a 15 times greater risk of fatal outcome and increased hospital stay [314]. Patients with hypovitaminosis D and secondary hyperparathyroidism compared to those without elevated PTH levels have increased bone turnover [315-318], increased fracture risk [315] and a higher mortality rate, especially cardiovascular deaths [316, 319]. Thus, secondary hyperparathyroidism should be recognised as a significant risk factor for poor outcomes in older adults and should be treated aggressively.
Patients in the lowest tertile of serum 25(OH)D levels had increased PTH and bone turnover marker (BTM) levels compared to those in the highest tertile. This can be seen in table 5 and figure 1, with older patients appearing to have the highest PTH levels for a given vitamin D tertile. Increased bone turnover, particularly bone resorption, can lead to bone loss and thus increased the likelihood of fracture. In some studies, baseline BTM levels correlated well with subsequent bone loss [241], which suggests that the bone turnover rate determines the subsequent bone loss. In the OFELY study, BMD was measured yearly for 4 years [242]. The BTM levels correlated negatively with the rate of bone loss at the distal radius where the precision error was comparable to the yearly bone loss. Increased bone turnover markers (BTM) levels predict fragility fractures independently of age, BMD and prior fracture. This association has been assessed in several prospective and case control studies [242, 244-246]. Thus, aggressive vitamin D replacement and anti-osteoporosis therapy should be encouraged to normalise PTH levels and reduce bone turnover to protect those at high risk of fractures.

There appeared to be a lack of correlation between serum vitamin D levels and bone heel ultrasound (QUS). As described in the previous chapter, heel ultrasound may be as useful as DXA to diagnose patients with osteoporosis or a prior history of fractures. A number of studies have examined QUS in older women, mainly in relation to its value as a predictor of osteoporotic fracture risk [298-304]. The relationship of QUS measures and 25(OH)D is not as clear. Previous evidence suggests neither vitamin D supplementation nor vitamin D receptor genotypes are associated with QUS measures in perimenopausal women [210, 308]. There is conflicting evidence about the usefulness of heel
ultrasound when used for follow-up. In a study of 18 patients with osteoporosis treated with an anti-resorptive agent, Ingle et al found that finger ultrasound was similar in clinical utility to DXA at the femoral neck for monitoring treatment [336]. Frost ML et al did a 2-year longitudinal study on 195 postmenopausal women to monitor the response to anti-resorptive therapy comparing QUS with BMD measurements at the lumbar spine, femoral neck, and total hip. They found that calcaneal QUS showed a highly significant response to anti-resorptive therapy, but their conclusion was that the precision of QUS was not good enough to allow QUS to be used for monitoring response to treatment [337]. Therefore, more studies are required for a definitive conclusion regarding the utility of QUS for monitoring osteoporosis management, particularly in those where vitamin D levels are being optimized and followed.

Vitamin D deficiency is being recognised as a major health problem for adults over the age of 50 years [95]. Previous cross-sectional studies have shown that low levels of serum 25(OH)D were associated with reduced muscle strength [320, 321], reduced ability to perform activities of daily living [322] and an increase in body sway and falls [322-325]. Vitamin D receptors for 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$) have been identified in human skeletal muscle [317], with higher levels of serum 1,25(OH)$_2$D$_3$ being associated with a reduced fall rate in older community-dwelling women [326]. Another study has shown that patients with vitamin D deficiency at baseline assessment were associated with a significantly increased risk of sarcopaenia [319, 327].
Treatment of vitamin D deficiency not only preserves bone and muscle strength but also prevents falls and fractures [338]. Falls are a major component in non-vertebral fracture risk including hip fractures. Low levels of 25(OH)D have been linked to frailty and poor health status, which could contribute to an association with fractures [339]. Previous studies have demonstrated the benefit of supplemental vitamin D₃ in preventing hip fractures, however, more recent meta-analyses have suggested that there is little benefit in preventing hip fractures in both cohort and case control studies. The mechanism by which vitamin D reduces falls is thought to be through improvement of muscular strength and function. In the NHANES III study, women > 60 years of age with higher 25(OH)D levels were associated with improved lower extremity function (faster walking and sit-to-stand speeds) [170]. There have been several meta-analyses that have shown the value of vitamin D supplementation in preventing falls [171, 340] and fractures [341].

This is a cross-sectional study with consecutive patients from a Dublin teaching hospital being analysed. All patients were assessed were from an urban setting and may therefore not be fully reflective of mixed rural and urban populations. This is a potential area for further research and it may be appropriate to repeat this study in several other centres to ascertain the “true” national prevalence of vitamin D deficiency in Irish patients with osteoporosis. It would have been expected that these people would be more aware of the importance of vitamin D to bone health and may have higher levels compared to general population.

Vitamin D deficiency has been associated with several other diseases including type 2 diabetes [342], some cancers (e.g. breast and colon) [342-344], cardiovascular
diseases [345] and infections [346-348]. Vitamin D receptors are present in the small intestine, colon, osteoblasts, activated T- and B-lymphocytes, B-islet cell and most organs in the body, including brain, heart, skin, gonads, prostate, breast and mononuclear cells [145]. Unfortunately, the vast majority of these associations are from cross-sectional studies and further randomised control trials and longitudinal studies are needed to assess these relationships and the optimal vitamin D dose required to achieve maximal benefit.

In conclusion, vitamin D deficiency is common in patients attending for assessment of their bone health, even in those who were taking vitamin D supplementation. It appears that higher doses of vitamin D are required to achieve optimal serum vitamin D levels in most patients.
Chapter 6. An Audit of Hip Fracture Care in St James’s Hospital compared to the UK National Hip Fracture Database (NHFD)

6.1 Introduction

Hip fractures are one of the most serious causes of morbidity and mortality in older adults [349, 350]. The incidence of osteoporotic related fractures is expected to increase over the next few decades as the number of older individuals increases [265]. The care of osteoporotic hip fractures presents a significant challenge to healthcare systems and to society in general. Older patients are a high risk group for hip fractures and a significant proportion of this group do not return to their premorbid level of mobility [351]. Hip fractures are associated with excess mortality up to 10% within 1 month and 33% within 1 year [352].

In Ireland, there is an estimated 3000 patients who suffer a hip fracture annually [353] and it is expected the rate and number of hip fractures will increase over the coming years [353]. Despite this, the care for hip fractures can vary from site to site. It has been estimated that hip fractures can cost 14,300 Euro per admission [61]. In order for health service providers to be able to allocate adequate resources for the management of hip fractures, accurate figures of hip fracture rates and outcomes should be measured.

Recently in the UK, the National Hip Fracture Database has been introduced as a collaborative venture by the British orthopaedic Association and the British Geriatrics Society to improve and standardise hip fracture care and secondary
prevention (http://www.nhfd.co.uk). This database collects information from patients admitted with an acute hip fracture, including the demographics, surgical intervention, outcomes and treatments of patients.

The aim of the study was to prospectively collect data on all hip fractures admitted to St James’s Hospital (SJH), Dublin and compare these results to the NHFD-UK. In St James’s Hospital, an orthogeriatric fracture liaison service was established in 2004. By analysing this data it is hoped that areas where improvements can be made will be identified with an aim to improve hip fracture outcomes.

6.2 Methods

Participants

From 1st January 2009 to 31st December 2009, all patients admitted with a fractured neck of femur to St James’s Hospital, Dublin 8 had a prospective collection of demographic, physiological and operative data. All patients were consented and the information was anonymised. Patients admitted from the community and those who sustained a hip fracture while as an inpatient in hospital.

Consecutive individuals who sustained a hip fractures were included. Sources of information included the St James’s Hospital Electronic Patient Record, orthogeriatric assessment forms completed by orthogeriatric clinical nurse specialist and discharge summaries. A proforma was developed to record information on hip fracture and aid clinical record-keeping and audit. The data was compared to the UK’s National Hip Fracture Database for 2009. St James’s Hospital has an Orthogeriatric Liaison team, which assesses and follows patients with hip fracture post discharge.
Hip fractures were defined as any fracture of the femur between the articular joint of the hip and 5 cm below the distal point of the lesser trochanter.

**Referral Criteria**

**Inclusion criteria:** Acute hip fracture associated with low trauma/spontaneously

**Exclusion criteria:**
- History of pathological fracture (Paget’s disease, metastatic cancer)
- Traumatic fracture i.e. road traffic accident, fall greater than standing height
- Patient/next of kin unable to give consent

Using the NHFD as a template, all patients admitted to our institution were assessed from time of hip fracture to discharge. Patients were reviewed from reviewing daily orthopaedic theatre lists, admission lists and inpatient fall data.

Patients are routinely admitted under the orthopaedic team with an orthogeriatric liaison team reviewing the patient while on the ward. The review of the orthogeriatric team includes risk factors for osteoporosis, history of falls, and current treatments.
6.3 Results

192 patients were referred to the orthogeriatric service over a 2 year period between January 2009 to December 2009. Table 1 demonstrates the baseline characteristics of patients admitted in 2009. Overall, there was a higher number of female patients (n=134, 69.4%) admitted with a hip fracture. The mean age of patients admitted with hip fracture was 76.2 (+/-11.5) years. The mean of female patients was 77.5 (+/-11.6) years and the mean age of male patients was 73.1 (+/-10.6) years. As can be seen in figure 2, 89.2% of patients who were admitted with a hip fracture were over 70 years of age.

Fracture Type
Fracture types by percentage included intracapsular 49% (56%), intertrochanteric 40% (38%) and subtrochanteric 11% (6%). Intertrochanteric fractures were common in female patients, whereas

Outcomes

167 (86.9%) patients went to theatre within 48 hours. The median length of stay was 18 days, with the mean length of stay being 39.3 (+/-54.5) days. 30% of patients were discharged home, 16% to residential care, 43% discharged to a rehabilitation unit, 1% discharged to an acute hospital, 6% were discharged to other facilities and there was a 4% in patient mortality rate.
<table>
<thead>
<tr>
<th></th>
<th>2009 (n=192)</th>
<th>NHFD 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (yrs) (SD)</td>
<td>76.2 (+/-11.5)</td>
<td>83.4</td>
</tr>
<tr>
<td>Median age (yrs)</td>
<td>79</td>
<td>84</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>69.4</td>
<td>75</td>
</tr>
<tr>
<td><strong>Type of Fracture</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracapsular</td>
<td>49%</td>
<td>54%</td>
</tr>
<tr>
<td>Intertrochanteric</td>
<td>40%</td>
<td>40%</td>
</tr>
<tr>
<td>Subtrochanteric</td>
<td>11%</td>
<td>6%</td>
</tr>
<tr>
<td><strong>Length of Stay</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>18</td>
<td>23</td>
</tr>
<tr>
<td>Mean</td>
<td>41.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Time to Surgery (&lt;2 days)</td>
<td>86.9%</td>
<td>75%</td>
</tr>
<tr>
<td><strong>Medications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-admission Bone Protection</td>
<td>19.6%</td>
<td>9%</td>
</tr>
<tr>
<td>Discharge Bone Protection</td>
<td>67%</td>
<td>60%</td>
</tr>
<tr>
<td>Pre-admission Calcium/Vitamin D supplementation</td>
<td>28%</td>
<td>N/A</td>
</tr>
<tr>
<td>Discharge Calcium/Vitamin D supplementation</td>
<td>86.8%</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Table 6.1. Comparison of characteristics in patients admitted in 2008 and 2009 to St James's Hospital compared to the 2009 National Hip Fracture Database UK (NHFD). N/A=not available**

**Medications**

36 (18.8%) patients were on bone protection (figure 4) and 54 (28%) patients were prescribed calcium and vitamin D supplementation prior to their hip fracture. Oral bisphosphonates were the most commonly prescribed osteoporosis medication on admission (n=34). Upon discharge, most patients were commenced on secondary bone protection, with 67% of patients being commenced on either a bisphosphonate or strontium ranelate. Calcium and vitamin D supplementation was 86.8%, however, there are no UK figures for this in the NHFD.
Figure 6.1 Distribution of Hip Fractures by Age

Figure 6.2 Fracture type by percentage according to gender
Figure 6.3 Osteoporosis medications prescribed prior to admission and at discharge.

Figure 6.4 Outcomes of hip fracture patients at 30 days compared to NHFD UK 2009.
6.4 Discussion

The evidence base for hip fracture is improving rapidly, and in general terms shows that prompt, effective, multidisciplinary management can improve quality and at the same time reduce costs. One of the main findings of this study was that the quality of hip fracture care in St James’s hospital appeared to be above the UK average in most areas, however, there are a few areas that need to be improved. Since 2004, we have had an orthogeriatric fracture liaison service available to all patients with a hip fracture admitted to St James's Hospital. Patients are reviewed post-operatively and the appropriate treatments are initiated by the orthogeriatric liaison team.

The mean age of patients admitted with hip fracture was 76.2 (+/-11.5) years. Almost one third of patients admitted with a hip fractures were male and tended to be younger than female patients. This mean age was lower than the mean given in United Kingdom and there may be several reasons for this, including vitamin D status, nutritional statues and socio-economic differences. The study was not designed to capture this information and may be an area of further research. Previous studies have shown that white women have a calculated 16% lifetime risk of suffering a hip fracture, while white men have a 5% lifetime risk [354]. Previous studies have suggested that male patients have poorer outcome after a hip fracture [355, 356].

The UK National Hip Fracture Database (NHFD) was developed in 2007 by the British Orthopaedic Association and the British Geriatrics Society (http://www.nhfd.co.uk/003/hipfractureR.nsf/). A number of large audits in Nottingham, Oxford, Leicester, Cardiff and Belfast had already established local hip fracture audits and these were used to develop the NHFD. A successful national audit
had already been established in Scotland and their experiences were used to develop an audit system for the rest of the population of the United Kingdom. The aim of the audit is to improve patient care using evidence based standards. The NHFD was established to improve the quality and cost-effectiveness of care for hip fracture patients. There are six evidence-based standards as set out by the BOA and BGS on the care of patients admitted with fragility fractures:

1. All patients with hip fracture should be admitted to an orthopaedic ward within 4 hours of hospital admission
2. All patients with hip fracture who are medically fit should have surgery within 48 hours and during normal working hours
3. All patients with hip fracture should be assessed and cared for with a view to minimising their risk of developing a pressure ulcer
4. All patients presenting with a fragility fracture should be managed on an orthopaedic ward with routine access to acute orthogeriatric medical support from time of admission
5. All patients presenting with fragility fractures should assessed to determine their need for anti-resorptive therapy to prevent further osteoporotic fractures
6. All patients presenting with fragility fractures following a fall should be offered a multidisciplinary assessment and intervention to prevent future falls.

