Bone Microstructure and Material Composition in Endocrine Disorders

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By

Thuy Dan Thuy Vu
MBBS FRACP
ORCID ID: 0000-0002-5768-1367

Department of Medicine
Austin Health
The University of Melbourne
West Heidelberg, Victoria
Australia

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Summary

In the general population, fractures are a significant healthcare issue. However, fractures also complicate some endocrine conditions. My work focused on investigating the possible changes in bone structure and material composition underlying bone fragility in a number of endocrine diseases such as type 1 and type 2 diabetes mellitus as well as patients with PTH excess and PTH deficiency.

Chapter one outlines the definition of osteoporosis as well as the epidemiology, burden and costs of hip, vertebral and non-hip, non-vertebral fractures in the general population. Although a lot of attention has focused on hip and vertebral fractures, non-hip, non-vertebral fractures have higher prevalence, significant morbidity and greater healthcare expenditure.

Chapter two describes the underlying pathogenesis of bone fragility. Bone modelling and remodelling during growth, adulthood and ageing are reviewed. The variability of growth within the skeleton is described with differences in the rate of growth of axial and appendicular bone highlighted. The mechanisms contributing to age-related bone loss is outlined. In particular, sex differences relating to trabecular bone loss and new insights relating to cortical bone loss are described.

Most bone loss is cortical and most occurs after 65 years of age through intra-cortical remodelling which can fragment cortical bone to the extent that it resembles trabecular bone. Important implications arise when these cortical fragments are erroneously included in the medullary (trabecular) compartment which causes age related trabecular bone loss as well as the age related increase in cortical porosity to be both under-estimated. The structural basis of fragility fractures in childhood and adulthood are also reviewed.

Chapter three introduces secondary osteoporosis as increased fracture risk occurs in some endocrine disorders with particular focus on type 1 and type 2 diabetes mellitus, primary hyperparathyroidism and hypoparathyroidism. The epidemiology of fractures, abnormalities in bone remodelling, structural basis of bone fragility, material basis of bone fragility are described in each of the endocrine conditions.
Chapter four outlines the methods employed to investigate changes in bone structure and material composition underlying bone fragility in cases (patients with type 1 and type 2 diabetes mellitus as well as patients with PTH excess and PTH deficiency) and controls. The limitations in densitometry are covered and advances in measuring bone microstructure in vivo using high resolution peripheral quantitative computed tomography (HR-pQCT) is described. HR-pQCT was used to acquire images of the distal radius and distal tibia in cases and controls. Bone structure was assessed by the standard analysis protocol from the manufacturers, which uses a threshold based algorithm in the type 1 and type 2 diabetes studies. A non-threshold based algorithm (StrAx) was used to measure bone structure and matrix mineral density in the PTH excess and PTH deficiency study.

Chapter five present the results of the thesis. Chapter 5.1 investigated patients with type 1 diabetes and examined whether the condition is associated with reduced bone formation and so lower total cross-sectional area and cortical area, higher medullary area, cortical porosity and tissue mineralization density and lower trabecular thickness in patients relative to controls in light of low bone formation observed in T1DM.

Chapter 5.2 presents data concerning patients with type 2 diabetes and explored whether the illness is associated with higher cortical area and cortical density due to lower cortical porosity as well as lower medullary area associated with better preserved trabecular morphology in patients relative to controls as bone turnover is thought to be low in T2DM.

Chapter 5.3 examined patients with untreated and treated primary hyperparathyroidism and investigated whether PTH excess is associated with (1) lower cortical area and density due to higher cortical porosity and lower tissue mineralization density; (2) higher transitional zone area, (3) lower, and not higher, true trabecular number, thickness and density and (4) whether surgical treatment only partly reverses the cortical and trabecular deficits.

Chapter 5.4 evaluated patients with hypoparathyroidism and examined whether PTH deficiency is associated with higher cortical area and density, lower cortical porosity
and increased tissue mineralization density as well as preserved trabecular morphology relative to controls.

Chapter six present the overall findings for patients with type 1 and type 2 diabetes as well as patients with PTH excess and deficiency. The evolution of detecting bone fragility was discussed and the recent advances in image acquisition and analysis of bone microstructure described along with their limitations.
Abstract

In the general population, fractures are a significant healthcare issue. However, fractures also complicate several endocrine disorders. The overall aim of my study was to investigate the changes in bone structure and material composition underlying bone fragility in a number of endocrine diseases such as type 1 and type 2 diabetes mellitus as well as patients with PTH excess and PTH deficiency.

High-resolution peripheral quantitative computed tomography was used to acquire images of the distal radius and tibia in cases and controls. Bone structure was assessed by the standard analysis protocol from the manufacturers that uses a threshold base algorithm in the type 1 and type 2 diabetes studies. A non-threshold base algorithm was used to analyze bone structure and matrix mineral density in the PTH excess and PTH deficiency study.

The main findings were that patients with type 1 diabetes had no significant deficits in cortical and trabecular bone relative to controls. Patients with T1DM had a tendency towards lower bone formation and bone resorption markers. Patients with type 2 diabetes had similar cortical morphology whereas trabecular bone was better maintained than controls. These results are consistent with higher BMD in the spine and hip as well as lower bone remodelling markers observed in diabetic patients.

PTH excess is deleterious to both cortical and trabecular bone. In patients with untreated primary hyperparathyroidism (PHPT), the normal or high trabecular density reported in several studies is likely to be the result of inclusion of cortical remnants in the medullary compartment. In patients with untreated PHPT, there was a left shift of the void-matrix mineralization density distribution curve relative to controls due to increased cortical porosity. Cortical and trabecular deficits in untreated PHPT may not be completely reversible with surgical treatment. Patients with PTH deficiency due to secondary rather than primary hypoparathyroidism have better maintained cortical and trabecular bone relative to controls as well as higher matrix mineral density. Similar results were found in women and in men. There was a rightward shift of the void bone matrix mineralization distribution curve for patients with hypoparathyroidism relative to controls.
In conclusion, patients with type 1 and type 2 diabetes may have underlying bone fragility predisposing to fractures, but my study failed to identify microstructural abnormalities. Further work is needed to determine whether abnormalities in the material properties of bone may account for the increased fracture risk seen in diabetes. Patients with PTH excess have deficits to both cortical and trabecular bone. The normal or high trabecular density reported in several studies is likely to be the result of inclusion of cortical remnants in the medullary compartment. Patients with PTH deficiency due to secondary hypoparathyroidism rather than primary hypoparathyroidism have better maintained cortical and trabecular bone morphology as well as higher matrix mineralization density relative to controls. Prospective studies are needed to see how these skeletal abnormalities affect bone strength and fracture risk.
Declaration

This is to certify that

i. The thesis comprises only my original work towards the PhD except where indicated in the Preface,

ii. Due acknowledgement has been made in the text to all other material used,

iii. The thesis is less than 100,000 words in length, exclusive of tables, figures, bibliographies and appendices.

Thuy Dan Thuy Vu

The Department of Endocrinology and Medicine
Austin Health
The University of Melbourne
West Heidelberg
Victoria 3081
Australia
Preface

The study presented in chapter 5.3 was designed by Professor Ego Seeman. I obtained ethics approval from the Austin Health Human Research Ethics Committee and was responsible for setting up the study, recruitment, patient assessment, running the study and all the data entry. I collaborated with researchers in the Endocrinology Department, Austin Health as well as Professor John Bilezikian’s group from Columbia University Medical Centre.