As can be seen in table 1, patients admitted with an acute hip fracture were younger than the UK average. A direct comparison is difficult to make as there are some differences in the demographics of patients, such as ethnicity, in the UK National Hip Fracture Database and the patients admitted to our hospital. The patients admitted to St James’s Hospital patients had a shorter median length of stay but were less likely to
be discharged directly home. In our study, the median length of stay was 18 days, whereas the median for the UK-NHFD was 23 days. Dodds et al [357] have reported reasons for delayed discharge included infections (32%), medico-social issues (22%) and chronic diseases (17%), such as chronic obstructive disease and cardiac failure, as significant contributors to the delayed progress of some patients after hip fracture surgery. This study is a cross-sectional study, although consecutive patients of a large Dublin hospital have been analysed, the urban setting would add bias for better care.

The involvement of the orthogeriatric liaison team appears to improve patient’s commencement on secondary bone treatment. Fewer patients required residential care and fewer deaths were recorded in St James’s Hospital compared to the UK NHFD in 2009. This may be explained by the younger mean age of the patients being admitted with hip fracture. It must be noted that there were higher numbers of patients at home at 30 days in the UK compared to St James’s Hospital, which may be a reflection of the implementation and more comprehensive community support networks in the UK, which facilitates early discharge. Previous studies have shown that integration of clinical and multidisciplinary care pathways have been shown to decrease mortality and length of stay in hospital [358, 359]. Daily intervention of orthogeriatric liaison teams have been shown to reduce the overall morbidity and mortality of patients admitted with hip fractures, although this intervention may not decrease inpatient length of stay [360, 361]. More recently, the Cochrane collaborative has reviewed the evidence for multidisciplinary care in older adults with hip fracture [362]. There was a tendency to have overall better results in a multidisciplinary approach to hip fracture care but they have recommended that future trials of multidisciplinary rehabilitation should aim to establish the cost effectiveness of the multidisciplinary approach.
There was a higher prevalence of men who sustained a hip fracture compared to the NHFD (table 1). Male patients are less likely to be assessed for osteoporosis compared to women although up to one in five men develop osteoporosis in their lifetime [363, 364]. The prospective MrOS study of older men confirmed that hip BMD is strongly associated with risk of non-vertebral fractures, particularly those of the hip (3.2 fold increased risk per SD decrease) [363, 364]. The spine BMD was a weaker predictor of fragility fractures in men. When the results are compared to the related study in women (Study of Osteoporotic Fractures (SOF)), women were more likely to fracture than men until age 80, after which the difference between the genders was no longer significant. In our study, 30% of all hip fractures occurred in men. This is an important finding as there is a fracture-related excess mortality, morbidity and institutionalisation may be greater for men than for women [363, 364].

Patients with fragility fractures are at risk of further fragility fractures. The rate of prescribing of calcium/vitamin D supplementation and bone protection both prior to admission and on discharge were higher compared to the UK average, however, they continue to remain suboptimal. There may be several reasons for patients not commencing on secondary bone protection i.e. unwilling or unable to attend for follow-up treatment or did not require anti-resorptive therapy based on risk factors and BMD levels [365, 366]. We have previously published that the primary-care prescribing of anti-osteoporotic medications after fragility fractures has increased since the introduction of the osteoporosis clinic in St James's Hospital [258]. One previous study have shown that the use of prescribed calcium and vitamin D
supplementation and anti-osteoporotic drugs in patients after hip fracture has been associated with lower mortality [367].

This audit has several limitations. It was conducted in a single university-affiliated teaching hospital, and our results may not reflect patterns in other hospitals. Our hospital has trained medical staff in osteoporosis with an interest in hip fractures. There are few centres in Ireland that have dedicated orthogeriatric liaison teams for the assessment of patients with fractures.

Many elderly patients who fracture are frail and have complex medical problems. Their needs for specialist medical care and early rehabilitation are best addressed when an orthogeriatrician is fully integrated in the work of the fracture service. Hip fractures are common, require treatment from a multidisciplinary team and result in significant mortality in older patients. The introduction of integrated care pathways for hip fracture management would benefit outcomes for all patients with hip fractures. The development of a similar National Hip Fracture Database in Ireland would allow us to assess the standard of care of hip fracture management nationwide and would highlight areas of deficits. It is imperative that a nationwide audit of hip fracture care be undertaken on an ongoing basis. Such an undertaking would be greatly facilitated by the routine collection of data pertaining to the quality of hip fracture care at each hospital site involved in acute care of patients with hip fractures. The formulation and implementation of such a strategy is crucial if we are to deliver efficient hip fracture care in future years and thus reduce complications and poor outcomes.
Chapter 7. The Prevalence of Vitamin D Deficiency in Irish Patients with Hip Fractures

7.1 Introduction

Hip fractures are a major public health issue, which will become more common with the ageing of the population. Hip fractures are a cause of significant morbidity and mortality of older individuals. In most populations, hip fracture incidence increases exponentially with age [368, 369] and is the most common clinical fracture for adults over the age of 75 years. At the end of the first year after a hip fracture, up to 50% of individuals have permanent functional disability, 15 to 25% require long-term nursing home care and up to 20% will have died [370-372]. In Ireland, it is estimated that 3500 to 4000 adults will sustain a hip fracture in a given year and this number is expected to increase in future years [353]. Identifying any potential treatable factor for a further hip fracture is of paramount importance and should be diagnosed and treated appropriately.

Vitamin D deficiency is common among older adults and it is increasingly being recognised as a significant contributing risk factor for an osteoporotic fracture. Vitamin D is required for the efficient absorption of calcium and for the normal mineralisation of bone. Serum 25-hydroxyvitamin D (25(OH)D) is the most common biomarker used to assess vitamin D status. Low 25(OH)D levels have been associated with an increased risk of fractures in older adults. This is thought to be mediated by its effects on bone metabolism and by contributing to an increase in the risk of falling. The risk of falling may be in part related to muscle weakness and to changes in balance [137, 311]. The importance of vitamin D status for optimal bone health has received increased recognition in recent years, with higher recommended intake levels
being proposed by some investigators [143]. There is a lack of consensus on the serum 25(OH)D concentration that reflects optimal vitamin D status, but a serum 25(OH)D concentration > 75nmol/L has been suggested as an optimal level for falls and fracture prevention [145, 340].

Falls are a major component in fracture risk and fall prevention is a core part of the management of patients with fragile bones. The prevalence of falls increases with advancing age and is higher in females than in males [373, 374]. Falls are a substantial factor in hip fractures. 95% of hip fractures are associated with a fall, with 10% of falls being associated with a fracture [285]. There have been several studies demonstrating the benefit of vitamin D in preventing falls and fractures [170, 171, 340, 341, 375]. This protective mechanism is thought to be related to improvements in muscle strength, as well as its effect on bone.

Subgroups of the Irish population, including older adults and adolescent girls, have been shown to have a high prevalence of vitamin D deficiency [376-381]. However, there is very little data on vitamin D status in patients with fractures in the Republic of Ireland. The aim of this study is to assess the prevalence of vitamin D deficiency in Irish adults who present acutely with a hip fracture.
7.2 Methods

This study was a prospective study of consecutive patients who presented acutely with an osteoporotic hip fracture admitted to St James's Hospital over a 12 month period between January 2008 to December 2008. Osteoporotic hip fracture cases were identified using the in-hospital Electronic Patient Record System and theatre lists. These records were reviewed on a daily basis with all patients being consented to physical examination and blood tests pre-operatively. We excluded patients who were not resident in Ireland or were unwell at the time of fracture. The study was approved by the St James's Hospital/AMNCH Ethics Committee. Written consent was obtained from all participants.

Hip fractures were defined as any fracture of the femur between the articular joint of the hip and 5 cm below the distal point of the lesser trochanter. Information on demographics, smoking, falls and medications (including vitamin D supplementation) was recorded.

Fasting early morning venous blood was collected from patients for the measurement of 25(OH)D, calcium, creatinine and parathyroid hormone (PTH) levels. Samples were stored at -20°C. Serum 25(OH)D was assayed with a radioimmunoassay (RIA) kit (Diasorin, Stillwater, MN, USA) at the Department of Biochemistry, St James's Hospital, Dublin 8. Serum vitamin D status was classified using the cut off criteria outlined by International Osteoporosis Foundation [328]. A serum 25(OH)D less than 50 nmol/L was considered to be vitamin D deficient, levels between 50-75 nmol/L were classified as vitamin D insufficient, and levels >75 nmol/L were considered optimal. The mean 25(OH)D levels are presented for type of residence and
season. The vitamin D levels are presented for patients who are not supplemented with vitamin D and on vitamin D supplementation in the results.

7.3 Results

Over the 12-month period, 158 individuals who presented acutely with a hip fracture were admitted to a large Dublin teaching hospital. 2 patients did not have serum 25(OH)D taken as both patients died in the pre-operative period. Overall 156 patients were assessed with a mean age of 77.55 (+/-10) years. 118 (75.6%) patients were female. The mean age of female patients was 78.3 (+/-10) years and the mean age of males was 75.3 (+/-9.7) years. 15 (9.6%) patients were admitted from a nursing home, with the remainder of patients being admitted from the community. 65 (41.7%) patients had a previous fracture at the time of admission. Characteristics of the study group are summarised in Table 1.

Table 7.1 Baseline Characteristics of Study Group (n=156)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (years (+/- SD))</td>
<td>77.6 (+/- 10)</td>
</tr>
<tr>
<td>Female (n=118, 75.6%)</td>
<td>78.3 (+/-10)</td>
</tr>
<tr>
<td>Male (n=38, 24.4%)</td>
<td>75.3 (+/-9.7)</td>
</tr>
<tr>
<td>Prior fracture history</td>
<td>41.7% (n=65)</td>
</tr>
<tr>
<td>Previous Wrist fracture</td>
<td>26.3% (n=41)</td>
</tr>
<tr>
<td>Previous Hip fracture</td>
<td>7.1% (n=11)</td>
</tr>
<tr>
<td>Vitamin D Supplementation</td>
<td>17.3% (n=27)</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
</tr>
<tr>
<td>Community dwellers</td>
<td>90.4% (n=141)</td>
</tr>
<tr>
<td>Nursing home</td>
<td>9.6% (n=15)</td>
</tr>
<tr>
<td>Number of Individuals admitted per season</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>49</td>
</tr>
<tr>
<td>Summer</td>
<td>29</td>
</tr>
<tr>
<td>Autumn</td>
<td>35</td>
</tr>
<tr>
<td>Winter</td>
<td>43</td>
</tr>
</tbody>
</table>

SD standard deviation
Distribution of Serum 25(OH)D

Mean 25(OH)D was 39.7 (+/-25.5) nmol/L for the overall group. 27 (16.7%) patients were on vitamin D supplementation at the time of fracture, with 16 (10.3%) patients on the 800IU/day and a further 11 (6.4%) patients on 400IU/day of cholecalciferol (Vitamin D3). The mean serum 25(OH)D for non-supplemented patients was 35.1 (+/-22.1) nmol/L and the mean serum 25(OH)D of supplemented patients was 65.4 (+/-28.2) nmol/L. Table 2 demonstrates mean serum 25(OH)D levels among subgroups of non-supplemented and supplemented individuals.

<table>
<thead>
<tr>
<th>&lt;20nmol/L</th>
<th>All patients (n=156)</th>
<th>Females (n=118)</th>
<th>Males (n=38)</th>
<th>Non-supplemented (n=129)</th>
<th>Supplemented (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30.1% (n=47)</td>
<td>28% (n=33)</td>
<td>36.8% (n=14)</td>
<td>36.4% (n=47)</td>
<td>-</td>
</tr>
<tr>
<td>20-50 nmol/L</td>
<td>37.2% (n=58)</td>
<td>35.6% (n=42)</td>
<td>42.1% (n=16)</td>
<td>37.2% (n=48)</td>
<td>37% (n=10)</td>
</tr>
<tr>
<td>50-75 nmol/L</td>
<td>23.7% (n=37)</td>
<td>26.3% (n=31)</td>
<td>15.8% (n=6)</td>
<td>21.7% (n=28)</td>
<td>33.3% (n=9)</td>
</tr>
<tr>
<td>&gt;75 nmol/L</td>
<td>9% (n=14)</td>
<td>10.2% (n=12)</td>
<td>5.3% (n=2)</td>
<td>4.7% (n=6)</td>
<td>29.6% (n=8)</td>
</tr>
</tbody>
</table>

Table 7.2 Distribution of Vitamin D Levels in subgroups of patients.

Severe vitamin D deficiency below 20 nmol/L was identified in 30.1% (47/156) and below 50 nmol/L was identified in 67.3% (105/156) of the overall group of patients. A greater percentage of male patients had serum vitamin D below 50 nmol/L compared to females (78.9% and 63.6%, respectively). Only 6 (4.7%) non-supplemented patients achieved an optimum level of serum vitamin D (>75nmol/L). 10 (37%) supplemented patients had a vitamin D level below 50 nmol/L.
Supplemented patients with a hip fracture had significantly higher serum 25(OH)D levels. They also had a higher prevalence of previous fractures and were more likely to be female. 73.6% of non-supplemented patients and 37% of supplemented patients had a serum 25(OH)D level <50 nmol/L.

Table 3 demonstrates 25(OH)D according to pre-admission residence. Patients admitted from a nursing home had a lower serum vitamin D level compared to community dwellers (p=0.096). Non-supplemented patients admitted from a nursing home had significantly lower levels of serum 25(OH)D compared to non-

### Table 7.3 Comparison of baseline characteristics between (1) supplemented and non-supplemented patients and between (2) type of residence prior to admission.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Non-supplemented (n=129)</th>
<th>Supplemented (n=27)</th>
<th>Community dwellers (n=141)</th>
<th>Nursing Home (n=15)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (SD)</td>
<td>77.4 (+/-10)</td>
<td>78.7 (+/-10)</td>
<td>77.6 (+/-10)</td>
<td>77.3 (+/-10)</td>
<td>0.92</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>72.3</td>
<td>92.3</td>
<td>75.2</td>
<td>80.0</td>
<td>0.68</td>
</tr>
<tr>
<td>Previous Fracture (%)</td>
<td>36.9</td>
<td>65.4</td>
<td>41.1</td>
<td>46.7</td>
<td>0.68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calcium Metabolism</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (nmol/L) (SD)</td>
<td>35.1 (+/-25.1)</td>
<td>65.4 (+/-24.3)</td>
<td>40.8 (+/-23.4)</td>
<td>29.3 (+/-25.2)</td>
<td>0.1</td>
</tr>
<tr>
<td>Non-supplemented</td>
<td>-</td>
<td>-</td>
<td>36.2 (+/-22.0)</td>
<td>17.8 (+/-7.9)</td>
<td>0.005</td>
</tr>
<tr>
<td>Supplemented</td>
<td>-</td>
<td>-</td>
<td>64.6 (+/-28.5)</td>
<td>75.6 (+/-9.0)</td>
<td>0.52</td>
</tr>
<tr>
<td>PTH (pg/mL) (SD)</td>
<td>55.5 (+/-45.5)</td>
<td>44.1 (+/-17.4)</td>
<td>53.2 (+/-43.5)</td>
<td>58.0 (+/-32.0)</td>
<td>0.69</td>
</tr>
<tr>
<td>Calcium (mmol/L) (SD)</td>
<td>2.3 (+/-0.1)</td>
<td>2.3 (+/-0.2)</td>
<td>2.3 (+/-0.1)</td>
<td>2.3 (+/-0.1)</td>
<td>0.54</td>
</tr>
<tr>
<td>Phosphate (mmol/L) (SD)</td>
<td>1.1 (+/-0.2)</td>
<td>1.1 (+/-0.2)</td>
<td>0.9 (+/-0.1)</td>
<td>1.0 (+/-0.1)</td>
<td>0.34</td>
</tr>
<tr>
<td>ALP (U/L) (SD)</td>
<td>83.2 (+/-29.6)</td>
<td>72 (+/-24.0)</td>
<td>74.2 (+/-36.6)</td>
<td>75.0 (+/-41)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

| < 50 nmol/L (%)               | 73.6                     | 37.0                | 66.0                      | 80.0                |         |
| 50-75 nmol/L (%)              | 21.7                     | 33.3                | 25.5                      | 6.7                 |         |
| > 75 nmol/L (%)               | 4.7                      | 29.7                | 8.5                       | 13.3                |         |

* Pearson’s Chi-square test  
* Independent samples t-test (2-sided)
supplemented community dwellers (p=0.005). 80% of nursing home residents had a serum 25(OH)D level less than 50 nmol/L.