From Austin Health, Dr Xiao Fang Wang and Dr Qingju Wang provided statistical assistance and advice, Ali Ghasem-Zadeh was responsible for all the scanning of patients and controls from Austin Health, Dr Roger Zebaze provided assistance with StrAx analysis, Professor Ego Seeman assisted with study design, recruitment of suitable patients, analysis of data and writing up of the manuscript and Professor George Jerums provided assistance with editing the manuscript. From Columbia University Medical Centre, Professor John Bilezikian was responsible for providing appropriate patients from his institution and editing of the manuscript. Stephanie Boutroy, Natalie E. Cusano, Dinaz Irani, Barbara C. Silva, Julia Udesky and Megan E. Romano assisted with providing patient files for analysis and patient demographic information.

The thesis contains published material presented in chapter 5.3. I wrote the publication with editorial assistance rendered by Professor Ego Seeman. It was published in Bone in March 2013.

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I would like to thank Professor Jeffrey Zajac and Professor George Jerums for use of the facilities in the Endocrinology Department. I am grateful to staff at the University of Melbourne IT department (Gina, Mary and Joey) who provided assistance with all matters relating to IT.

I am grateful to all the staff at the Bone Mineral and Research Unit and in particular Geoff Roff, Helen Patterson and Ali Ghasem-Zadeh who willingly scanned all the patients and controls recruited for the study. Ali gave up his time to scan some patients after hours as well as prepare and upload patient files for analysis by StrAx.

I would also like to thank Dr Xiao Fang Wang who provided assistance with statistics, advise on writing up the thesis and obtaining relevant patient information.

I am very appreciative to staff at Endocrinology Department and University of Melbourne for administrative assistance, namely Lynda Smyth and Jo Mayall. Kylie King is a research nurse in the department and willingly contributed her time to help with recruiting patients and data entry.

Finally, without the unfaltering support of my husband, Sean, my parents, Phung and Phuong, my sisters, Tran and Trang and other members of my family I would not be where I am today.

My children, Loc and Lan were born during this tumultuous period and have brought so much joy, wonder and enrichment into my life.
Publications arising from this thesis


Presentations relating to this thesis

Oral Presentations


Poster Presentation

Abbreviations

aBMD  Areal bone mineral density
AGE(s)  Advanced glycation end product(s)
ALP  Alkaline phosphatase
BMD  Bone mineral density
BMU  Bone multicellular unit
BV/TV  Bone volume/total volume
CSA  Cross-sectional area
CTx  C-terminal telopeptide of type I collagen
CV  Coefficient of variation
DPP4  Dipeptidyl peptidase-4
DXA  Dual energy x-ray absorptiometry
GIP  Gastric inhibitory polypeptide
GLP-1  Glucagon-like peptide
HR-pQCT  High resolution peripheral quantitative computed tomography
HypoP  Hypoparathyroidism
NS  Not significant
OPG  Osteoprotegerin
PINP  Procollagen type I N-terminal propeptide
PHPT  Primary hyperparathyroidism
PPARγ  Peroxisome proliferator activated receptor γ
PTH  Parathyroid hormone
RANKL  Receptor activator of nuclear factor kappa β ligand
RUNX2  Runt-related transcription factor 2
SD  Standard deviation
SEM  Standard error of the mean
T1DM  Type 1 diabetes mellitus
T2DM  Type 2 diabetes mellitus
µCT  Micro-CT
µm  Micrometres
Table of contents

Summary .......................................................................................................................... ii
Abstract ......................................................................................................................... v
Declaration ..................................................................................................................... vii
Preface .......................................................................................................................... viii
Acknowledgements ....................................................................................................... ix
Publications arising from this thesis .............................................................................. x
Abbreviations ................................................................................................................ xi
Table of contents .......................................................................................................... xii
List of Figures ............................................................................................................... xv
List of Tables ................................................................................................................. xvii

Literature Review

Chapter 1: Definition of Osteoporosis and Epidemiology of Fractures ............... 2
  1.1 Definition of osteoporosis ....................................................................................... 2
  1.3 Epidemiology of fragility fractures ......................................................................... 4
      1.3.1 Hip fractures ................................................................................................. 4
      1.3.2 Vertebral fractures ....................................................................................... 5
      1.3.3 Non-hip, non-vertebral fractures ................................................................. 6
  1.4 Morbidity and mortality .......................................................................................... 9
      1.4.1 Hip fractures ................................................................................................. 10
      1.4.2 Vertebral fractures ....................................................................................... 12
      1.4.3 Non-hip, non-vertebral fractures ................................................................. 13
  1.5 Cost ....................................................................................................................... 13
      1.5.1 Hip fractures ................................................................................................. 14
      1.5.2 Vertebral fractures ....................................................................................... 16
      1.5.3 Non-hip, non-vertebral fractures ................................................................. 16

Chapter 2: Pathogenesis of Bone Fragility .............................................................. 20
  2.1 Introduction .......................................................................................................... 20
  2.2 Bone modelling and remodelling during growth .................................................. 21
      2.2.1 Bone Modelling .......................................................................................... 21
      2.2.1 Bone Remodelling ..................................................................................... 23
      2.2.3 Growth in bone size, mass and microstructure .......................................... 25
      2.2.4 Axial growth ............................................................................................... 26
      2.2.5 Appendicular growth ................................................................................. 27
  2.3 Bone modelling and remodelling during advancing age and menopause .......... 28
      2.3.1 Abnormalities in bone material composition with ageing ......................... 33
      2.3.2 Abnormalities in bone microstructure with ageing .................................... 33
### Chapter 2: Structural Basis of Fragility Fractures Across Life

2.3.3 Sex steroid deficiency ................................................................. 35
2.3.4 Secondary hyperparathyroidism .................................................. 36
2.4 Structural basis of fragility fractures across life .................................... 37
  2.4.1 Fractures in childhood .............................................................. 38
  2.4.2 Fractures in adulthood .............................................................. 42

### Chapter 3: Secondary Osteoporosis ..................................................... 52
3.1 Definition of secondary osteoporosis ............................................... 52
  3.1.1 Epidemiology of fractures associated with known causes of bone fragility ....... 53
3.2 Diabetes mellitus and bone fragility ............................................... 53
  3.2.1 Epidemiology of fractures .......................................................... 53
  3.2.2 Material and structural basis of bone fragility .................................. 60
  3.2.3 Abnormalities in bone remodelling ............................................. 69
  3.2.4 Local and systemic factors influencing bone fragility ......................... 72
  3.2.5 Effect of treatment on bone fragility ............................................ 77
3.3 Primary hyperparathyroidism and bone fragility .................................. 82
  3.3.1 Epidemiology of fractures in primary hyperparathyroidism .................... 82
  3.3.2 Increased bone turnover in primary hyperparathyroidism ...................... 85
  3.3.3 Structural basis of bone fragility in primary hyperparathyroidism ............. 86
  3.3.4 Material basis of bone fragility in primary hyperparathyroidism .............. 88
  3.3.5 Effect of treatment in primary hyperparathyroidism .......................... 90
3.4 Skeletal abnormalities in hypoparathyroidism .................................... 95
  3.4.1 Epidemiology of fractures in hypoparathyroidism .............................. 95
  3.4.2 Impaired bone turnover in hypoparathyroidism ............................... 97
  3.4.3 Structural basis of skeletal abnormalities in hypoparathyroidism ............. 99
  3.4.4 Material basis of skeletal abnormalities in hypoparathyroidism .............. 103

### Methodology

Chapter 4: Methodology ................................................................. 108
  4.1 Dual energy x-ray absorptiometry ............................................... 108
  4.2 Image acquisition using high resolution peripheral quantitative computed tomography ................................................................. 110
  4.3 Image analysis using StrAx ................................................................ 113
  4.4 Study design .................................................................................. 115

### Results

Chapter 5: Bone Microstructure and Material Composition in Endocrine Disorders ..................................................... 118
  5.1 Type 1 diabetes mellitus ............................................................... 118
  5.2 Type 2 diabetes mellitus ............................................................... 130
  5.3 Primary hyperparathyroidism ......................................................... 142
  5.4 Hypoparathyroidism ...................................................................... 164
Discussion and Conclusions