**Relationship between Serum 25(OH)D and Parathyroid Hormones levels**

The relationship between parathyroid hormone and 25(OH)D is demonstrated in figure 1. Lower levels of serum 25(OH)D were associated with higher levels of parathyroid hormone with a significant correlation between the two variables, $r^2 = -0.0784$, $p<0.01$ (Figure 1).

![Figure 7.1 Relationship between 25(OH)D and parathyroid hormone.](image)

There was a significant correlation between the two variables, $r^2=0.0784$, $p<0.01$.

When comparing all individuals with a vitamin D level below 50 nmol/L, only 23 of 105 (21.9%) had evidence of secondary hyperparathyroidism (PTH >65pg/mL, hospital standard range between 15-65). The remaining 82 patients with a 25(OH)D <50 nmol/L had a PTH level within the hospital standard reference range.
Seasonal Variation of Serum 25(OH)D Levels

The seasonal variation in 25(OH)D levels was assessed in the group as demonstrated in table 4. Serum 25(OH)D levels did increase in Summer and Autumn months compared to Winter months in both the supplemented and non-supplemented groups. This did not achieve significance (p<0.05).

Non-supplemented patients had low serum 25(OH)D levels throughout all seasons, indicating a high prevalence of vitamin D deficiency throughout the year. 86.8% of non-supplemented patients had serum 25(OH)D in winter below 50 nmol/L. In Autumn months, 63.3% of non-supplemented patients had a serum 25(OH)D level <50 nmol/L.

<table>
<thead>
<tr>
<th>Overall Patients (n=156)</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 25(OH)D (nmol/L) (SD)</td>
<td>37.6 (+/-27.3)</td>
<td>44.0 (+/-28.3)</td>
<td>43.8 (+/-23.6)</td>
<td>36.0 (+/-22.7)</td>
<td>0.4</td>
</tr>
<tr>
<td>PTH (pg/mL) (SD)</td>
<td>48.8 (+/-28.1)</td>
<td>51.3 (+/-28.1)</td>
<td>49.5 (+/-42.3)</td>
<td>63.4 (+/-56.4)</td>
<td>0.4</td>
</tr>
<tr>
<td>Serum 25(OH)D &lt; 50 nmol/L (%)</td>
<td>77.6</td>
<td>58.6</td>
<td>57.1</td>
<td>67.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-supplemented Patients (n=129)</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 25(OH)D (nmol/L) (SD)</td>
<td>29.9 (+/-22.6)</td>
<td>36.5 (+/-23.6)</td>
<td>40.8 (+/-21.3)</td>
<td>32.9 (+/-19.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>PTH (pg/mL) (SD)</td>
<td>49.2 (+/-29.7)</td>
<td>53.5 (+/-38.7)</td>
<td>51.1 (+/-45.5)</td>
<td>65.4 (+/-58.8)</td>
<td>0.4</td>
</tr>
<tr>
<td>Serum 25(OH)D &lt; 50 nmol/L (%)</td>
<td>86.8</td>
<td>69.6</td>
<td>63.3</td>
<td>71.8</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Supplemented Patients (n=27)</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 25(OH)D (nmol/L) (SD)</td>
<td>64.0 (+/-26.3)</td>
<td>72.9 (+/-28.2)</td>
<td>61.4 (+/-31.7)</td>
<td>66.1 (+/-31.9)</td>
<td>0.9</td>
</tr>
<tr>
<td>PTH (pg/mL) (SD)</td>
<td>47.4 (+/-22.5)</td>
<td>40.0 (+/-9.2)</td>
<td>40.5 (+/-15.5)</td>
<td>44.1 (+/-14.4)</td>
<td>0.9</td>
</tr>
<tr>
<td>Serum 25(OH)D &lt; 50 nmol/L (%)</td>
<td>45.5</td>
<td>16.7</td>
<td>20.0</td>
<td>50.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Table 7.4* Comparison of mean 25(OH)D levels, parathyroid hormone levels and 25(OH)D percentage below 50 nmol/L in the overall, supplemented and non-supplemented patients using ANOVA. (SD: Standard deviation. PTH: Parathyroid hormone. 25(OH)D: 25-hydroxyvitamin D.)
7.4 Discussion

This study demonstrates that there is a high prevalence of vitamin D deficiency (25(OH)D <50nmol/L) in Irish patients admitted with a hip fracture to a large Dublin hospital. The high prevalence of vitamin D deficiency is in agreement with previous studies on the prevalence of vitamin D and altered vitamin D-PTH homeostasis in hip fracture populations, both in European countries and in the USA [95, 382-385].

Vitamin D deficiency is being recognised as a major health problem for adults over the age of 50 years [95]. Low levels of serum 25(OH)D and higher levels of parathyroid hormone have been documented as having a negative impact on functional recovery of patients who have sustained a hip fracture [314]. Previous cross-sectional studies have shown that low levels of serum 25(OH)D were associated with reduced muscle strength [320, 321], reduced ability to perform activities of daily living [322] and an increase in body sway and falls [322-325]. Vitamin D receptors for 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) have been identified in human skeletal muscle [317], with higher levels of serum 1,25(OH)₂D₃ being associated with a reduced fall rate in older community-dwelling women [326]. Another study has shown that patients with vitamin D deficiency at baseline assessment were associated with a significantly increased risk of sarcopenia [319, 327].

Several studies have observed an increased serum PTH concentration in older people with or without hip fracture associated with vitamin D deficiency [141, 312, 313]. In our study, serum PTH correlated with levels of serum 25(OH)D, with higher levels of PTH being observed in those with lower vitamin D levels. Some studies have suggested that patients with the highest levels of PTH post hip fracture have poorer
outcomes [314]. Patients with an elevated PTH compared to those with serum PTH in the normal range have a 15 times greater risk of fatal outcome and increased hospital stay [314]. Patients with hypovitaminosis D and secondary hyperparathyroidism compared to those without elevated PTH levels have increased bone turnover [315-318], increased fracture risk [315] and a higher mortality rate, especially cardiovascular deaths [316, 319]. Secondary hyperparathyroidism should be recognised as a significant risk factor for poor outcomes and should be treated.

In this study, there was a non-significant seasonal variation of serum 25(OH)D levels in non-supplemented patients, with lowest levels being observed in spring months and highest in autumn months. This seasonal variation has been observed in previous studies of healthy older adults [334, 335] and hip fracture patients [386], although it has not been seen in all hip fracture patients [315]. This observation should be noted when prescribing vitamin D supplementation and is another area for further study in older patients. Seasonality and latitude are well documented causes of vitamin D deficiency and can influence levels [333]. However, in older adults the ability of the skin to produce vitamin D is reduced by several factors as previously mentioned. In countries above a latitude of 35°N, there is virtually no production of vitamin D from October to April, with populations being more reliant on oral supplementation for maintainence of adequate vitamin D levels.

Treatment of vitamin D deficiency not only preserves bone and muscle strength but also prevents falls and fractures [338]. Falls are a major component in non-vertebral fracture risk including hip fractures. Low levels of 25(OH)D have been linked to frailty and poor health status, which could contribute to an association with fractures
Several studies have demonstrated the benefit of supplemental vitamin D in preventing hip fractures. Almost 20 years ago, Chapuy et al [387] showed the benefit of 1200 mg calcium and 800 IU vitamin D₃ in reducing the risk of hip fractures by 43% and the risk of non-vertebral fractures by 32% in institutionalised patients. The mechanism by which vitamin D reduces falls is thought to be through improvement of muscular strength and function. More recently, the NHANES III study showed that women > 60 years of age with higher 25(OH)D levels had improved lower extremity function (faster walking and sit-to-stand speeds) [170]. There have been several meta-analyses that have shown the value of vitamin D supplementation in preventing falls [171, 340] and fractures [341]. However, not all meta-analyses have shown benefit in vitamin D supplementation post hip fracture [388], suggesting a variation in response due to confounders.

We noted that 1 in 6 (17.3%) of all patients were on vitamin D supplementation at the time of admission in the study group, with 10% of all the study group taking a recommended dose of 800 IU of cholecalciferol (Vitamin D₃). A substantial portion of patients (41%) had a previous history of fracture at the time of admission. Previous fractures are a risk factor for having a hip fracture – this is particularly true for wrist and vertebral fractures. This under-treatment of previous fractures is similar to a recent audit in the UK. [389] Better education of healthcare professionals, more consistent recording of fractures in primary care and the use of clearly defined care pathways that involve patients and their carers provide rational approaches to reducing this care gap.
There is much debate on the optimum level of serum vitamin D (25(OH)D). Recently, the International Osteoporosis Foundation (IOF) have published recommendations for the optimal serum vitamin D level to be above 75nmol/L [143]. This level is based on maximal PTH suppression, reduced rate of bone loss and falls to optimise overall bone health. The dose of vitamin D supplementation required by a patient would depend on several factors including the baseline level of serum 25(OH)D, body mass index, sun exposure and vitamin D metabolism. For individuals with effective sunlight exposure, a dose of 800 IU/day vitamin D<sub>3</sub> may be sufficient. Patients with obesity, a history of falls, known osteoporosis and limited sun exposure may require higher doses of vitamin D<sub>3</sub> up to 2000 IU/daily [143]. In our study, less than a third of the study patients achieved an optimal level of vitamin D on 800 IU vitamin D<sub>3</sub>/day and therefore required a higher vitamin D supplementation to achieve an optimal serum level of vitamin D. The rate of replacement, mode of administration and the total dose required for reaching optimal levels still remains to be fully established.

This is a cross-sectional study with consecutive patients from a Dublin teaching hospital being analysed. All patients were admitted from an urban setting and may therefore not be fully reflective of mixed rural and urban populations. This is a potential area for further research and it may be appropriate to repeat this study in several other centres to ascertain the national prevalence of vitamin D deficiency in Ireland in patients with hip fracture. A strength of the study is the assessment of seasonality and living situation (community versus nursing home) as additional important determinants of 25(OH)D status in patients admitted with acute hip fractures.
In conclusion, this study shows a high prevalence of vitamin D deficiency in Irish patients with hip fractures. In addition, these patients also had a high prevalence of previous fractures, with 41% reporting a previous clinical fracture. The results indicate that most community dwelling patients who have a hip fracture are severely vitamin D deficient at the time of the fracture. Identifying and treating patients who have vitamin D deficiency may reduce falls and fractures, and also reduce the incidence of secondary hyperparathyroidism with improvements in bone health. There is a need for a heightened awareness of the high prevalence of vitamin D deficiency and the need to optimise vitamin D therapy among this older at risk group of patients.
Chapter 8. The Relationship of Bone Turnover Markers and Bone Mineral Density in Patients with Hip Fracture using MicroCT Analysis

8.1 Introduction

Bone quality describes specific aspects of the tissue composition and structure that contribute to strength independently of bone mineral density (BMD). Fragility fractures, including hip fractures, can occur in patients who are considered to have normal or osteopaenic bone densities based on DXA. The underlying microarchitecture of bone is an important structural property and it also has a major impact on bone quality. Microarchitecture can be understood in terms of the trabecular microstructure, which encompasses the orientation, thickness and spacing of the trabeculae as well as the extent to which they are interconnected. Cortical bone microstructure is complex and hierarchical and encompasses porosity, distribution of porosity and cortical thickness among other factors.

MicroCT has been shown as an alternative non-destructive method of measuring the 3 dimensional structures in cortical and trabecular bone. In recent years, the use of high resolution micro-computed tomography (microCT, uCT) imaging to assess bone morphology in animal and human specimens has grown immensely. There are now several different commercially available microCT systems, and as a result there are various approaches to image acquisition, image evaluation and reporting of outcomes.

Understanding the concept of bone turnover is important in the diagnosis and treatment of osteoporosis. High bone turnover for any given BMD increases the
fracture risk compared to bone turnover that is low [390]. Previous studies have shown that accelerated bone resorption was associated with an increased incidence of osteoporotic fractures, independent of BMD [391]. Thus, combining measurements of BMD and bone resorption improve the future fracture predictions. There are several theories to explain why increased bone turnover negatively affects bone strength. Parfitt et al [392] proposed that high bone turnover constitutes a mechanical threat to bone strength as trabeculae are removed, leading increased bone fragility.

This chapter describes the relationship between bone markers taken from patients at the time of fracture and compare these results to the microarchitecture of femoral bone based on microCT analysis. The specific aims of the study were to:

- To compare bone turnover with microCT parameters
- To compare Bone Mineral Density at both the hip and spine with microCT parameters
8.2 Materials and Methods

Bone Samples

Human femoral heads were collected from patients who were undergoing total hip arthroplasty for a hip fracture. Patients who were suspected of having secondary osteoarthritis, Paget's disease, congenital hip dysplasia, rheumatoid arthritis or other inflammatory joint disease which may have affect the bone architecture and quality were excluded from the study. A total of 25 femoral heads were collected for the present study (18 females and 7 males). Written informed consent was obtained from the patients. This study was approved by the local Ethics Committee (AMNCH Ethics Committee). Samples were stored at -20°C and all samples were processed within 8 weeks of collection.

MicroCT analysis

The bone samples were scanned using a microCT scanner (Scanco, uCT-40, Bassersdorf, Switzerland). Samples were placed in a cylindrical specimen holder and fixed into the scanner. During the scanning process, X-rays are directed towards the samples and, after passing through the samples.