Chapter 6: Discussion and Conclusions .............................................................. 182
Bibliography......................................................................................................... 186
Appendix One ..................................................................................................... 231
List of Figures

Figure 1.1 Incidence of hip fracture in the Dubbo Osteoporosis Epidemiology Study. 4
Figure 1.2 Incidence of vertebral fractures between the period 1989-1991 and 2009-2011 in Rochester, USA ................................................................. 6
Figure 1.3 Incidence of distal radial fracture between the period 1989-1991 to 2009-2011 in Rochester, USA ................................................................. 7
Figure 1.4 Incidence of humeral fracture in Rochester, USA ........................................ 8
Figure 1.5 Incidence of pelvic fractures in Rochester, USA ........................................... 9
Figure 1.6 Burden of disease in America and Europe .................................................. 10
Figure 1.7 Survival curves for different fractures ....................................................... 11
Figure 1.8 Length of stay in hospital, rehabilitation and residential care according to fracture types ................................................................. 17
Figure 2.1 Modelling at the distal radial metaphysis during growth ..................... 22
Figure 2.2 Phases of bone remodelling .................................................................... 25
Figure 2.3 (A) Total height, (B) sitting height and (C) leg length growth velocity in boys and girls .......................................................................................... 26
Figure 2.4 Reversible and irreversible bone deficits caused by bone remodelling ......... 30
Figure 2.5 Biomechanical effects of periosteal apposition ........................................ 32
Figure 2.6 Annual rates of change in endocortical resorption, periosteal apposition, net cortical width and section modulus ................................................. 32
Figure 2.7 Age related bone loss ............................................................................. 34
Figure 2.8 Implications of cortical remnants being erroneously assigned to trabecular bone .............................................................................................. 35
Figure 2.9 Incidence of distal radial fracture across the lifespan ............................... 38
Figure 2.10 Mother daughter correlations in bone traits ......................................... 40
Figure 2.11 Changes in cortical traits during puberty ............................................... 42
Figure 2.12 Age- and sex-related changes in trabecular morphology ....................... 44
Figure 2.13 Incidence of hip fracture ...................................................................... 45
Figure 2.14 Regional cortical deficits associated with hip fractures ......................... 46
Figure 2.15 Age related changes in mean trabecular plate density in fracture cases and controls ...................................................................................... 48
Figure 3.2.1 Incidence of hip fracture in patients with and without type 1 diabetes .. 55
Figure 3.2.2 Incidence of hip fracture in patients with and without type 2 diabetes .. 57
Figure 3.2.3 Reference point indentation .................................................................. 61
Figure 3.2.4 Trabecular morphology in patients with and without type 2 diabetes ... 63
Figure 3.2.5 Cortical morphology in patients with and without type 2 diabetes ...... 64
Figure 3.2.6 Formation of advanced glycation end products ................................... 65
Figure 3.2.7 Serum osteocalcin levels patients with in type 1 diabetes ................. 71
Figure 3.2.8 Correlation between serum sclerostin and β-catenin in patients with (A) and without type 2 diabetes (B) ...................................................... 74
Figure 3.2.9 Impaired bone formation in diabetes model ........................................... 75
Figure 3.2.12 Effect of metformin on bone marrow stem cell differentiation ............ 78
Figure 3.2.13 Renal handling of glucose ................................................................... 80
List of Tables

Table 1.1 World Health Organization classification of bone fragility using areal bone mineral density measurements.................................................................2
Table 1.2 Healthcare cost according to fracture site and services utilized in Australia. ..................................................................................................................15
Table 1.3 Healthcare cost according to fracture site and services utilized in the U.S.A. ........................................................................................................15
Table 3.1.1 Diseases and drugs causing secondary osteoporosis ..................52
Table 3.2.1 Incidence and relative risk of hip fracture stratified by diabetes type and duration of diabetes ........................................................................58
Table 5.1.1 Baseline characteristics of patients with type 1 diabetes mellitus...122
Table 5.1.2 Bone microstructure in patients with type 1 diabetes and controls ......124
Table 5.2.1 Baseline patient characteristics.........................................................134
Table 5.2.2 Bone microstructure in patients with type 2 diabetes and controls ......136
Table 5.4.1 Baseline patient characteristics..........................................................169
Table 5.4.2 Bone morphology in hypoparathyroidism at the distal radius........173
Table 5.4.3 Bone morphology in hypoparathyroidism at the distal tibia ..........174
Table 5.4.4 Bone morphology in primary vs. secondary hypoparathyroidism .....175
Review of the Literature

Epidemiology of Fractures

Chapter One
Chapter 1: Definition of Osteoporosis and Epidemiology of Fractures

1.1 Definition of osteoporosis

Osteoporosis is defined as a ‘systemic skeletal disease characterized by low bone density, micro-architectural deterioration of bone tissue leading to enhanced bone fragility, and a consequent increase in fracture risk’ (European Foundation for Osteoporosis, 1991). Although, bone mineral density (BMD) is frequently employed as a measure of bone strength, other skeletal factors are equally important such as bone size, microstructure and material composition. Non-skeletal risk factors such as falls may also contribute towards fracture risk.

1.2 Diagnosis of osteoporosis

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>T SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>A value for BMD that is greater than 1 standard deviation below the young adult female reference mean (T score &gt; -1 SD)</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>A value for BMD that is more than 1 standard deviation below the young female adult mean, but less than 2.5 standard deviation below this value (T score &lt; -1 and &gt; -2.5 SD)</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>A value for BMD that is 2.5 standard deviations or more below the young adult mean (T score &lt; -2.5 SD)</td>
</tr>
<tr>
<td>Severe osteoporosis</td>
<td>A value for BMD that is 2.5 standard deviations or more below the young female adult mean in the presence of one or more fragility fractures</td>
</tr>
</tbody>
</table>

Table 1.1 World Health Organization classification of bone fragility using areal bone mineral density measurements

(World Health Organization, 2007).

The WHO defines osteoporosis in post-menopausal women and older men (>50 years of age) as areal bone mineral density that is 2.5 standard deviations below the mean for young Caucasians at the femoral neck (Table 1.1). The reference range used is calculated from the database for femoral neck measurements in young Caucasian
females (20 – 29 years) in the third National Health and Nutritional Examination Survey (NHANES III). The database is thought to be fairly representative of the North American population.

There are different methods available to assess bone mineral density but dual energy x-ray absorptiometry (DXA) has been widely used clinically and provides areal bone mineral density (aBMD) measurements. It is unclear as to whether the WHO diagnostic criteria can be appropriately used in other populations such as men and non-Caucasian females. However, the studies that have investigated the risk of hip fractures and BMD as well as the risk of vertebral fractures and BMD were no different in both sexes. There is marked variability in fracture risk between different regions around the world. This is greater than ten fold for hip fractures, in comparison to BMD, which varies by about one standard deviation between different sites globally. Therefore, it is recommended to use the NHANES III database to derive hip T-score in non-Caucasian populations until more studies are conducted (International Society for Clinical Densitometry, 2013).

Osteoporosis in younger individuals (aged 20 to 50 years and who have achieved peak bone strength) have been defined as T score ≤ -2.5 at the spine or hip by the International Osteoporosis Foundation. This definition is used since in young individuals the T- and Z-scores are very similar. Furthermore, this definition is consistent with the WHO definition of osteoporosis. However, in individuals who have not yet achieved peak bone strength then a low bone mass is defined by Z-score < -2.0 (Ferrari et al., 2012).