The specimens were scanned using a µCT scanner (Scanco, µCT-40, Bassersdorf, Switzerland). Samples were placed in a cylindrical specimen holder and fixed into the scanner. During the scanning process, X-rays are directed towards the sample and, after passing through the sample, they are detected by a 2048 x 256 element CCD array that is controlled by a dedicated workstation. A specific measurement protocol (control file) was created before scanning began so that all parameters, such as source energy, scan time and resolution, were identical for every sample in the study. Source
energy was 70 kV, scan time for each sample was less than 40 minutes and the scan resolution was 8μm. Samples were wrapped in moist gauze for the duration of scanning. When a full 3D reconstruction of the sample had been created, Image Processing Language (IPL, Scanco, Bassersdorf, Switzerland) was used to manipulate the bone image so that, using a ‘negative’ of the image, the intracortical porosity could also be reconstructed. This image was then combined with the original so that the distribution of the porosity throughout the cortex could be visualised. Porosity was calculated using a program, which counted the number of voxels in the pores within the cortex and divided that number by the bone volume.

Numbers given on the printout include total volume (TV), bone volume (BV), BV/TV ratio, mean density of TV and BV, trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), connective density (Conn.D) and Structure Model Index (SMI). A program was created on the connected computer so that the results got from the scans could be compared. (See Appendices 11.11)

The computer automatically reconstructs the bone in 2D, which then must be contoured and morphed for 3D reconstruction. This involved drawing around the edge of one slice and the next followed by a morphing tool, which gels them together for the 3D task.
Statistical Analysis

Data are presented as mean ± standard deviation (SD). For statistical analyses, groups were assessed for normal distribution and then compared using ANOVA and t-tests. For those variables failing the normality test, a nonparametric Mann-Whitney rank sum test was used. JMP 8.0.2 statistical package was used for statistical analyses. A p value of <0.05 was considered to be significant.

8.3 Results

25 patients were assessed using MicroCT, bone turnover and DXA scanning. Mean age of the group was 79.67 (+/-8.17) years. The baseline demographics are given in table 1.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>79.7 (+/-8.2)</td>
<td>Range: 59 – 93</td>
</tr>
<tr>
<td>Female: Male (% Female)</td>
<td>18:7 (72%)</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>61.7 (+/-16.8)</td>
<td>Range: 46 – 113</td>
</tr>
<tr>
<td>Height</td>
<td>158.8 (+/-7.2)</td>
<td>Range: 149 – 175</td>
</tr>
<tr>
<td>BMI</td>
<td>24.3 (+/-5.0)</td>
<td>Range: 17.7 – 36.8</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>36.2 (+/-20.3)</td>
<td></td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>57.4 (+/-32.9)</td>
<td></td>
</tr>
<tr>
<td>Femoral Neck BMD (g/cm2)</td>
<td>0.777 (+/-0.1)</td>
<td></td>
</tr>
<tr>
<td>Hip BMD (g/cm2)</td>
<td>0.762 (+/-0.1)</td>
<td></td>
</tr>
<tr>
<td>Spine BMD (g/cm2)</td>
<td>0.881 (+/-0.1)</td>
<td></td>
</tr>
</tbody>
</table>

Table 8.1 Descriptive statistics of patients included in the study.

25 femoral heads were analysed and divided into 6 sections as described in appendices. Wedges 1 and 4 were analysed in this study with 50 bone samples in total being analysed.
Correlation of Bone Mineral Density and MicroCT Results
Table 2 and 3 demonstrate the correlations of bone turnover and microCT findings. In wedges 1 and 4, we found that hip BMD correlated with bone mass and bone density and

Bone Turnover markers
Table 2 and 3 demonstrate the correlations of bone turnover and microCT findings.

In wedge 1, we found that vitamin D and PTH levels correlated with bone mass. Higher PTH levels were associated with reduced BV/TV (figure 1; $R^2=0.14$), reduced trabecular number (figure 2; $R^2=0.22$), increased trabecular spacing and reduced trabecular thickness (figure 3; $R^2=0.17$). Low vitamin D levels correlated with lower bone volume/total volume ratio (BV/TV) and bone mass. Bone resorption was measured using C-telopeptide (CTx). Higher CTx levels were associated with reduced BV/TV and bone density. It was noted that higher CTx levels were associated with reduced trabecular number and thickness but this was not clinically significant.

![Figure 9.1 Correlation between serum PTH Levels and BV/TV in Wedge 1.](image)
Figure 9.2 Correlation of Trabecular Number and PTH Levels in Wedge 1.

Figure 9.3 Correlation of Trabecular Thickness and PTH Levels in Wedge 1.
Figure 9.4 Comparison of BV/TV and Serum PTH Levels in Wedge 1 and Wedge 4.

Higher PTH Levels were associated with non-significant reduction in BV/TV in both Wedges 1 and 4.

Figure 9.5 Comparison of Trabecular Number and PTH Levels in Wedge 1 and Wedge 4.

Higher PTH levels were associated with a non-significant reduction in
In wedge 4 (table 9.2), we found that vitamin D and PTH levels correlated with bone mass. Higher PTH levels were associated with reduced BV/TV, reduced trabecular number, increased trabecular spacing and reduced trabecular thickness. Low vitamin D levels correlated with lower bone volume/total volume ratio (BV/TV) and bone mass. Bone resorption was measured using C-telopeptide (CTX). Higher CTX levels were associated with reduced BV/TV and bone density.

**PTH Levels and Cortical Bone**

As can be seen in figure 9.5, patients with the highest PTH levels had the thinnest cortical depths, with a mean difference of 0.12 mm$^2$. Although, there was a trend to have reduced cortical depths with increased PTH levels, this was not statistically significant.

![Cortical bone dimensions according to PTH levels](image-url)
<table>
<thead>
<tr>
<th></th>
<th>BV/TV</th>
<th>Connective Density</th>
<th>SMI</th>
<th>Trabecular Number</th>
<th>Trabecular Thickness</th>
<th>Trabecular Spacing</th>
<th>Mean Density Total Volume</th>
<th>Mean Density Bone Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip BMD</td>
<td>0.40</td>
<td>-0.18</td>
<td>-0.53</td>
<td>-0.1656</td>
<td>0.2883</td>
<td>0.0678</td>
<td>0.3130</td>
<td>0.4248</td>
</tr>
<tr>
<td>Neck BMD</td>
<td>0.25</td>
<td>0.10</td>
<td>-0.35</td>
<td>0.0814</td>
<td>-0.0492</td>
<td>-0.2451</td>
<td>0.1476</td>
<td>0.1379</td>
</tr>
<tr>
<td>Spine BMD</td>
<td>0.24</td>
<td>0.12</td>
<td>-0.35A</td>
<td>0.3188</td>
<td>-0.0732</td>
<td>-0.4590A</td>
<td>0.0800</td>
<td>0.4248</td>
</tr>
<tr>
<td>CTx</td>
<td>-0.36</td>
<td>-0.04</td>
<td>0.16</td>
<td>-0.2040</td>
<td>-0.1759</td>
<td>0.2669</td>
<td>0.3534A</td>
<td>0.3642A</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>-0.29</td>
<td>-0.18</td>
<td>-0.05</td>
<td>-0.2559</td>
<td>-0.1229</td>
<td>0.3568</td>
<td>-0.3505</td>
<td>-0.2060</td>
</tr>
<tr>
<td>PTH</td>
<td>-0.31</td>
<td>0.13</td>
<td>-0.03</td>
<td>0.0141</td>
<td>-0.4125A</td>
<td>0.1529</td>
<td>-0.5419A</td>
<td>-0.4676A</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0.46A</td>
<td>-0.26</td>
<td>-0.37</td>
<td>-0.3254</td>
<td>0.3432</td>
<td>0.0794</td>
<td>0.6129</td>
<td>0.6648</td>
</tr>
</tbody>
</table>

A = p<0.05
<table>
<thead>
<tr>
<th></th>
<th>BV/TV</th>
<th>Connective Density</th>
<th>SMI</th>
<th>Trabecular Number</th>
<th>Trabecular Thickness</th>
<th>Trabecular Spacing</th>
<th>Mean Density Total Volume</th>
<th>Mean Density Bone Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip BMD</td>
<td>-0.0579</td>
<td>-0.4318 ^A</td>
<td>-0.0958</td>
<td>-0.5225</td>
<td>0.2017</td>
<td>0.3434</td>
<td>0.0012</td>
<td>0.3513</td>
</tr>
<tr>
<td>Neck BMD</td>
<td>0.2407</td>
<td>-0.2072</td>
<td>-0.3258</td>
<td>-0.2531</td>
<td>0.3472</td>
<td>0.0530</td>
<td>0.2276</td>
<td>0.5128 ^A</td>
</tr>
<tr>
<td>Spine BMD</td>
<td>-0.0280</td>
<td>-0.0659</td>
<td>-0.1664</td>
<td>-0.1081</td>
<td>0.0180</td>
<td>-0.0311</td>
<td>0.0113</td>
<td>0.1210</td>
</tr>
<tr>
<td>CTx</td>
<td>-0.1623</td>
<td>-0.3603</td>
<td>-0.0510</td>
<td>0.1855</td>
<td>-0.3757</td>
<td>-0.1599</td>
<td>-0.1838</td>
<td>-0.4229 ^A</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>-0.2905</td>
<td>-0.0051</td>
<td>0.0593</td>
<td>-0.1003</td>
<td>-0.2944</td>
<td>0.1897</td>
<td>-0.3299</td>
<td>-0.3206</td>
</tr>
<tr>
<td>PTH</td>
<td>0.0120</td>
<td>0.2655</td>
<td>-0.0905</td>
<td>0.1146</td>
<td>-0.1973</td>
<td>-0.1293</td>
<td>-0.0752</td>
<td>-0.1728</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>-0.0160</td>
<td>-0.5158 ^A</td>
<td>0.0956</td>
<td>0.4537 ^A</td>
<td>0.2981</td>
<td>-0.4574 ^A</td>
<td>0.0509</td>
<td>0.4157 ^A</td>
</tr>
</tbody>
</table>

\(^{A}p<0.05\)
8.4 Discussion

In this study of femoral bone specimens in patients with hip fractures, we found a strong relationship between serum parathyroid hormone and vitamin D levels and the BV/TV estimated by microCT, trabecular structural parameters and cortical measurements. Higher PTH levels were associated with reduced BV/TV, reduced trabecular number and reduced cortical depths. We also found that BMD did correlate with bone mass, it poorly identified patients with reduced cortical and trabecular parameters.

In this study, almost 1/3 of patients did not have osteoporosis based on standard BMD testing. MicroCT did demonstrate that BMD correlated with trabecular number and bone volume. Patients with lower BMD had less bone mass and trabeculae implying reduced bone strength and thus an increased risk of fracture. Increased bone turnover appeared to also reduce bone volume and trabecular number, indicating a bone more susceptible to fracture.

The majority of studies that investigate osteoporotic bone quality tend to focus on trabecular bone tissue. This is because of the prevailing belief that the rate of bone turnover is higher in areas of trabecular bone; thus, most of the deterioration in bone quantity and quality, including microarchitecture, will be found in these areas. Neither of these suppositions is necessarily true. There is several studies which have emphasised the rapid loss of trabecular bone mass following menopause in women at the primary determinant of bone fragility, both old and new studies point to an important role for the cortex, particularly when the trabecular bone volume is low. We found in addition to trabecular bone loss, there was a reduction in cortical bone mass
and cortical depths. Trabecular bone appears clinically to have a higher turnover than cortical bone, however, recent studies have suggested that up to 70% of bone strength may be explained by cortical bone mass [393]. This suggests an important relationship between compact bone microarchitecture and fracture risk in osteoporosis.

There is increasing interest in the use of in-vivo micro-CT for drug related research and in human based research. Bone quality is difficult to quantify as it encompasses several aspects including bone architecture. There is need for caution when using in vivo micro-CT due to the influence of ionizing radiation on bone metabolism. Previous studies report radiation doses between 0.2 and 1.0 Gy per scan. Although this may be relatively low, such a dose is still about 10,000 times greater than that generated by a general chest x-ray. A single dose as low as 0.25 Gy negatively affects bone marrow in humans [394], and thus this caution is justified.

A limitation of the present study is the size of the data set, which was relatively small (25 subjects). However, the number of the here investigated patients is similar to that of other studies reporting age-related changes in cancellous bone: 28 arthritic patients and 69 controls [395], 44 donors [396], 31 donors [397], 40 donors [15], 36 arthritic patients and 36 controls [21], 33 arthritic patients and 12 controls [20].

To the authors’ knowledge, this is the first study showing the influence of bone turnover on the structural parameters in human femoral heads from patients who have sustained hip fractures. Increased bone turnover, particularly increased serum parathyroid hormone and reduced serum vitamin D levels, leads to alterations in bone
microarchitecture. These alterations in bone microarchitecture may have been as important as low BMD for these patients sustaining hip fractures. Further studies may focus on the influence of reducing bone turnover and vitamin D replacement has on improving these microCT findings associated with bone quality.
Chapter 9. The Influence of Bone Turnover and Serum Vitamin D Levels on Bone Elasticity Moduli and Hardness measured by Nanoindentation

9.1 Introduction

The characterisation of the biomechanical properties of bone as a living biomaterial is important in understanding the behaviour of bone. Bone is not a homogenous material but a composite of proteins, crystals and calcium organized into various structures and compartments. Several physiological processes can lead to variations in these composite materials and thus lead to an alteration in the behaviour of the underlying bone tissue. The ability to assess the risk of fracture, evaluate new therapies and assess the influence of bone remodelling disorders requires specific measurement of these local bone micromechanical properties and their changes to various physiological states.

Nanoindentation is widely used in the materials science community for the probing and assessment of the mechanical properties of thin films, small volumes and small microstructural features. Nanoindentation has been established as an effective method to measure the mechanical properties of various hard tissues, including bone, at the micron and sub-micron scale. Using this technique, there is the possibility of determining the elastic and hardness properties, which reflects the degree of mineralisation of the bone tissue. There is clinical and laboratory evidence that in addition to BMD, the mechanical properties of bone tissue may play a critical role in bone strength [398, 399]. These mechanical properties would be expected to play a significant role in bone fracture.
Physiological processes such as increased bone remodelling, increased bone turnover and vitamin D deficiency can affect bone quality and may lead to fracture, independent of bone mineral density.

We performed measurements of tissue elastic and hardness moduli using nanoindentation of femoral bone tissue from patients who had sustained a hip fracture. We compared cortical and trabecular bone and we postulated that there would be variation in the elastic and hardness moduli corresponding to bone turnover and bone mineral density. To date, there has been little published on the effect of bone turnover on the parameters of nanoindentation, particularly in relation to vitamin D metabolism.

9.2 Materials and Methods

Bone Samples

Human femoral heads were collected from patients who were undergoing total hip arthroplasty for a hip fracture. Patients who were suspected of having secondary osteoarthritis, Paget's disease, congenital hip dysplasia, rheumatoid arthritis or other inflammatory joint disease which may have affect the bone architecture and quality were excluded from the study. A total of 25 femoral heads were collected for the present study (18 females and 7 males). A written informed was obtained from the patients. This study was approved by the local Ethics Commitee (AMNCH Ethics Committee). The samples were stored at -20C and all samples were process within 8 weeks of collection.
Specimens were prepared as per cutting, embedding and polishing protocols described in appendices 11.

Figure 9.1 Each indent produced a loading and unloading curve, as shown in the following diagram.

The elastic modulus was determined from the unloading portion of the curve. This unloading portion represents the elastic recovery of the material as the load is removed. The Testworks software recorded the force-displacement data continuously and all the parameters were exported to Microsoft Excel for further analysis. We carried out multiple indents in the cortical (osteonal and interstitial lamellae) and trabecular parts of the samples.
In total, 675 indents were performed in this study. Twenty-seven indents per specimen were performed: 9 indents in interstitial and 9 indents in trabecular bone. Three osteons were identified per sample with 3 indents performed in the longitudinal direction (i.e. perpendicular to the transverse section). Osteonal bone consists of
layers/lamellae that alternated in thick and thin lamellae. All indents were made on the thick lamellae. Interstitial bone consists of primary interstitial bone and old osteons.

Figure 9.4 and 9.5 Displacement curves generated for 2 separate OP3 and OP4.
Statistical Analysis

An important objective of the project was to correlate findings with vitamin D and bone markers levels. Data are presented as mean ± standard deviation (SD). For statistical analyses, groups were assessed for normal distribution and then compared using ANOVA and t-tests. For those variables failing the normality test, a nonparametric Mann-Whitney rank sum test was used. JMP 8.0.2 statistical package was used for statistical analyses. A p value of <0.05 was considered to be significant.
9.3 Results

25 patients were consented in the study. The baseline characteristics of the group are described in table 9.1.

<table>
<thead>
<tr>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>79.7 (+/-8.2)</td>
</tr>
<tr>
<td>Female: Male</td>
<td>18:7 (72%)</td>
</tr>
<tr>
<td>Weight</td>
<td>61.7 (+/-16.8)</td>
</tr>
<tr>
<td>Height</td>
<td>158.8 (+/-7.2)</td>
</tr>
<tr>
<td>BMI</td>
<td>24.3 (+/-5.0)</td>
</tr>
</tbody>
</table>

Table 9.1 Baseline Characteristics of Study Population.

A summary of the elastic moduli (E) and hardness moduli (H) of the trabecular, osteonal and lamellar bone are represented in table 1. Tissue elastic modulus and tissue hardness varied between specimens. Mean values for the elastic modulus and hardness of the trabecular bone were calculated for each of the samples. Mean trabecular hardness was 0.23 (+/-0.11) GPa, mean cortical osteonal bone was 0.25 (+/-0.10) GPa and mean cortical interstitial bone was 0.29 (+/-0.10) GPa. Using ANOVA, the overall analysis was significant (p=0.03).

<table>
<thead>
<tr>
<th>Trabecular</th>
<th>Osteonal</th>
<th>Interstitial</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elasticity</td>
<td>0.23 (+/-0.11)</td>
<td>0.25 (+/-0.1)</td>
<td>0.29 (+/-0.1)</td>
</tr>
<tr>
<td>Hardness</td>
<td>6.37 (+/-3.25)</td>
<td>9.38 (+/-2.48)</td>
<td>11.5 (+/-3.5)</td>
</tr>
</tbody>
</table>

Table 9.2 shows correlation co-efficients of bone turnover markers and elastic and hardness moduli.
Figure 9.3 Vitamin D tertiles and elasticity measured in 3 types of bone tissue (trabecular, osteonal and interstitial).

Figure 9.4 Vitamin D tertiles and hardness measured in 3 types of bone tissue (trabecular, osteonal and interstitial). N/S=non-significant
Vitamin D and Bone Turnover Markers influence on Nanoindentation Results

Table 3 shows the $R^2$ correlation co-efficients between bone turnover markers, vitamin D, parathyroid hormones levels and nanoindentation derived parameters. We found that there was a significant correlation between serum C-telopeptide levels and measured hardness moduli in trabecular ($r^2=0.31$, $p<0.05$), cortical osteonal ($r^2=0.28$, $p<0.05$) and cortical interstitial bone ($r^2=0.21$, $p<0.05$). There was no significant correlation between bone formation (osteocalcin) and tissue elasticity and hardness.

In figure 9.3, we compared serum vitamin D tertiles and elasticity measured by nanoindentation in the three bone tissue types. The lowest vitamin D tertile (10-22 nmol/L) had the lowest elasticity results in all bone types, but particularly in trabecular bone. There was an increase in all elasticity moduli with increasing serum vitamin D levels, with the highest being observed in the highest tertile of vitamin D (44-69 nmol/L).

In figure 9.4, we compared serum vitamin D tertiles and hardness measured by nanoindentation in the three bone tissue types. The lowest vitamin D tertile (10-22 nmol/L) had the lowest hardness results in all bone types. For each vitamin D tertile group, there was an increase in hardness from trabecular, osteonal and interstitial bone in that order; however, this was not statistically significant ($p<0.05$).
<table>
<thead>
<tr>
<th></th>
<th>Trabecular Elasticity</th>
<th>Trabecular Hardness</th>
<th>Cortical Elasticity (Inter)</th>
<th>Cortical Hardness (Inter)</th>
<th>Cortical Stiffness (Osteon)</th>
<th>Cortical Hardness (Osteon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTx (ng/mL)</td>
<td>0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11</td>
<td>0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.12</td>
<td>0.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08</td>
<td>0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.07</td>
<td>0.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin D (nmol/L)</td>
<td>0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Table 9.3 Correlations of Bone Turnover Markers and Nanoindentation Results*

<sup>a</sup>p<0.05  <sup>b</sup>p<0.01
Figure 9.5 Correlation of serum 25(OH)D levels and mean elastic moduli.

\[ y = 0.0044x + 0.0701 \]
\[ R^2 = 0.75135 \]

Figure 9.6 Correlation of serum 25(OH)D levels and mean hardness.
9.4 Discussion

The characterisation of the biomechanical properties of bone as a living biomaterial is important in understanding bone's behaviour. Mature human bone is made composed of densely packed osteons and interstitial bone and therefore not homogenous in its mechanical behaviour. Advanced bone imaging and analysis technologies may lead to better assessment of bone strength [400] but rely on potentially inaccurate assumptions about the tissue-level mechanical properties. One of the great advantages of the nanoindentation technique is its ability to probe a surface and map its properties on a spatially resolved basis, often with a resolution of better than $1\mu m$. Since many of the microstructural features of interest in bone are several micrometres or more in dimension, the nanoindentation technique offers a means by which their intrinsic mechanical properties can be measured directly.

In this study, the elastic moduli and hardness of bone types from patients who had a hip fracture were shown to have variations in the moduli of older bone compared to newer bone found in the osteons. This is demonstrated in table 2. Cortical bone was harder and stiffer when compared to trabecular bone, with interstitial bone (older bone) showing the highest elastic moduli and hardness levels. Rho et al [401] have shown variations in the individual thick lamellar properties within osteons by nanoindentation. These variations in elasticity and hardness are thought to be related to changes in mineral content of the bone, organisation of collagen fibres and arrangements of mineral crystals [402]. Nanoindentation offers a continuous stiffness measurement enables a direct study of the viscoelastic properties of human cortical bone down to lamellar
level and can demonstrate individual variations of lamellar bone between bone tissue types.

Several studies have given a range of results for the elastic modulus of bone when measured by nanoindentation. In our study, serum 25(OH)D and parathyroid hormone levels strongly correlated with both hardness and elastic moduli. Patients with higher levels of 25(OH)D levels had higher elastic and hardness moduli i.e. stiffer and stronger bone. As we have previously discussed in chapter 7, vitamin D deficiency (defined by a serum 25(OH)D level <50nmol/L) is highly prevalent in patients with hip fractures being admitted to our hospital. It is unclear if prior vitamin D supplementation would have altered the biomechanical properties seen in our study population. Thus, any further work with nanoindentation in human should acknowledge underlying bone turnover, particularly vitamin D deficiency as influencing indenting results.

It is well documented that the mechanical properties of macroscopic bone specimens vary depending on whether the samples are tested dry or wet, nanoindentation is generally conducted on dehydrated bone tissue at room temperature. The reason for this is primarily due to nanoindentation systems being extremely sensitive to changes in environmental conditions such as humidity and temperature. Rho et al have shown that the elastic and viscoelastic properties from cortical tibia bone at lamellar level using nanoindentation varied depending on environmental and preparation conditions [234]. In our study, samples were dehydrated initially and embedded in a resin. The testing room was strictly kept at the same temperature and humidity at all times so as to reduce the external factors that would cause variation in the sample
results. It must be noted that some studies have reported an elevation of the estimates in elastic modulus and hardness using nanoindentation when samples have been dehydrated and embedded [236]. As all samples in our study underwent the same processing in dehydration and embedding, it is unlikely that the heterogeneity in mechanical properties within the bone has altered nor the relationship between the elasticity and hardness reported here. We do have to acknowledge that samples were obtained from a standardised location. There would have been some heat due to friction generated by cutting, radiation exposure and embedding of the bone specimens. It was impossible to prevent dehydration of the bone samples during the nanoindentation technique, as it was obviously needed for embedding of the bone samples. The effects of soaking the samples in alcohol, and exposing them to air and heat during storage and testing would have affected the elastic modulus and hardness results. It is known that drying increases the elastic modulus value recorded, so the actual figures for this parameter would be slightly lower. Townsend et al [235] found that drying increases Young's moduli of individual trabeculae by about 24%.

Recently, one study has reported the use of bone micro-indentation (BMT) of periosteal bone of the tibia in patients with and without osteoporotic fractures [403]. The technique may be a clinical option in assessing patients with fragility fractures. Preclinical studies in human cadavers suggest that the BMT technique induces separation of mineralised collagen fibrils and the initiation of microcracks, which is the likely the basic mechanism of fracture. This method directly measures the mechanical competence of bone tissue to resist fracture [403].
In summary, we report a novel technique that measures an aspect of bone quality not routinely being evaluated. This represents a direct assessment of bone tissue's mechanical strength in patients with hip fractures, which is an important component of the properties encompassed under the umbrella of "bone quality." Vitamin D deficiency appears to have a negative effect on the mechanical strength of bone with patients having the lowest vitamin D levels having reduced hardness and elasticity.
Chapter 10. Summary and General Discussion

10.1 Introduction

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength resulting in an increased risk for fractures. Dual X-ray absorptiometry (DXA) is the standard test to assess for osteoporosis in fracture patients, although there is a significant overlap between patients diagnosed with normal and osteoporotic bones who subsequently fracture. Bone quality and fragility are terms used to describe how bone mineral density, microarchitecture, mineralisation and propensity to fall interact causing fracture. By analysing osteoporotic bone samples, we hoped to reveal alterations in bone that would not be routinely available with DXA. New techniques of assessment of bone quality and structure have been developed such as high-resolution computerized tomography (microCT) and nanoindentation assess the microarchitecture and mineralisation of bone respectively, which may account for the variance in patients who fracture.

More recently, there has been interest in the effect vitamin D has on reducing falls, bone quality and muscle strength. Falls are often the cause of fracture in the elderly. Higher levels of vitamin D have been associated with reduced incidence of falls but also can reduce bone turnover, improve muscle strength, and improve balance, although the level of vitamin D required is uncertain. Accelerometers are a new technique that can measure improvements in balance with increasing levels of serum vitamin D.
Fractures that result from osteoporosis are a major and growing concern for future health systems. As the population ages, the number of fractures worldwide will double, and in some places triple, in the next 50 years. Osteoporosis is a common problem in older adults patients and we have seen that a high percentage of patients being reviewed in our osteoporosis service have had a fracture (chapter 2). All osteoporotic fractures may lead to a significant physical impairment, with fractures of the proximal femur (hip) being a major cause of morbidity and mortality and often contribute to the patient’s loss of independence and of quality of life. The ability of bone to resist fracture depends not only on the bone mass but also on the bone distribution and the intrinsic properties of the materials that constitute bone. In addition, older patients are more likely to experience an osteoporotic fracture as they have the highest prevalence of osteoporosis and highest risk of falling.

The theme of this study was to determine the reasons and risk factors for fractures in patients with reduced bone mass (either osteoporosis or osteopaenia).

10.1 Risk Factors for Osteoporosis

Osteoporosis is a disease characterised by low bone mineral density (BMD) and structural deterioration of bone tissue leading to bone fragility and increased susceptibility to fractures, particularly of the hip, spine and wrist [404]. Although BMD should not be used in isolation to predict fractures, BMD predicts osteoporotic fractures independently of age, body weight and prevalent fractures, and regardless of the site of measurement [405].
Advancing age and prior fragility fractures are markers of low bone mass in our patients (Chapter 2). In addition, low body mass index, height loss, smoking, rheumatoid arthritis, steroid use and a history of thyroid disease were strong predictors for low bone mass in our study population. These factors should be given consideration in assessing the urgent need for bone mass assessment and may lead to an increase in fracture risk if left untreated.

10.2 The Prevalence of Vertebral Fractures

In Chapter 3, we reviewed the prevalence of vertebral fractures in patients attending for DXA. Bone mineral density (BMD) alone underestimates the severity of osteoporosis in patients with previously undiagnosed vertebral fractures. Lateral vertebral assessment (LVA) was used as a diagnostic tool for the identification of vertebral fractures in patients attending for DXA and highlighted patients who required additional assessment and treatment for optimal bone health. High risk groups of patients, such as patients who have had a hip fracture or those who are receiving glucocorticoid therapy, appear to have a higher prevalence of vertebral fractures when compared to control patients with similar BMDs. We looked at the overall prevalence of vertebral fractures in the DXA cohort and included two high risk groups of patients i.e. patients with hip fractures and patients who had a history of glucocorticoid use >5.0 mg for more than 3 months. The overall percentage of vertebral fractures identified were 16.9%. Younger male patients (50-59 years) had a higher prevalence of vertebral fractures compared to similar aged female patients (10.47% vs 4.00% respectively, p<0.05). Older females patients had a higher prevalence of vertebral fractures, with 48.51% of women over 90 years old having at least one vertebral fracture. Patients with a history of a hip fracture had the highest
prevalence of vertebral fractures (38.1%), although they were significantly older than
the general population. Patients with a history of glucocorticoid usage had a higher
vertebral fracture prevalence compared to the overall group and the risk increased
two-fold over similar age-matched patients who were not on glucocorticoid therapy.
There is a high prevalence of vertebral fractures in patients who attended our unit for
a DXA examination, particularly in those with significant risk factors for osteoporosis
such as a previous hip fracture or a history of glucocorticoid use. It is our
recommentation that lateral vertebral assessments should be performed on all patients
attending for a DXA scan.