In contrast, other organisations have recommended using Z-score in pre-menopausal females and males ≤50 years of age (International Society for Clinical Densitometry, 2013). A Z-score of ≤ -2.0 is defined as 'below the expected range for age’ while a Z-score of ≥2.0 is ‘within the expected range for age’. Unlike post-menopausal osteoporosis, the relationship between aBMD and fracture risk is not well substantiated for young individuals. Furthermore, fracture prediction tools such as FRAX are not validated.
1.3 Epidemiology of fragility fractures

Fragility fractures result from low energy trauma (World Health Organization, 2007). Trauma that normally would not cause fracture in healthy individuals or a fall from standing height or less is referred to as ‘low energy’. The lifetime risk for a hip, spine or forearm fracture is thought to be ~30–40% in developed countries (World Health Organization, 2007). There were ~9 million fragility fractures globally in 2009 of which 1.6 million were hip fractures, 1.4 million were vertebral fractures and 6.0 million were non-hip, non-vertebral fractures (Cooper et al., 2011). Hence the majority of fragility fractures are non-hip, non-vertebral.

1.3.1 Hip fractures

The risk of falling increases with age. As such, most hip fractures are due to a fall. It is estimated that one third of elderly individuals fall every year with 5% sustaining a fracture and 1% sustaining a hip fracture (World Health Organization, 2007). For Caucasian females >50 years of age, the lifetime risk of hip fracture is ~17%, whereas for Caucasian males it is ~6% in USA (Cummings et al., 2002). Due to the ageing population, hip fractures are expected to rise from 1.66 million in 1990 to 6.26 million by 2050 worldwide (Cooper et al., 1992).

![Incidence of hip fracture in the Dubbo Osteoporosis Epidemiology Study](image)

Figure 1.1 Incidence of hip fracture in the Dubbo Osteoporosis Epidemiology Study (Chang et al., 2004).
Hip fracture incidence varies significantly between different populations. Northern Europe and North America have one of the highest incidences of hip fracture. Whereas Southern Europe have lower incidence by seven fold (Sambrook et al., 2006). Asia and Latin America have lower hip fracture rates (Cummings et al., 2002). While in Australia, hip fractures rates are comparable to North America and Europe. The Dubbo Osteoporosis Epidemiology Study recruited individuals in the community (>60 years of age) and reported that the rate of hip fracture increased with age with a greater female preponderance. The overall female and male incidence is 759 and 329 per 100,000 person-years respectively (Figure 1.1) (Chang et al., 2004).

1.3.2 Vertebral fractures

The actual incidence of vertebral fractures is difficult to determine accurately as only one third of all radiological vertebral fractures are symptomatic and thus able to be detected. Furthermore, only a small minority of patients with vertebral fractures require admission to hospital (Sambrook et al., 2006; World Health Organization, 2007). Unlike hip fractures, falls cause approximately one third of clinical vertebral fractures, while the majority are due to everyday activities such as lifting or bending (Sambrook et al., 2006). Caucasian females have 16% whereas Caucasian males have 5% lifetime risk of clinical vertebral fracture (Cummings et al., 2002).

The prevalence of vertebral fractures can vary significantly depending on the criteria used to identify the fracture (Cummings et al., 2002). Currently there is no consensus on the best diagnostic criteria. The Genant semi-quantitative method appears to achieve higher prevalence rates in comparison to algorithm-based qualitative method or fully quantitative morphometric methods (Schousboe, 2016).
There is an increase in the incidence of clinical vertebral fractures with increasing age. In women, from 50 years onwards the increase is gradual and accelerates after 70 years of age. Whereas in men from 50 years there is a slight increase with the incidence accelerating after 75 years of age. Overall, the incidence of radiological vertebral fracture in women and men is 1,092 and 798 per 100,000 person-years respectively in Rochester, USA (Amin et al., 2014). The secular trends in the incidence of vertebral fractures appear to be highly variable. In Iceland, the incidence decreased from 1989 to 2008 whereas in Rochester, Minnesota, USA the incidence increased between 1989-1991 to 2009-2010 (Figure 1.2) (Amin et al., 2014).

1.3.3 Non-hip, non-vertebral fractures
Falling onto out-stretched hand is the most frequent cause of a wrist fracture. In females, the incidence of wrist fracture rises between 45 – 60 years of age and plateaus thereafter (Cooper et al., 2011). Whereas in males, the incidence of wrist fractures appear stable between 20 to 80 years of age. Most wrist fractures occur in females with the overall incidence in the Rochester cohort reaching 328 per 100,000 person-years (Amin et al., 2014). The secular trend in the incidence of distal forearm
fractures between 1989-1991 to 2009-2011 in Rochester decreased in women by 26% and in men increased without achieving significance (Figure 1.3) (Amin et al., 2014). Whereas, in Australia the rates were stable between 1992 to 1996 (Cooper et al., 2011).

![Graph of incidence of distal radial fracture between 1989-1991 to 2009-2011 in Rochester, USA](image)

Figure 1.3 Incidence of distal radial fracture between the period 1989-1991 to 2009-2011 in Rochester, USA (Amin et al., 2014).

In the Rochester cohort, children and young adults had the highest incidence of distal humeral fractures, which stabilized thereafter in both sexes. In contrast, the incidence of humeral shaft fractures is low and stable for both males and females. The incidence of proximal humeral fractures is highest during puberty then diminished thereafter and remained stable in both sexes until 50 years of age when the incidence rises again in both sexes with the rate attaining 439.4 per 100,000 person-years for the elderly cohort (>80 year age). The female to male ratio is 4:1 for those >80 years (Figure 1.4) (Rose et al., 1982).
In females, the incidence of pelvic fracture is almost double that of males with the overall incidence reaching 37.0 per 100,000 person-years in the Rochester cohort (Melton et al., 1981). During puberty and young adulthood there is a slight increase in the incidence, which is higher in males compared to females. In males, the rate of fractures diminished in the 25–34 year old cohort and remained stable until the 55–64 year old cohort, thereafter increasing sharply to 220.3 per 100,000 person-years in the elderly (>85 years of age). In females, the incidence increases from 35 years of age to reach rates of 446.3 per 100,000 person-years in the elderly (>85 years of age) (Figure 1.5).
1.4 Morbidity and mortality

Fragility fractures are associated with significant morbidity due to chronic pain, functional decline, loss of independence and early admission into residential care. On the global stage, fragility fractures comprise 83% of the burden of non-communicable disease. While in America and Europe, fragility fractures account for 2.8 million disability adjusted life years annually, which is more than common cancers such as breast and prostate but less than chronic illnesses such as diabetes mellitus and chronic obstructive pulmonary disease (Figure 1.6) (World Health Organization, 2007).
Figure 1.6 Burden of disease in America and Europe

Disability-adjusted life years (DALYs) (World Health Organization, 2007).

1.4.1 Hip fractures

Hip fractures are associated with significant functional impairment. A prospective study investigating disability outcomes following fractures report that for hip fractures, 94% of patients required a mean of 21 days of bed rest and 100% of patients experienced a mean of 101 days of restrictions on everyday tasks (Fink et al., 2003). A study in the northern suburbs of Sydney reported that 12 months following a hip fracture in patients living in the community, 76% were not able to walk to pre-morbid levels, 45% needed additional home help, 22% required new nursing home admissions and 18% died (March et al., 1996). In patients that survived a hip fracture only 1 in 3 were able to return to their pre-morbid level of function (International Osteoporosis Foundation, 2007).
Figure 1.7 Survival curves for different fractures

Major fractures comprised pelvis, distal femur, proximal tibia, \( \geq 3 \) rib fractures and proximal humerus. Minor fractures comprised the remaining fractures (Bliuc et al., 2009).

Increased mortality following a hip fracture is well known. The Dubbo Epidemiological Study reported in their 18 year follow up that the age-adjusted mortality rate and standardized mortality ratio for hip fracture in women is 15.42 (95% CI, 12.88-18.52) per 100 person-years and 2.43 (95% CI, 2.02-2.93) and in men is 25.67 (95% CI, 19.46-33.87) per 100 person-years and 3.51 (95% CI, 2.65-4.66) respectively. Compared to the general population, the higher mortality in the Dubbo cohort persisted for up to 10 years (Figure 1.7) (Bliuc et al., 2009).
However, the duration of excess mortality has been variably reported. Some studies suggest increased mortality in the first 6–12 months post-hip fracture, while other studies report 5 and 10 years excess mortality (Cooper et al., 1993; Jacobsen et al., 1992; Magaziner et al., 1997; Parker et al., 1991; Schroder et al., 1993). The cause of death has been attributable to underlying co-morbidities in some studies while in other studies it has been attributable to complications associated with the fracture event (Cummings et al., 2002; Vestergaard et al., 2007).

### 1.4.2 Vertebral fractures

Vertebral fractures may be associated with significant pain and functional impairment. Though improvements in pain largely occur by three months, in some patients significant pain may persist for 6 to 12 months following the vertebral fracture (Suzuki et al., 2009). Other studies have indicated that the pain may be more prolonged, lasting 2 years after the acute fracture in some patients (Klazen et al., 2010). Furthermore, chronic pain and pain related functional impairment increases with the number of vertebral fractures (Nevitt et al., 1998; Silverman et al., 2001). In the study by Fink et al, lumbar vertebral fractures appear to cause a longer period of disability compared to those with hip fractures. As such, 53% of patients required a mean of 25 days bed rest and 97% of patients experienced a mean 158 days of restricted activities. Whereas thoracic vertebral fractures caused a mean of 12 days of bed rest in 47% of patients and 73 days of restricted activities in 74% of patients (Fink et al., 2003). Progression of age related kyphosis has been associated with vertebral fractures, which may worsen pulmonary function and limit routine daily activities (Kado et al., 2013; Schlaich et al., 1998).

Vertebral fractures are also well known to cause increased mortality. The cohort in Dubbo had increased age-adjusted mortality rate and standardized mortality ratio for vertebral fractures in women with 8.97 (95% CI, 7.57-10.63) per 100 person-years and 1.82 (95% CI, 1.52-2.17) respectively and in men with 15.16 (95% CI, 11.89-19.33) per 100 person-years and 2.12 (95% CI, 1.66-2.72) respectively. Unlike hip fractures, increased mortality for vertebral fractures lasted 5 years in this cohort (Figure 1.7) (Bliuc et al., 2009). Other studies using populations in community have similarly reported increased mortality following clinical vertebral fractures with
relative risk estimated at 8.64 (95% CI, 4.45-16.74) (Cauley et al., 2000). The increased mortality immediately post-fracture may be associated with fracture event, while the increased mortality at 12 months may be related to underlying co-morbidities (Schousboe, 2016).

1.4.3 Non-hip, non-vertebral fractures

Distal radial fractures may cause some difficulty with returning to work, functional impairment and persistent disability. In younger adults, 1 in 40 were unable to return to their prior occupation as a result of the fracture (Nellans et al., 2012). While in older adults, >50% experienced major functional deterioration. These patients describe difficulties associated with meal preparation, heavy housework, walking up 10 steps, shopping and transferring in and out of the car (Edwards et al., 2010).

Pelvic fractures can greatly impact level of independence as well as mobility. Following hospitalization for pelvic fracture, all patients were reliant on a walking aide and more than half needed assistance with walking upon discharge. Although most patients (~70%) were able to return home, the majority that did (84%) needed extra services. Requirements for residential care also increased from 20% on admission to 35% on discharge (Morris et al., 2000).

Non-hip, non-vertebral fractures, which accounts for a large proportion of fractures, also resulted in increased mortality. In the Dubbo cohort, the age adjusted standardized mortality ratio for women is 1.50 (95% CI, 1.30-1.73) and for men is 1.48 (95% CI, 1.18-1.85). The increased mortality lasted for 5 years in women in the younger age group (60-74 years) and in both sexes in the older age group (>75 years) (Figure 1.7) (Bliuc et al., 2009).

1.5 Cost

Due to the ageing population and the accompanying greater burden of fragility fractures, there is greater use of medical resources and expenditure. In USA, an estimated ten million have osteoporosis with two million fragility fractures every year, which is estimated to cost $19 billion annually and is predicted to rise to $25.3 billion by 2025 (Benjamin, 2010).
In Australia, ~3% of the population (726,000) have osteoporosis, which was self reported as part of the National Health Survey conducted by the Australian Bureau of Statistics in 2011-2012 period. The study population included participants greater than 15 years of age or under 15 years of age if they reported having gout, rheumatism or arthritis (Australian Bureau of Statistics, 2013). The National Health Survey may underestimate the true prevalence of osteoporosis.

Other studies have reported that osteoporosis (defined by WHO bone mineral density classification) affects over one million people over the age of 50 years. This prevalence was based on the Geelong Osteoporosis Study, which assessed areal bone mineral density in a random sample of volunteers residing in Geelong. This data was subsequently extrapolated to the Australian population (Watts et al., 2012). More recently, the authors have published state and territory specific data on the prevalence with NSW/ACT having the highest number of people affected ~365,000; then Victoria with ~263,000; Queensland ~195,000; Western Australia ~99,000; South Australia ~87,000; Tasmania ~27,000 and Northern Territory ~5,000. The authors also calculated the costs associated with osteoporosis including fragility fractures.

In 2012, the total cost of osteoporosis and fragility fractures was estimated to be $2.75 billion in patients >50 years of age which included ambulance services, emergency department admissions, hospital admissions, rehabilitation, outpatient services, aged care and community services. In 2022, the projected total cost is $3.84 billion (Watts et al., 2012). Inpatient hospital admissions constitute almost half of the total direct costs relating to fracture treatment. The inpatient hospital admission costs associated with hip, vertebral, wrist and other fractures were $502, $93, $75 and $466 million respectively in 2012, (Table 1.2).

1.5.1 Hip fractures
In Australia, the healthcare cost relating to hip fracture is highest in comparison to other fractures. In the younger age group (50-69 years) the mean length of stay in hospital and mean hospital cost is 6.9 days and $17,123 respectively, whereas in the older age group (>70 years) it is 11.5 days and $22,532 respectively. The rate for
rehabilitation in the younger and older age group is similar at 39% and 32% respectively. The rate for residential care is 11% (Watts et al., 2012).

In USA, greater than one third of the total healthcare expenditure of $20 billion is spent on hip fractures every year. The total cost associated with hip fracture is $8.7 billion with the majority due to inpatient hospital stay. The remaining costs include: emergency department, outpatient doctor, outpatient hospital, community services/ambulance services/medical equipment and residential care (Table 1.3) (Cummings et al., 2002).

<table>
<thead>
<tr>
<th>Type of service (millions of Australian $)</th>
<th>Inpatient</th>
<th>Emergency department and outpatient services</th>
<th>Ambulance</th>
<th>Rehabilitation</th>
<th>Community services (general practitioner, radiology, physiotherapy, analgesia)</th>
<th>Home help</th>
<th>Nursing home</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip</td>
<td>502</td>
<td>4</td>
<td>15</td>
<td>93</td>
<td>3</td>
<td>9</td>
<td>65</td>
</tr>
<tr>
<td>Spine</td>
<td>93</td>
<td>9</td>
<td>10</td>
<td>26</td>
<td>3</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Forearm</td>
<td>76</td>
<td>6</td>
<td>3</td>
<td>21</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Other sites</td>
<td>466</td>
<td>24</td>
<td>22</td>
<td>89</td>
<td>14</td>
<td>11</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 1.2 Healthcare cost according to fracture site and services utilized in Australia.