10.3 Quantitative Heel Ultrasound

Heel ultrasound appears to perform similarly to DXA in discriminating prevalent
fractures however there were differences in gender and fracture type (Chapter 5). We
found that QUS was able to discriminate hip and wrist fractures better than DXA.
However, in male patients BMD measurements were better at discriminating hip
fractures compared the heel ultrasound measurements. Heel ultrasound (QUS) has
been used as an alternative to the assessment of bone health and to predict future risk
of fracture in several studies [298-304]. Several prospective studies have shown QUS
predicts future fracture risk, including hip fractures, independently of BMD, and
nearly as well as DXA [210-218]. The use of clinical risk factors and calcaneal
quantitative ultrasound can correctly identify more women at low risk fracture for hip
fracture compared to either the stiffness index on QUS or the clinical risk factors
alone [219]. More recently, the EPISDEM study demonstrated that the combination of
the predictive power derived from clinical risk factors (CRFs) and QUS
measurements can be used to predict 10-year probability of hip fracture [305, 306].
10.4 Vitamin D Deficiency, Secondary Hyperparathyroidism and Increased Bone Turnover

Vitamin D is well recognised to be suboptimal in older patients and patients with fragility fractures are more likely to have lower serum vitamin D levels when compared to age-matched controls. Vitamin D deficiency is considered to be a serum level of 25-hydroxyvitamin D [25(OH)D] <50 nmol/L and a serum level >75 nmol/L is considered to be an optimal level. In Chapters 5 and 7, we found that the prevalence of vitamin D deficiency was high in patients attending our osteoporosis clinic and in patients admitted with a hip fracture. We found that a large proportion of patients attending our hospital were vitamin D deficient, even though they were taking vitamin D supplementation. For example, 67.3% of patients admitted with hip fractures were vitamin D deficient (<50 nmol/L), with only 9% of patients having an optimal vitamin D level (>75 nmol/L). Approximately one quarter of our patients with hip fractures demonstrated secondary hyperparathyroidism.

In Chapter 5, we found that patients with the lowest serum 25(OH)D levels had increased PTH and bone turnover marker (BTM) levels compared to those in the higher levels. Older patients appeared to have the highest PTH levels for a given vitamin D tertile level. Increased bone turnover, particularly bone resorption, can lead to bone loss and thus increased the likelihood of fracture. In some studies, baseline BTM levels correlated well with subsequent bone loss [241], which suggests that the bone turnover rate determines the subsequent bone loss. This association has been assessed in several prospective and case control studies [242, 244-246]. Thus, aggressive vitamin D replacement and anti-osteoporosis therapy should be promoted
to normalise PTH levels and reduce bone turnover to protect those at high risk of fractures.

Vitamin D deficiency can lead to secondary hyperparathyroidism which has an adverse affect on bone health by increasing bone turnover. Several studies have observed an increased serum PTH concentration in older people with or without fractures were associated with vitamin D deficiency [141, 312, 313]. In our study, serum PTH correlated with levels of serum 25(OH)D, with higher levels of PTH being observed in those with lower vitamin D levels. In our study, mean PTH levels increased with decreasing serum vitamin D levels. Some studies have suggested that patients with the highest levels of PTH in older patients have poorer outcomes post fracture [314]. Patients with an elevated PTH compared to those with serum PTH in the normal range have a 15 times greater risk of fatal outcome and increased hospital stay [314]. Patients with hypovitaminosis D and secondary hyperparathyroidism compared to those without elevated PTH levels have increased bone turnover [315-318], increased fracture risk [315] and a higher mortality rate, especially cardiovascular deaths [316, 319]. Thus, secondary hyperparathyroidism should be recognised as a significant risk factor for poor outcomes in older adults and should be treated aggressively.

10.6 Micro-architectural Changes

Bone quality encompasses micro-architecture of bone and the intrinsic properties of bone, along with bone mineral density. The use of microCT has been mainly used in-vitro for the assessment of bone microarchitecture. MicroCT may offer an avenue of assessment of biomechanical relationships in bone, which
is routinely not captured on DXA. We noted that patients with increased PTH levels had reduced bone mass, trabeculae and cortical thinning, particularly in the superior portions of the femoral neck. As we have previously discussed, up to one quarter of patients being admitted with hip fractures have secondary hyperparathyroidism related to vitamin D deficiency. However, it is unclear if reducing the secondary hyperparathyroidism caused by vitamin D deficiency will lead to a reverse in the micro-architectural changes that were observed.

10.7 The Measurement of the Intrinsic Properties of Bone

Nanoindentation is an established tool for the assessment of micromechanical properties of hard biological tissues. In Chapter 9, we measured the elastic moduli and hardness of human bone samples. Samples were obtained from femoral heads of patients who had sustained hip fractures. We found that patients with vitamin D deficiency had reduced hardness and stiffness of their bones. This represents a direct assessment of bone tissue’s mechanical strength in patients with hip fractures, which is an important component of the properties encompassed under the umbrella of bone quality. Vitamin D deficiency appears to have a negative effect on the mechanical strength of bone with patients having the lowest vitamin D levels having reduced hardness and elasticity.

10.8 Further Considerations

Our study was conducted on patients admitted with falls and fractures with a high prevalence of vitamin D deficiency. There is much debate on the optimal level of serum vitamin D (25(OH)D). Recently, the International Osteoporosis Foundation (IOF) have published recommendations for the optimal serum vitamin D level to be
above 75nmol/L [143]. This level is based on maximal PTH suppression, reduced bone loss and a reduced rate of falls. The dose of vitamin D supplementation required by a patient would depend on several factors including the baseline level of serum 25(OH)D, body mass index, sun exposure and vitamin D metabolism. For individuals with effective sunlight exposure, a dose of 800 IU/day vitamin D₃ may be sufficient. However, patients with obesity, a history of falls, known osteoporosis and limited sun exposure may require higher doses of vitamin D₃ up to 2000 IU/daily [143]. The rate of replacement, mode of administration and the total dose required for reaching optimal levels still remains to be fully established and is another area for research in the future.

As previously discussed, vitamin D deficiency and high PTH levels appear to be associated with detrimental alterations in the microarchitecture and the material properties of bone. It is unclear if correcting these biochemical abnormalities will lead to an improvement in the micro-architectural and intrinsic bone material properties that were observed. Further studies should establish if reduction of PTH levels and optimising vitamin D levels have beneficial effects on bone quality parameters.

There are some very promising concepts of orthogeriatric co-management in older fracture patients, most of which have shown to improve the patient’s outcome in different areas. By establishing a high level of care for patients who have sustained a hip fracture, it would be hoped that the morbidity and mortality associated with hip fracture would be reduced. Highlighting and treating patients at risk of fracture will ultimately lead to a reduction in the strain placed on health systems in the future.
10.9 Conclusion

Fragility fractures are one of the main contributors to mortality, morbidity and diminished quality of life in older adults. Comprehensive assessment of bone quality, including assessment of the risk of fall, may improve the outcome in these patients. Better understanding of the ability of bone to resist fracture (i.e. risk factors of low bone mass, underlying microarchitecture and intrinsic material properties of the bone tissue) may lead to the improved ability to assess the risk of fracture, evaluate new therapies and assess the influence of bone remodelling disorders. The assessment of all these parameters encompassed by bone quality would greatly add to predicting future fracture predictions.
Chapter 11. Appendices

11.1 Bone Sample Analysis Flowchart
11.2 Serum Biochemistry
11.3 Bone Mineral Density Measurement
11.4 Protocol for Theatre Specimen Collection
11.5 Safety Protocol for Coring and Cutting of Human Femoral Heads
11.6 Preparation of Bone Specimens
11.7 Coring and Cutting
11.8 Storage and Cleaning
11.9 MicroCT Scanning
11.10 Preparing and Embedding the Samples
11.11 Polishing Protocol
11.12 Nanoindentation Protocol
11.13 MicroCT Images
11.14 Nanoindentation Images
11.15 Examples of MicroCT analysis forms
11.16 Vitamin D Assessment Form
11.17 DXA Patient Questionnaire Form
11.18 Bone Health Assessment Form for Patients with Fractures
11.1 Bone Sample Analysis Flowchart

1. Consent and collection of bone from theatre
2. Bone markers and vitamin D analysis
3. Bone Coring
4. Quantitative CT of bone
5. Bone Cleaning
6. Fixation and Storage
7. Preparation and polishing of bone
8. Nanoindentation

- Consent and Collection of Bone Sample from Theatre → Collection of Bone Markers, Vitamin D and PTH
- Bone Coring → HIV, Hepatitis B and C collection
- Quantitative CT Scanning of Samples → Histology/Histomorphometry of Bone Samples
- Cleaning of Bone Samples → Department of Pathology St James Hospital
- Embedding and Nanoindentation of Bone Samples → Bioengineering Department in Trinity College Dublin

239
11.2 Serum Biochemistry

Serum and 24 hour urine samples will be collected at baseline and again at each evaluation. Biochemical markers of bone turnover were measured at each evaluation and were collected before 1pm.

Routine measurements include:
- Renal profile, Liver profile, Thyroid profile, FBC, Magnesium
- Albumin
- Estimated Creatinine Clearance

Calcium Homeostasis
- Calcium
- Phosphate
- Alkaline Phosphatase
- Parathyroid hormone: endogenous
- 25 (OH₂) D₃
- 24 Hour urinary calcium

Immunological
- TTG and EMA
- Serum immunoglobulins and Protein electrophoresis

Markers of Bone Formation
- Procollagen Type 1 Amino terminal Peptide (PINP)
- Osteocalcin

Markers of Bone Resorption
- C-telopeptide
**Serum Vitamin D**

Serum 25(OH)D (in nmol/l) was analysed at St James’s Hospital Biochemistry Department using the DiaSorin LIAISON® 25-OH Vitamin D TOTAL (http://www.diasorin.com/en/productsandsystems/view/20), a chemiluminescence immunoassay. Inter- and intra-assay coefficients of variation were <12%. Internal quality control was determined using kit controls of two different concentrations. The laboratory participates in the International Vitamin D External Quality Assessment Scheme (DEQAS, subgroup Liaison users) as a means of determining accuracy of results.

Serum vitamin D status was classified using the cut off criteria outlined by International Osteoporosis Foundation. A serum 25(OH)D less than 50 nmol/L was considered to be vitamin D deficient, levels between 50-75 nmol/L were classified as vitamin D insufficient, and levels >75 nmol/L were considered optimal. The mean 25(OH)D levels are presented for type of residence and season. The vitamin D levels are presented for patients who are not supplemented with vitamin D and on vitamin D supplementation in the results.
11.3 BMD Measurement

BMD and total body composition will be measured at baseline using dual x-ray absorptiometry (Lunar prodigy).

**DXA Measurements will include:**

- Hip BMD
- Spine BMD
- Lateral Vertebral morphometry*

*For patients unable to lie on their side, lateral spine x-rays will be performed
Bone mineral density (BMD) was measured by a Lunar Prodigy (GE) primarily at 2 sites, the hips and the L1-L4 spine. In cases where one of these sites was not available (e.g. patients with bilateral hip replacements), the distal one third of the radius was assessed. The hip measurements included the total hip i.e. neck and intertrochanteric and the femoral neck. This technique analyses the attenuation of X-rays as they pass through an area of the body. DXA cannot detect the depth of the bone being measured, therefore the density that was measured was an “areal” density in g.cm$^{-2}$ rather than a “volumetric” density in g.cm$^{-3}$.

Patient BMD was measured at the lumbar spine (antero-posterior projection at L1-L4) and at the femurs (i.e. femoral neck and total hip). The World Health Organisation (WHO) classification system was applied, defining osteoporosis as a T score $\leq-2.5$ and osteopenia as $-2.5$ to $-1$. Study participants were categorized by the lowest T-score of the L1–4 lumbar spine, femoral neck, or total femur.

Quality control procedures for bone densitometers

The protocol for quality control of densitometers at the Osteoporosis Unit consisted of twice daily scans of a known-density hydroxyapatite spine phantom and a once-daily scan of a known-density hydroxyapatite right hip phantom. Spine data were entered into the machine quality control algorithm and had to fall within manufacturer-specified limits. The manufacturer-accepted coefficient of variation was 1.5 %. The measured coefficient of variation over the time frame of usage was 0.3 %. Daily quality control of the hip phantom was referenced to the initial hip phantom scan performed when the densitometers were installed by service engineers using the
compare mode. Data for BMD of the NOF had to fall between 0.72 and 0.76 g.cm\(^{-2}\) prior to patient scanning. These procedures were in place to ensure longitudinal stability and accuracy of patient scans. To assess longitudinal linearity of the densitometers, a Hologic block phantom containing known high, medium and low-density blocks was scanned in replicate every three months. The densitometer is independently checked by qualified service engineers twice per annum.

The cushion was placed under the legs so that the hips and knees were both flexed to 90° and the lumbar spine was flattened and flushes with the table, to ensure that the spine was parallel to the longitudinal axis of the table. The C-arm was moved so that the laser cross hairs were 5 cm distal to the umbilicus and ran parallel with and horizontal to the table. The patient was asked to lie still and breathe normally throughout the scan.

Patient positioning was verified using either the fast array mode or turbo array mode. The image was reviewed on screen and the cross hairs were moved so that the scan began in the middle of the fifth lumbar vertebra, ended at the twelfth thoracic vertebra at least, and included the intermediary vertebrae in their entirety.

When the image was satisfactory, the spine scan was performed using the array mode configuration. The array scan width and length were set at 11.36 and 20.36 cm, respectively. Point resolution was 0.09 cm and line spacing was 0.1 cm. When the array scan was completed, L1 to L4 were selected and each intervertebral space was marked using the inbuilt computer software. The scan was then analysed and a report screen was displayed and printed.
Hip Scan Procedure

The following was the procedures used in the Osteoporosis Unit for a hip scan performed. The patient was positioned on the table as shown in Figure 6. Positioning the left foot as shown in the diagram rotated the left leg inward 25° and also kept the left leg in a slight degree of abduction. The C-arm was then manoeuvred so that the laser cross hairs were 5 cm below the level of the greater trochanter of the left hip. The thigh was positioned so that its long axis was parallel with the longitudinal component of the cross hairs. The patient was instructed to lie still and breathe normally throughout the scan.

Patient positioning was verified using the fast array mode. The resulting image was then reviewed to ensure that all of the components of the proximal femur and hip had been included. If the image was satisfactory, the positioning hairs were aligned on the midpoint of the lateral edge of the greater trochanter and the hip scan was then carried out using the array mode configuration. Point resolution was 0.09 cm and line spacing was 0.1 cm.

After scanning, the image was analysed. Firstly, the areas of interest were selected, using demarcation bars provided by the computer software. A box was moved to the edge of the greater trochanter and covered only the femoral neck. This marked the position of the femoral neck. The computer then marked Ward’s triangle and the base of the greater trochanter. The computer then began to analyse the scan. When the analysis had been completed, a report screen was displayed and was printed out.
Spine and LVA scan procedure

The following was the procedure used in the Osteoporosis Unit for a spine scan performed using the Hologic QDR-4500™ Elite X-ray Bone Densitometer.