(Watts et al., 2012)

<table>
<thead>
<tr>
<th>Type of service (millions of US$)</th>
<th>Inpatient hospital</th>
<th>Emergency room</th>
<th>Outpatient Doctor</th>
<th>Outpatient hospital</th>
<th>Community services, ambulance, medical equipment</th>
<th>Residential care</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip</td>
<td>5576</td>
<td>130</td>
<td>67</td>
<td>9</td>
<td>90</td>
<td>2811</td>
<td>8682</td>
</tr>
<tr>
<td>Spine</td>
<td>575</td>
<td>20</td>
<td>13</td>
<td>3</td>
<td>10</td>
<td>126</td>
<td>746</td>
</tr>
<tr>
<td>Forearm</td>
<td>183</td>
<td>55</td>
<td>93</td>
<td>8</td>
<td>4</td>
<td>41</td>
<td>385</td>
</tr>
<tr>
<td>Other sites</td>
<td>2259</td>
<td>362</td>
<td>297</td>
<td>45</td>
<td>91</td>
<td>899</td>
<td>3953</td>
</tr>
</tbody>
</table>

Table 1.3 Healthcare cost according to fracture site and services utilized in the U.S.A.

(Cummings et al., 2002).
1.5.2 Vertebral fractures

Although vertebral fractures are not as costly compared to hip fractures, it also uses up significant medical resources. In Australia, the mean length of stay and mean hospital cost is 5.4 days and $6,684 respectively (Watts et al., 2012). In USA, the total cost for vertebral fractures is $746 million with most of the cost due to inpatient stay. The remainder of the cost comprised: emergency room, outpatient doctor, outpatient hospital, community services/ambulance services/medical equipment and residential care (Table 1.3) (Cummings et al., 2002). In Manitoba province, Canada, vertebral fracture in the first year accounts for 11% of the total costs (Leslie et al., 2013). Whereas in Europe, vertebral fracture accounts for 5% of the total cost (Hernlund et al., 2013).

1.5.3 Non-hip, non-vertebral fractures

The Global Longitudinal Study of Osteoporosis in Women recruited ~50,000 post-menopausal women. After a one year follow-up, 1898 women reported new fragility fractures. Use of medical resources was calculated from questionnaires. For non-hip, non-vertebral fractures, the average length of stay was ten days and the total length of stay in hospital was 3,805 days. The mean length of stay in rehabilitation was twenty-seven days and the total length of stay in rehabilitation was 4,083 days. The mean length of stay in nursing home was twenty-nine days and total length of stay in nursing home was 1,103 days (Figure 1.8) (Ioannidis et al., 2013). Thus, due to the higher prevalence of non-hip, non-vertebral fractures, the total length of stay in hospital, rehabilitation and nursing home was ~3 fold higher than for hip fractures.
A study using different databases to estimate healthcare costs in America on different fractures reported that in a younger cohort (50-64 years) the non-hip, non-vertebral fracture costs for inpatient, outpatient and medication was $5910, $7967 and $2899 respectively during the first year (totaling $16,775). Similarly, the total cost in an older cohort (>65 years) during the first year was $15,262. Though the average costs for non-hip, non-vertebral fracture was lower than for hip fracture ($38,662) and vertebral fracture ($25,662) in younger individuals (aged 50-64 years), it constituted 66% of the total fracture costs in comparison to 21% for hip fracture and 13% for vertebral fracture. However, in older individuals, hip fractures comprised 52% of the total fracture cost, whereas non-hip, non-vertebral fractures constituted 36% and vertebral fractures accounted for 12% (Shi et al., 2009).

**Summary**

Osteoporosis has been defined as a ‘systemic skeletal disease characterized by low bone density, micro-architectural deterioration of bone tissue leading to enhanced bone fragility, and a consequent increase in fracture risk’. There were ~9 million fragility fractures in 2009 globally; hip fractures accounted for 1.6 million, vertebral fractures accounted for 1.4 million and non-hip, non-vertebral fractures accounted for the remaining 6.0 million. Hence the majority of fragility fractures were non-hip, non-
vertebral. The incidence of hip fractures varies greatly from one region to another. While the true incidence of radiologic vertebral fractures are difficult to determine as only one third seek out medical attention. Fragility fractures are associated with significant morbidity and mortality. They can lead to issues with chronic pain, functional decline and admission to residential care. Both hip fractures and vertebral fractures are known to be associated with increase mortality. Due to the ageing population and the accompanying greater burden of fragility fractures, there is greater use of medical resources and healthcare expenditure.
Review of the Literature

Pathogenesis of Bone Fragility

Chapter Two
2.1 Introduction
Throughout growth, adulthood and ageing, bone modelling and remodelling work continuously on the skeleton. During growth bone modelling predominates, whereas, once peak bone strength is attained bone remodelling becomes more dominant. Bone modelling changes the size, shape and length of bone and involves osteoblasts depositing bone on one surface and osteoclasts resorbing bone on a different surface. In contrast, bone remodelling renews and repairs bone and involve the coupling of bone resorption followed by bone formation by basic multicellular units (BMUs) at the same site.

Bone cells

Osteoblasts, osteocytes and lining cells
Pluripotent mesenchymal stem cells give rise to osteoblasts. The differentiation of osteoblasts requires a number of transcription factors including Runx2 and osterix as well as signalling pathways such as Wnt and Notch (Long, 2012). Osteoblasts not only produce and deposit type 1 collagen but are also involved in its post-translational modification and subsequent mineralization. They are also involved in the differentiation of osteoclasts. Osteoblasts can either develop into osteocytes trapped within the bone matrix with cytoplasmic processes connecting them to other lining cells and nearby osteocytes, lining cells or apoptose (Noble et al., 2000). Osteocytes also assist with regulating the onset of bone remodelling, mineralization and phosphate metabolism (Schaffler et al., 2012).

Osteoclasts
Osteoclasts are large multi-nucleated cells that arise from haematopoietic stem cells. The differentiation of osteoclasts require a number of signaling pathways that include: macrophage colony stimulating factor (m-CSF), receptor activator nuclear factor κ-B ligand (RANKL), vascular endothelial growth factor (VEGF) and nitric oxide (Sims et al., 2014). Osteoclasts resorb bone by attaching to the bone surface and secreting hydrogen ions and lysosomal enzymes to degrade the bone matrix.
2.2 Bone modelling and remodelling during growth

During growth, bone modelling and remodelling facilitate the development of peak bone strength.

2.2.1 Bone Modelling

Bone modelling involves osteoblasts and osteoclasts working independently of one another. Unlike bone remodelling, there is no temporal or spatial relationship between bone resorption and bone formation. Bone modelling is dominant during growth to facilitate changes in bone size, shape and length to achieve peak bone strength but continues to a lesser degree throughout adulthood and old age in response to mechanical loading (Dempster et al., 2015).

Modelling long bones

During growth, bone is predominantly gained through bone modelling which encompasses both endochondral and intramembranous bone formation (Figure 2.1). Linear growth of cortical bone is achieved through endochondral bone formation, where a cartilage intermediate is subsequently replaced by bone. The trabeculae formed around the periphery of the growth plate thicken and integrate to become cortical bone at the metaphysis (Cadet et al., 2003).

At the metaphysis, bone modelling on the periosteal as well as the endocortical surfaces shape this region to fit the diaphysis. As such resorptive modelling on the periosteum and formative modelling on the endocortical surface occur concurrently to reduce the diameter of bone at this region (Martin et al., 2015). While at the diaphysis, radial expansion of the cortex is accomplished through intramembranous bone formation on the periosteum, a process whereby bones are formed from condensation of mesenchymal cells without the need of a cartilage model (Frisancho et al., 1970). In long bones, trabeculae are formed from the central portion of the growth plate. As the bone grows longitudinally, the trabeculae are resorbed before reaching the diaphysis as this site contains no trabeculae (Rauch, 2005).
Modelling at the distal radial metaphysis during growth

(i) Longitudinal growth of long bones is achieved through endochondral bone formation. (ii) The metaphysis is shaped to fit the diaphysis with bone resorption and bone formation taking place on the periosteal and endocortical surfaces respectively. (iii) Central metaphyseal trabeculae are resorbed before reaching the diaphysis as this region contains no trabeculae. Radial growth of long bones results from (iv) intra-membraneous bone formation on the periosteum and (v) endocortical bone resorption to expand the medullary cavity (Rauch, 2012).

Modelling flat bones

During growth, the flat bones located in the skull, ribs and pelvis enlarge to accommodate the growth of the various organs.

The ilium has both an outer and an inner cortex. During growth, modelling on the ilium causes an outward drift of both cortices due to periosteal bone formation and endocortical bone resorption on the outer cortex and periosteal bone resorption and endocortical bone formation occurring concurrently on the inner cortex. Iliac cortical width also expands but is similar between the two cortices.
Unlike long bones where trabeculae are predominantly located in the metaphysis, axial bone contains trabeculae throughout. During growth in the ilium, increases in trabecular width is due to concurrent increases in trabecular thickness (not number) as well as wall thickness (Parfitt et al., 2000). Further growth causes trabecular bone volume to expand and be displaced outwardly. The outward displacement of the inner cortex causes some trabeculae to be incorporated, which ‘corticalizes’ the trabeculae. Some of the new trabeculae are formed from cortical remnants arising from endocortical resorption ‘trabecularising’ the outer cortex. While the remainder are produced from endochondral bone formation (Parfitt et al., 2000).

2.2.1 Bone Remodelling

Bone remodelling occurs throughout life and is required to maintain bone’s material composition. This is accomplished through renewing and repairing old bone to prevent the accumulation of fatigue damage and fragility fracture (Parfitt, 1994; Verborgt et al., 2000). Bone remodelling is coupled; that is bone resorption is followed by bone formation in the same location.

Bone remodelling is surface dependent and takes place on all three endosteal surfaces (endocortical, intra-cortical and trabecular) and to a lesser degree on the periosteum (Eriksen, 1986; Martin et al., 2008). Bone remodelling is undertaken by the basic multicellular units (BMUs), which comprise mainly osteoclasts and osteoblasts.

Bone remodelling consists of 6 phases (Figure 2.2):
(i) Activation: Bone remodelling can be initiated through different mechanisms. Damaged osteocytes may signal to their healthy counterparts regarding an area of bone that needs to be replaced (Schaffler et al., 2014). Alternatively, the death of osteocytes or lining cells from micro-damage can signal for osteoclast precursors and osteoblasts to be recruited (Martin et al., 2015).

(ii) Resorption: Osteoclasts are recruited and resorb bone, a process that takes ~3 weeks. They accomplish it by secreting hydrogen ions (which dissolves the mineral component of the matrix) and lysosomal enzymes such as tartrate resistant acid phosphatase and cathepsin K (which degrades the protein component of the matrix)
through the ruffled border and into the resorption pit (Baron et al., 1988; Bromme et al., 1996).

(iii) Reversal: The reversal period begins after bone resorption and lasts for a number of weeks until bone matrix is deposited by osteoblasts. It may contribute to the coupling mechanism between bone resorption and bone formation (Delaisse, 2014). Several processes take place during this period to optimize the bone surface for bone formation and include removing debris, smoothing over the resorptive surface and depositing cement substance (Delaisse, 2014). It is thought that osteoclasts release osteogenic factors, which can interact with cells in the excavated cavity such as bone lining cells, mononuclear cells and mesenchymal cells which forms a canopy over the site. The majority of mononuclear cells appear to be of osteoblast lineage since they express osteoblastic marker (Runx2) and not monocytic nor osteoclastic markers (Andersen et al., 2013). The mononuclear cells may be able to differentiate into osteoblasts (Nakashima et al., 2002). However, others have reported that mesenchymal cells may also be able to differentiate into osteoblasts (Bi et al., 2008).

(iv) Formation: Osteoblasts are recruited and subsequently deposit bone. Unlike bone resorption, bone formation lasts ~3 months (Dempster et al., 2015).

(v) Mineralization: Primary mineralization is initiated 5 to 10 days after bone matrix is laid down. This delay produces a layer of osteoid between the osteoblasts and mineralized bone. Primary mineralization accounts for 60–70% of the mineralization in the first 3 weeks (Martin et al., 2015). In contrast, secondary mineralization is a more gradual process that results in enlargement of crystals and lasts 24 to 30 months (Boivin et al., 2009).

(vi) Quiescence: A period of inactivity ensues after bone resorption and bone formation is finalized.
2.2.3 Growth in bone size, mass and microstructure

Growth within the skeleton is highly variable. As such, there are not only differences in the growth of axial bone and appendicular bone but also differences in the growth of bone types, bone regions and bone surfaces. Furthermore, within a long bone, the rate of growth is faster at the distal than proximal end for distal long bones such as the radius and faster proximally than distally at proximal long bones such as the humerus (Pritchett, 1991). Thus, during growth, conditions or illnesses affecting bone will depend on what stage of development the bone segment is at, as bone that is further from their predicted peak for adulthood are more severely affected (Bradney et al., 2000).

Bone size

The post-natal growth rate accelerates then slows down during the initial year of life but increases again due to growth in leg length (Fredriks et al., 2005; Karlberg et al., 1987; Parfitt, 1994). As such, growth in the legs is almost two fold more rapid than

Figure 2.2 Phases of bone remodelling

(i) **Quiescence**: A period of inactivity on the bone surface. (ii) **Activation**: bone remodelling is initiated through different mechanisms. (iii) **Resorption**: osteoclast precursors are recruited and differentiate into osteoclasts and resorb bone, a process that takes ~3 weeks. (iv) **Reversal**: mononuclear cells prepare the bone surface for bone formation. (v) **Bone formation**: osteoblast deposit bone, a process that takes ~3 months. (vi) **Mineralization**: primary mineralization begins 5–10 days after bone formation (http://belajar.us/bone-remodeling.html).
growth in the trunk prior to puberty and was comparable in both sexes (Iuliano-Burns et al., 2009). By early to mid-puberty, axial growth increases similarly in both sexes. Whereas leg growth is steady but similar in both sexes. By late puberty, growth in the legs in males remains steady but in females decelerate. Following puberty, growth at all sites slows down, though the deceleration in leg length in females may be more rapid than in males (Iuliano-Burns et al., 2009) (Figure 2.3). Hence, the sex related differences in leg length may be due to differences in the onset of puberty and the rate of growth during late puberty and post-puberty.

![Figure 2.3 (A) Total height, (B) sitting height and (C) leg length growth velocity in boys and girls](image_url)

(Iuliano-Burns et al., 2009).

### 2.2.4 Axial growth

Sex has differential effects on vertebral body size as measured using QCT. Despite adjustment for body size, vertebral body cross-sectional area and vertebral volume was higher in boys than girls throughout growth (Gilsanz et al., 1994; Gilsanz et al., 1997). The favourable bone geometry in males confers biomechanical advantages and may partly contribute to the lower vertebral fragility in men compared to women later in life (Gilsanz et al., 1994).

In contrast, vertebral body height and both trabecular and cortical vBMD were comparable between boys and girls evaluated using QCT (Gilsanz et al., 1994; Gilsanz et al., 1997). Before the onset of puberty, trabecular vBMD was similar...
between boys and girls (Gilsanz et al., 1988; Gilsanz et al., 2009). Although puberty produced trabecular vBMD increases of 18–23% in both sexes, for girls this started and peaked ~2 years before boys. Thickening of trabeculae and not increases in trabecular number may be responsible for the rise in trabecular vBMD (Gilsanz et al., 2009; Glorieux et al., 2000). In contrast, the increase in cortical vBMD was similar in boys and girls (Gilsanz et al., 1994).