The patient was positioned on the table as shown in Figure 5. The cushion was placed under the legs so that the hips and knees were both flexed to 90° and the lumbar spine was flattened and flushes with the table, to ensure that the spine was parallel to the longitudinal axis of the table. The C-arm was moved so that the laser cross hairs were 5 cm distal to the umbilicus and ran parallel with and horizontal to the table. The patient was asked to lie still and breathe normally throughout the scan.

Patient positioning was verified using either the fast array mode or turbo array mode. The image was reviewed on screen and the cross hairs were moved so that the scan began in the middle of the fifth lumbar vertebra, ended at the twelfth thoracic vertebra at least, and included the intermediary vertebrae in their entirety.

When the image was satisfactory, the spine scan was performed using the array mode configuration. The array scan width and length were set at 11.36 and 20.36 cm, respectively. Point resolution was 0.09 cm and line spacing was 0.1 cm. When the array scan was completed, L1 to L4 were selected and each intervertebral space was marked using the inbuilt computer software. The scan was then analysed and a report screen was displayed and printed.
Calcaneal Ultrasound (Achilles Insight) will be performed at each evaluation:

Measurements will include:

Stiffness Index

Broadband Ultrasound Attenuation

Speed of Sound

We used the Achilles Insight (General Electric) for QUS. This system consists of two transducers, one being the transmitter and the other the receiver; ultrasound passes through the centre of the heel. The acoustic coupling between the two transducers is obtained by placing gel between the two membranes, at lateral sites, containing water at a temperature of 35°C.

The primary QUS variables are speed of sound (SOS) and broadband ultrasound attenuation (BUA), but the Achilles uses these to calculate a stiffness index based on normalized BUA and SOS, where \( n_{BUA} = 0.67 \times (BUA - 50) \) and \( n_{SOS} = 0.28 \times (SOS - 1,380) \). Stiffness is the addition of \( n_{BUA} \) and \( n_{SOS} \). Stiffness corresponds to \( 0.67 \times BUA + 0.28 \times SOS - 420 \).
11.4 Protocol for Theatre Specimen Collection

1. Patients who have consented will only be included in the study. Patients may undergo hemiarthroplasty or bipolar arthroplasty.

2. At the time of consent, a serum sample will be sent to the Department of Microbiology in St. James's Hospital for screening of Hepatitis B and C, along with HIV.

3. Theatre will be informed of the patients consent and collection and contact details will be relayed to the theatre staff i.e. bleep numbers, mobile numbers, etc.

4. The collected sample will be placed in a dry sterile container, without the use of formalin. The container will be labelled with MRN initially, but this will be changed on storage in fridge in Hospital 4. The patient will be allocated an identifier label (e.g. OP Hip 1, OP Hip 2, etc.).

5. Theatre will contact either Dr Joe Browne or a member of the osteoporosis team for collection of the sample.

6. Samples will be collected from theatre and moved directly to the storage fridge in Hospital 4, St James's Hospital. It will be stored at a temperature of –20 degree Celsius.

7. For out of hours sample collection, Dr Joe Browne will be contacted on his mobile. At that stage, a person will be nominated to collect the sample. This may be done by the on call MedEL Senior House Officer or Registrar.

8. If the sample cannot be collected directly from theatre, the sample will be stored in the specimen fridge within theatre. The sample will be clearly
labelled with the patient's MRN. The person collecting the sample must sign the specimen collection book within theatre.
11.5 Safety Protocol for Coring and Cutting of Human Femoral Heads

1. The fresh bone samples are to be labelled and stored individually in sealed, plastic containers in the freezer. The samples are to be stored at -20°C or -80°C, depending on the college at which they are being stored. In TCD, samples are stored at -20°C and in RCSI they are to be stored at -80°C. For RNA preservation, the samples would ideally be stored at -80°C, but samples previously brought to TCD, which does not have this facility, are stored at -20°C, the closest available alternative.

2. The samples are to be screened for HIV, Hepatitis B and Hepatitis C prior to testing and any samples returning positive results are to be disposed of accordingly and will not be used for testing.

3. When handling fresh tissue of any kind, great care needs to be taken not to contaminate working areas or risk spreading any infections or bacteria present. All work surfaces, equipment and any areas in contact with the tissue are to be cleaned using Virkon cleaner (For use in cleaning and disinfecting industrial, animal and agricultural facilities, active ingredients: 20.4% Potassium peroxymonosulfate and 15% Sodium Chloride) before and after use for each sample using disposable towels. This includes the outside of the sample containers and the freezers in which they are to be stored. It is vital that every piece of equipment is disinfected between samples to avoid cross contamination. The holesaws used to core the bone samples are to be soaked in Virkon for 5 minutes between the coring of samples. The blade used to divide the samples is to be wiped down with Virkon between samples also. (Possibility of soaking here also with rotation of blades).

4. For the purpose of RNA preservation, all work surfaces, equipment and areas in contact with the samples should be sprayed or wiped down with
anti-RNase wipes and/or spray before, after and in between coring and cutting of samples, as should the outside of the sample containers.

5. Surgical masks with attached eye-shield or safety glasses are to be worn to avoid inhalation of particles and to protect from splashes from the coring procedure. Nitrile surgical gloves, surgical gowns, caps and shoe-covers should be worn at all times when handling and processing the tissue. Care should be taken not to touch skin, hair, eyes etc. while working with the samples. Gloves should be changed between samples.

6. Between the coring and cutting procedures, the samples are to be returned to the freezer to avoid excessive thawing and possible degradation of the RNA within the samples.

7. All gowns, gloves, shoe-covers, eye-shields and paper towels used are to be disposed of in a separate hazardous waste container. Any human tissue waste is to be placed in its own hazardous waste container. These containers should be returned to St. James Hospital on a regular basis (after each batch?) for incineration by hazardous waste disposal. All persons using the work space for other purposes should be made aware of these containers and they should not be opened or used for any other purposes than those outlined. In the same way, all persons using the space should also be made aware of where and how the samples are being stored and that they constitute a potential risk of infection and so to minimise contact.

8. The room in which coring and cutting of samples will take place should not be accessible to others for the duration of these procedures. The relevant people will need to be informed of when this will be happening. The window and door are to be kept shut for the duration of the procedures to prevent contamination of the area from outside or vice versa.
9. All cut parts of the samples need to be marked and coded carefully before and after coring and cutting (to ensure the pieces taken are orientated correctly), and placed in individually labelled containers.
11.6 Preparation of Bone Specimens

Human femoral heads were obtained from patients undergoing hemiarthroplasty procedures at St. James Hospital. Ten of the heads were obtained in 2008 and a further fifteen retrieved in 2009. The samples were labelled to protect patient confidentiality.

The femoral heads were stored in St James’s Hospital until they were ready to be transported to Bioengineering Laboratory in Trinity College Dublin for further analysis. The safety protocol was of great importance in this part of the project. The measures taken to ensure safety throughout the project are outlined in the Safety section in Appendices 11.5.
11.7 Coring and Cutting

We used a milling machine to core the samples. Depending on the size of the femoral head, a different sized hole-saw was used accordingly. The hole saws were between 23 and 27mm in diameter. We used a vice and emery paper to prevent the femoral head from slipping. Before we placed the femoral head in the vice, we identified the inferior and superior surfaces of the head, and marked a line between the two using a hacksaw to divide the head into anterior and posterior regions. We chose the fovea on the proximal cortical surface as the reference point for cutting. We clamped and aligned the femoral head and began the process of lowering and raising the rotating hole-saw into the bone. A coolant was used to minimise damage to the bone.

Figure 11.1. Images showing the coring procedure.
When we finished this process we removed the core and remaining ring of bone from the vice. The cores were cut in half longitudinally and labelled Core A and B. Core A was kept for this project and B was given to RCSI for RNA extraction. The ring of bone was then divided into sections, as shown in the following diagram.

![Diagram](image)

Figure 11.2 Schematic diagram demonstrating the division of femoral head into cores and wedges.

Wedges 1 and 4 were kept at TCD for testing in this study; 2 and 5 were given to RCSI for RNA extraction; 3 and 6 were given to the Histology Department at St. James’s Hospital for analysis. All samples were then replaced in the freezer awaiting further testing and cleaning.

Wedge 1 corresponded to the superior portion of the femoral neck, whereas wedge 4 corresponded to the lower portion.
11.8 Storage and Cleaning

The bones samples were stored at -20°C in the Trinity Bioengineering Laboratory, and -80°C in RCSI and St James’s. The samples should be stored at -80°C to better preserve the RNA within the samples.

The bone samples were initially cleaned using distilled water. The bone marrow, blood, and soft tissue had to be removed to avoid decomposing and interference with the testing.
11.9 MicroCT Scanning

The first testing done on the samples was the microCT scanning (Scanco,μCT 40, Switzerland).

![Image of scanner](image)

**Figure 11.3: The inside of the scanner with the place for the container to slot into positioned in the centre of the picture**

Numbers given on the printout include total volume (TV), bone volume (BV), BV/TV ratio, mean density of TV and BV, trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), connective density (Conn.D) and Structure Model Index (SMI). A program was created on the connected computer so that the results got from the scans could be compared.

There is a plastic holding tube that the sample is placed in to the machine that rotates while the machine scans. The tube was 30.7mm in diameter that most samples fit snugly into. If there is space to move, sponge is placed around the sample so that it cannot move, as this would cause the scan to be blurred. The
sponge does not appear on the scan so it does not interfere with the results. The first step in the scan is to take a ‘scout view’ of the bone. This is a quick scan so that reference lines can be created as parameters for how much the machine will scan. This reference line is measured in relation to slices and time. 409 slices were chosen which took approximately 40 minutes to scan.

The computer automatically reconstructs the bone in 2D that then must be contoured and morphed for 3D reconstruction. This involved drawing around the edge of one slice and the next followed by a morphing tool that gels them together for the 3D task.
A program is then created to make the 3D reconstruction. The computer then calculates the structural indices and prints out the final image with results.

<table>
<thead>
<tr>
<th>Indices</th>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Areal bone mineral density</td>
<td>aBMD</td>
<td>2D derivation of mineral density (g/cm²) as derived by DXA</td>
</tr>
<tr>
<td>Volumetric bone mineral density</td>
<td>vBMD</td>
<td>3D derivation of mineral density (g/cm²)</td>
</tr>
<tr>
<td>Bone volume fraction</td>
<td>BV/TV</td>
<td>Relative percentage of bone</td>
</tr>
<tr>
<td>Connectivity density</td>
<td>Conn.D</td>
<td>Relative connectedness of one trabeculae to the next</td>
</tr>
<tr>
<td>Structural model index</td>
<td>SMI</td>
<td>Relative shape of trabeculae from rod-like to plate-like</td>
</tr>
<tr>
<td>Trabecular Number</td>
<td>Tb.N</td>
<td>Relative number of individual trabeculae</td>
</tr>
<tr>
<td>Trabecular Thickness</td>
<td>Tb.Th</td>
<td>Relative thickness of individual trabeculae</td>
</tr>
<tr>
<td>Trabecular Separation</td>
<td>Tb.Sp</td>
<td>Relative spacing between individual trabeculae</td>
</tr>
<tr>
<td>Mean intercept length</td>
<td>MIL</td>
<td>Quantification of 3D anisotropy of the bone (provides eigenvectors based on the fabric tensor that defines the principal direction of the bone</td>
</tr>
<tr>
<td>Degree of anisotropy</td>
<td>DA</td>
<td>Ratio of largest to smallest MIL value</td>
</tr>
</tbody>
</table>

*Table 1. Definitions of microstructural indices measured by Micro-CT*
11.10 Preparing & Embedding the Samples

The samples had to be embedded in a holding material so that they fit into the nanoindentation holder where they were to be indented. They also had to be dehydrated in alcohol and acetone mixtures to clean any bone marrow and blood still remaining in the samples. In the past different materials have been tried and tested. Although this process does definitely affect the results it is a necessary step to do the test.

The method used for embedding used a styrene based Kleer Set Polyester Casting Resin (MetPrep Ltd). This casting resin was chosen as it has a relatively fast settling time of 6 hours compared to PMMA resin, which takes about 8 days. A concern regarding the use of PMMA is that high temperatures may be required to properly embed the samples. These temperatures may denature and alter the structural proteins within the sample and thus lead to a result, which is different from in vivo. The Kleer Set Polyester Casting Resin is mildly exothermic, with samples rising 5-10 degrees on average for a short period of time. It was felt that this would not be sufficiently hot or prolonged so as to alter the proteins. Polymerisation of the resin uses a catalyst consisting of methyl ethyl ketone peroxide, di-isobutyl phthalate and 4-hydroxy-4-methylpentan-2-one. A wide variety of moulds are available to be used with this Kleer Set Resin, including oven safe glass, metal, latex and plastics including polypropylene and polyethylene. The moulds used in this study were plastic[406].

Before the hip samples were embedded in the resin they had to be cleaned and dehydrated with mixtures of alcohol and acetone to get bone marrow and blood out of the samples.
The procedure had 4 steps to it. The samples got placed in 4 sets of alcohol baths using an 80W ultrasonic cleaner for 20 minutes each. This dehydration process is necessary as it allows the Kleer set resin to stick to the bone. The samples were cleaned in this order:

- Bath 1 - 80% Alcohol and 20% Acetone
- Bath 2 - 50% Alcohol and 50% Acetone
- Bath 3 - 20% Alcohol and 80% Acetone
- Bath 4 - 100% Acetone

Ultrasound cleaning is very effective at removing debris the bone. It vibrates the molecules of the bone letting alcohol in and out of the bone. Small amounts of damage may occur to the collagen fibres in the bone but would not affect the indenting results.

A solution of Kleer Set resin and hardener is then made up with 6 drops of hardener being added for every 10ml of resin. The mixture was stirred carefully with a flat-based glass stirrer to avoid bubble formation. The next step was to place the hip sample into a labelled plastic polyethylene mould and then add the resin. Each sample was placed in an upright position. The embedded samples were put in a fumigation press for 6 hours. When set the samples have a transparent appearance. They were easily extracted from the moulds and labelled again.

Care should be taken when making the resin. Anything that came in contact with the resin needed to be cleaned in acetone immediately or it would stick to anything around it. When pouring resin from it's container it is a good idea to wipe the mouth
of the container with acetone as well as the cap with acetone as this prevents them sticking together causing difficulty when opening the next time. Once the samples were out of the moulds and labelled they were ready to be polished.

Figure 11.4 A finished embedded hip sample
11.11 Polishing Protocol

Specimen surface preparation is a critical step that ensures accurate and repeatable nanoindentation measurement. Hand polishing of samples can be difficult but the following protocol was developed for human femoral tissue embedded in a polyester Resin (Kleer-Set, available from Metprep Ltd.).

Samples should be polished in the Department of Anatomy Laboratory in Royal College of Surgeons in Ireland. *Permission should be sought for polishing from Mr John O Brien, Chief Technician in Department of Anatomy, RCSI.* There are 8 cycles in total with polishing of bone samples – each cycle should be at least 6 minutes in length. This time was achieved by comparing the outcomes of various lengths of time and polishing under microscope.

The direction of rotation was recorded. After 40s samples should be rotated 180 degrees.

<table>
<thead>
<tr>
<th>Grit Paper</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>6 minutes</td>
</tr>
<tr>
<td>800</td>
<td>6 minutes</td>
</tr>
<tr>
<td>1200</td>
<td>6 minutes</td>
</tr>
<tr>
<td>2500</td>
<td>6 minutes</td>
</tr>
<tr>
<td>4000</td>
<td>6 minutes</td>
</tr>
<tr>
<td>Nylap Cloth</td>
<td>10 minutes</td>
</tr>
<tr>
<td>(1 micron diamond Abrasive)</td>
<td></td>
</tr>
<tr>
<td>Alphacloth M</td>
<td>6 minutes</td>
</tr>
<tr>
<td>(Synthetic short Nap fibres bonded to a woven cotton backing)</td>
<td></td>
</tr>
</tbody>
</table>

*Table 11.1 Cycles of Polishing with Grit Paper size and Recommended Time for each cycle*
Polishing Procedure

1. Set up Polishing wheel
2. Ensure that correct Grit Paper Disc is on the spinning wheel, as per Table 1.
3. Samples are hand held with light pressure on the polishing wheel for the allotted time. After 40 seconds, the samples was rotated a full 180° - this was to prevent over grinding in one direction.
4. After each cycle, the sample is reviewed under a microscope to ensure that the polishing marks are in one direction.
5. If there are polishes marks on the sample at 90° the cycle should be repeated as per point number 3.
6. If the sample is satisfactory, the next cycle should be repeated as per table 1, with the sample being held at 90°
7. This process should be completed until all cycles have been completed.

Figure 11.6 Badly polished sample imaged under the microscope
11.12 Nanoindentation Protocol

11.12.1 Theory

Nanoindentation evolved from the traditional Vickers microhardness testing used in engineering. The test involves a hard tip whose mechanical properties are known (often a very hard material like diamond) being pushed into a sample whose properties are unknown. The load placed on the indenter tip is increased as the tip penetrates further into the specimen and soon reaches a user-defined value. In our test the load is held constant for a period and removed three times for accuracy. Elastic and plastic deformation occur which result in a hardness impression on the surface of the material, conforming to the indenter shape. As the tip is withdrawn, the elastic portion of the displacement is recovered, allowing the elastic properties of the material to be determined. The values of interest got from this machine are the Young's modulus of the bone and the hardness both measured in GPa. There are a few methods to extract these values from the load-displacement data.

The Oliver and Pharr (1992) method is the most widely used. In their experiment they indented six materials with a Berkovich indenter and looked at their load-displacement behaviour. It was shown that the load-displacement curves during unloading in these materials was not linear, even in the initial stages, thereby suggesting that the flat punch approximation used so often in the analysis of unloading data was not entirely adequate. They present an analysis technique that accounts for the curvature in the unloading data and provides a physically justifiable procedure for determining the depth which should be used.
in conjunction with the indenter shape function to establish the contact area at peak load [407].

The first step in the procedure is to determine the contact stiffness $S$, which is the bone's resistance to elastic deformation. It is measured from the load displacement data as the slope of the upper portion of the unloading curve [234]. Below is a graph showing the load-displacement curve. As the load increases so does the displacement. Release of the load will let the material return to its original state.

![Figure 11.7 Schematic of load-displacement curve for an instrumented nanoindentation test.](image)

A load is applied to a sample causing displacement of the material. As the load increases, the displacement increases. Release of the load will result in the material returning to the original state [408]
The stiffness $S$ is related to the reduced modulus, $E_r$, by:

$$S = \frac{\partial P}{\partial H} = \beta \frac{2}{\sqrt{\pi}} E_r \sqrt{A}$$

where $A = \text{projected area of contact (a function of depth, } h)$

$\beta = \text{empirical shape factor} = 1.034 \text{ for Berkovich tip.}$

There is an assumption when this equation was derived that the elastic properties of the material are homogeneous and isotropic which bone is now. $A$, the contact area can be estimated from the load-displacement data. The reduced modulus accounts for a non-rigid indenter and is determined by the following equation:

$$\frac{1}{E_r} = \frac{1-v_s^2}{E_s} + \frac{1-v_i^2}{E_i}$$

where $E_s = \text{Bone modulus}$

$E_i = \text{Indenter modulus}$

$v_s = \text{Poisson's ratio for bone}$

$v_i = \text{Indenter Poisson's ratio}$

The projected area of contact, $A$ is a nonlinear function of the contact depth,

$$A(h) = 24.5h^2 + C_1h + C_2h^2 + \frac{1}{C_3}h^4 + \ldots + \frac{1}{C_8}h^{128}$$

The hardness of the bone can be determined by:

$$H = \frac{P_{\text{max}}}{A}$$

Where:

$P_{\text{max}} = \text{the peak load applied}[407]$. 

267
11.12.2 Testing Protocol

The testing was carried out using a Nanoindenter XP (MTS Systems, Oakridge, TN) in the Bioengineering Testing Laboratory, Trinity College Dublin. The room temperature is kept at 20°C and is relatively vibration free. This is important as the machine is enclosed in an insulated box and suspended on a pneumatic anti-vibration table. Errors can occur in the testing if vibration levels rise or if there are variations in temperature that cause thermal expansion inside the box. The samples must then be placed in the nanoindenter stage ensuring they are all on a level flat plane to avoid any possibility of the tip colliding with the holder or the sample as this could cause the tip to break.

![Nano Indenter XP](image)

**Figure 11.8 Nano Indenter XP.**
The above picture demonstrates the inside of the enclosed compartment. The stage can be seen slotted in under the silver cylinder with a blue band (the microscope).

Various nanoindentation tips can be used depending on the type of test and materials are involved. The tip selected for this experiment was the Berkovich diamond indenter tip. It has three sides and is shaped like a pyramid. Its aspect
ratio is similar to that of the four-sided Vickers tip. It has an elastic modulus of 1147 GPa and a Poisson's ratio of 0.07. The benefits of using this tip is that because of the tip's small radius of curvature (~100 nm) it allows for fairly good resolution of the testing images. The shape of the tip is quite straightforward so it's shape constant k can easily be found. It also produces plasticity at very low loads and minimizes the influence of friction. The tip has been manufactured well and is very durable making it able to withstand a large amount of use.

Figure 11.9 The Berkovich diamond-indenting tip [409]

The first task when indenting is to calibrate the indenter so that it is indenting exactly where it has been told to indent. This is done by indenting the centre test piece of silica and then making sure the point under the microscope is the same as the indent. Fused silica is used for calibration in this type of testing as it has a low modulus-to-hardness ratio that leads to a large amount of elastic recovery during unloading that improves the measurement accuracy.
The next step was to establish a testing protocol on the machine for the test so that each test was the same. A multi-load programme was used which has been producing good results for indents in bone. The load chosen is 20mN, which worked best and gave the least amount of displacement. At the end of each test it is important for the indenter to be held at constant load for at least 50 seconds for accuracy of results. The allowable thermal drift rate in this programme was 0.2nm/s. Thermal drift correction adjusts the measured displacement of the indenter to account for small amounts of thermal expansion or contraction in the material and/or the equipment, arising from a lack of thermal equilibrium between the indenter apparatus and the test specimen. It is generally held to be constant throughout the course of the test. Therefore, if the rate of displacement owing to thermal effects can be measured at a single point during a test, then its effect over the whole test can be accounted for. Variations in thermal drift and vibrations could have been caused by the opening and closing of the door in the testing room [410]. Poisson's ratio of 0.3 was used in this study, which is consistent with reported values for human femoral cortical and trabecular bone [408].
Table 11.3 Parameters for the programme used in the nanoindentation experiment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage to unload</td>
<td>90%</td>
</tr>
<tr>
<td>Max Load</td>
<td>20mN</td>
</tr>
<tr>
<td>Load rate for multiple unload</td>
<td>1</td>
</tr>
<tr>
<td>No. Times to load</td>
<td>3</td>
</tr>
<tr>
<td>Peak hold time</td>
<td>90s</td>
</tr>
<tr>
<td>Time to load</td>
<td>90s</td>
</tr>
<tr>
<td>Poisson’s ratio</td>
<td>0.3</td>
</tr>
<tr>
<td>Drift Rate</td>
<td>0.2nm/s</td>
</tr>
<tr>
<td>Surface approach distance</td>
<td>800nm</td>
</tr>
<tr>
<td>Surface approach sensitivity</td>
<td>25%</td>
</tr>
<tr>
<td>Surface approach velocity</td>
<td>10nm/s</td>
</tr>
<tr>
<td>Approach distance to store</td>
<td>800nm</td>
</tr>
</tbody>
</table>

The sample holder moves in the x, y plane, while the indenter moves in the z-plane. The holder is then placed under the microscope so that the microstructure of the bone can be seen. The most clear areas of cortical and trabecular bone were then found and indents were done.
Figure 11.10 Clear image of osteons with surrounding lamellae in cortical bone.

The above image was taken by the nanoindenter’s microscope. This shows two osteons with surrounding lamellae in the cortical bone of this sample. The magnification of the microscope was X40.

One problem encountered was dehydration of the bone samples, which was unavoidable in this part of the experiment. The soaking in alcohol and exposure to air and heat during storage and testing would have affected the values obtained for elastic modulus and hardness.
11.13 MicroCT Images

OP1

OP2

OP3
11.14 Nanoindentation Images

Figure 11.11 Trabecular Bone

Figure 11.12 Trabecular Bone
Figure 11.13 Cortical bone

Figure 11.14 Cortical Bone
Figure 11.15 Interstitial Cortical Bone

Figure 11.16 Interstitial bone
# Vitamin D Assessment Form

## Patients Details

<table>
<thead>
<tr>
<th>Name:</th>
<th>Date of Birth:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Address:</td>
<td>Contact No:</td>
</tr>
<tr>
<td>Chart Number</td>
<td>Mobile:</td>
</tr>
</tbody>
</table>

## GP Details

<table>
<thead>
<tr>
<th>Name:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Address:</td>
</tr>
</tbody>
</table>

## Study Check list

- Consent form signed
- Bone markers taken
- GP Letter sent
- Consultant Letter sent
- Information Given to Patient
**Fracture History:**

Has there ever been a **fracture?** Yes ☐  No ☐

Details: __________________________

Have you ever **broken a hip?**  Yes ☐  No ☐

Right ☐  Left ☐

**Right Hip**

<table>
<thead>
<tr>
<th>Year: _______________</th>
<th>Region: Subcapital ☐</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: _______________</td>
<td>Transcervical ☐</td>
</tr>
<tr>
<td></td>
<td>Intertrochanteric ☐</td>
</tr>
<tr>
<td></td>
<td>Subtrochanteric ☐</td>
</tr>
</tbody>
</table>

Surgical Procedure: __________________________

Date: __________________________

Have you ever broken a wrist or lower arm? Yes ☐  No ☐  Right ☐  Left ☐

Year right wrist broken? ___________  Year left wrist broken? ___________

Comment: ___________________________________________________________

Have you ever broken any of the following bones?

- Upper arm ☐
- Pelvis ☐
- Skull ☐
- Lower leg ☐
- Ribs ☐
- Backbone ☐

Have you broken any other bones?  Yes ☐  No ☐

If yes, details: _____________________________________________________
**Current Drug Therapy:**

<table>
<thead>
<tr>
<th>Name and dosage</th>
<th>Length of time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Steroid Usage**

Steroid use:
Have you ever taken steroids? (Prednisolone)
- Never □
- By inhaler only □
- Tablets □
- Inhaler and tablets together □

Length of time on steroids?
__________________________ months

For how long, altogether, did you take steroids?
__________________________

What was the largest daily dose during that time?
__________________________

Have you ever taken more than 7.5 mg of steroid daily, for 3 months or more?
- Yes □
- No □

If you are still taking steroids what is your daily dose?
__________________________
### Risk factors for Osteoporosis

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early natural/surgical menopause &lt;45yrs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low trauma fracture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family Hx of Osteoporosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal Hx of hip fracture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertebral deformity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low body weight (57 kgs/9 st or BMI &lt;19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steroid Use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption (&gt;3IU/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kyphosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late Menarch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray evidence of Osteoporosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current Smoking</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Medical History

<table>
<thead>
<tr>
<th>List all medical diagnoses</th>
<th>Diagnosis Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Secondary causes for Osteoporosis.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addisons Disease</td>
<td>Hypogonadism</td>
</tr>
<tr>
<td>Anorexia Nervosa</td>
<td>Immobilisation</td>
</tr>
<tr>
<td>Cushings Syndrome</td>
<td>Lymphoma/leukaemia</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>Malabsorption</td>
</tr>
<tr>
<td>Gastrectomy</td>
<td>Multiple Myeloma</td>
</tr>
<tr>
<td>Haemochromatosis</td>
<td>Nutritional Disorders</td>
</tr>
<tr>
<td>Haemophilia</td>
<td>Osteogenesis Imperfecta</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>Pernicious Anaemia</td>
</tr>
<tr>
<td>Renal Failure</td>
<td>Anticonvulsants</td>
</tr>
<tr>
<td>Rh. Arthritis</td>
<td>Cytotoxic Drugs</td>
</tr>
<tr>
<td>Sarcoïdosis</td>
<td>Lithium</td>
</tr>
<tr>
<td>Severe Liver Disease</td>
<td>Heparin</td>
</tr>
<tr>
<td>Malabsorption</td>
<td>Proton pump inhibitors</td>
</tr>
</tbody>
</table>
**DIET**

Present daily milk intake: (e.g. in drinks, cereal, cooking)

<table>
<thead>
<tr>
<th>Amount</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;½ pint</td>
<td>□</td>
<td></td>
<td></td>
</tr>
<tr>
<td>½ to 1 pint</td>
<td>□</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1 pint</td>
<td>□</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cups of tea/ coffee per day?  

<table>
<thead>
<tr>
<th>Amount</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4</td>
<td>□</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-9</td>
<td>□</td>
<td>□</td>
<td></td>
</tr>
<tr>
<td>&gt;9</td>
<td>□</td>
<td>□</td>
<td></td>
</tr>
</tbody>
</table>

**Frequency of following foods**

<table>
<thead>
<tr>
<th>Food</th>
<th>Never</th>
<th>Once/week</th>
<th>Once or twice/week</th>
<th>Most Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Dark Green Veg</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Milk Pudding</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Oily Fish (Mackerel/Sardine)</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>
Chapter 12. References


182. Lloyd, B.D., et al., Recurrent and injurious falls in the year following hip fracture: a prospective study of incidence and risk factors from the