2.2.5 Appendicular growth

Growth in bone size

The dimensions of the appendicular skeleton were similar in boys and girls prior to puberty – total CSA, cortical and medullary areas at the radius and tibia (Gilsanz et al., 1997; Hogler et al., 2003; Kontulainen et al., 2006). However, during puberty the increase in bone size was greater in boys than in girls. Consequently, boys have bigger total and cortical area but comparable cortical thickness relative to girls as a result of greater periosteal apposition (Hogler et al., 2008; Loro et al., 2000; Macdonald et al., 2006; Nieves et al., 2005).

Growth on the bone surfaces

The rate of bone modelling on the periosteal and endocortical surfaces determines cortical thickness. During growth, periosteal and endocortical diameters both increase but the rate is greater at the periosteal surface, which results in cortical thickening (Bass et al., 1999; Bradney et al., 2000). Variable findings on whether endocortical apposition takes place during puberty have been reported. Studies using DXA and pQCT in girls have reported that following menarche at the femur and tibia, there was a decrease in endocortical diameter suggesting endocortical apposition (Bass et al., 1999; Wang et al., 2005). Whereas studies utilizing QCT, pQCT, HR-pQCT and MRI demonstrated either no change to endocortical diameter at the radius in females or increased endocortical diameter at the radius, tibia and femur in both males and females (Gilsanz et al., 1998; Hogler et al., 2003; Kirmani et al., 2009; Neu et al., 2001).


**Cortical vBMD**

At the appendicular skeleton prior to puberty, cortical vBMD is comparable in boys and girls (Gilsanz et al., 1997; Kirmani et al., 2009; Neu et al., 2001). During puberty, cortical vBMD increased in both boys and girls (Loro et al., 2000; Wang et al., 2005). However, both prospective and cross-sectional studies report greater increases in cortical vBMD in girls over boys at the radius and tibia using pQCT and HR-pQCT (Kontulainen et al., 2006; Neu et al., 2001; Nishiyama et al., 2012; Schoenau et al., 2002). This may be due to lower cortical porosity in girls compared to boys (Nishiyama et al., 2012). More recent studies using HR-pQCT have reported transient decreases in cortical vBMD in mid-puberty in girls accompanied by a rise in cortical porosity (Kirmani et al., 2009). Similar findings of a transient decrease in cortical vBMD in boys during mid-puberty have also been demonstrated (Wang et al., 2010). Studies using micro-finite element analysis have reported that during growth, boys have better total strength as well as load to strength ratios compared to girls (Kirmani et al., 2009; Nishiyama et al., 2012).

**Trabecular vBMD**

Inconsistent results have been reported at the distal radius for sex related differences in trabecular bone volume prior to puberty. Studies using pQCT observed higher trabecular vBMD in boys compared to girls (Neu et al., 2001). Whereas studies using HR-pQCT reported no sex differences in trabecular parameters prior to puberty (Kirmani et al., 2009; Nishiyama et al., 2012; Wang et al., 2010). Throughout puberty trabecular morphology (BV/TV, number, thickness) did not differ significantly in girls. In boys, trabecular BV/TV increased due to trabecular thickening, while trabecular number did not change during late puberty (Kirmani et al., 2009; Nishiyama et al., 2012). Consequently boys have higher trabecular vBMD, BV/TV and thickness but comparable trabecular numbers to girls post-puberty (Khosla et al., 2006; Kirmani et al., 2009; Nishiyama et al., 2012).

**2.3 Bone modelling and remodelling during advancing age and menopause**

Throughout adulthood, bone modelling and remodelling maintains bone strength. Once skeletal maturity is attained, bone modelling is largely restricted to periosteal
apposition. Bone remodelling serves to repair and replace old and damaged bone which helps maintain the strength of bone.

*Reversible and irreversible bone deficits*

Prior to menopause, bone density is stable as bone remodelling is slow, balanced and in steady state, as the number of cavities undergoing resorption is the same number undergoing refilling. The onset of menopause causes a rapid net loss in bone density as there is perturbation in the steady state of bone remodelling with many more cavities being resorbed than prior to menopause. Oestrogen deficiency markedly increases the rate of bone remodelling. Animal studies have demonstrated that loss of gonadal function causes cytokines known to promote osteoclastogenesis and osteoblastogenesis to be up-regulated (Manolagas, 2000). In addition to these changes, there is the normal delay and slowness of refilling the cavities created prior to menopause, which results in a reversible remodelling deficit (Seeman et al., 2015).

Later on the bone deficit becomes irreversible as menopause causes negative BMU balance in addition to the higher rate of bone remodelling. Oestrogen deficiency prolongs the lifespan of osteoclasts but shortens the lifespan of osteoblasts (Manolagas, 2000; Weitzmann et al., 2006). Consequently this produces a greater erosion depth after each remodelling cycle and leads to an imbalance between bone resorption and bone formation. There is attainment of a new but still rapid steady state for bone remodelling. The creation of the many new resorption pits is now matched by refilling of the earlier excavated sites. Although this phase of bone loss is slower, any microstructural deterioration that occurs is irreversible due to the negative BMU balance (Figure 2.4) (Seeman et al., 2015).
Figure 2.4 Reversible and irreversible bone deficits caused by bone remodelling

Effect of oestrogen deficiency on bone remodelling. Prior to menopause, bone remodelling is balanced, as the sites excavated are matched by refilling. (A) However, with the onset of menopause, more sites are resorbed and each resorption pit is deeper. (B) The fewer resorption pits created prior to menopause begin to incompletely refill. Unlike bone resorption, which takes 3 weeks, bone formation takes 3 months. As a consequence of this delay in refilling, bone loss is accelerated. (C) Later in menopause a new but still rapid steady state is reached with the many new sites resorbed now matched by the incomplete refilling of earlier sites excavated causing bone loss to continues but at a slower rate (Seeman, 2008).

Although, skeletal decay has been reported at midlife or prior in both sexes in cross sectional and prospective studies, there is still uncertainty regarding the onset of age-related bone loss.

Several studies have described trabecular bone loss prior to midlife in men and women (Khosla et al., 2006; Riggs et al., 2004; Riggs et al., 2008; Riggs et al., 1986).
However, for this to occur there needs to be a net negative bone balance at the level of the BMU which has not yet been demonstrated. One possible explanation may be that the loss of bone is an artifact of a reduction in cellularity of the marrow with increased marrow fat (Bolotin et al., 2001; Genant et al., 1977). Marrow fat attenuates photons less than water or cells and so it seems like trabecular BMD decreases when in fact it does not. Cortical bone loss appears to occur around midlife in both sexes (Riggs et al., 2004; Riggs et al., 2008).

A further factor contributing to the structural decay may be the reduction in periosteal apposition once skeletal maturity is achieved (Epker et al., 1966). Periosteal apposition provides biomechanical advantages to bone, as it partly offsets endocortical bone loss, since expanding the cortex further from the centre of the bone increases bending strength of long bones (Bouxsein, 2005; Lauretani et al., 2008) (Figure 2.5).

The prospective study by Szulc et al highlights the important role of periosteal apposition to bone strength. More than 800 women (age range 30-89 years) were followed up for 7 years with radial bone geometry measured using DXA. Pre-menopausal females appeared to have increased periosteal expansion offsetting endocortical resorption with outward displacement of the cortex producing greater bending strength. In contrast, peri-menopausal females were seen to have increased endocortical resorption with reduced periosteal expansion accompanied by slight outward displacement of the cortex with subsequent preservation of bending strength. Moreover, in post-menopausal females endocortical resorption was further increased while periosteal expansion was further diminished and associated with negligible outward displacement of the cortex with decline in bending strength (Szulc et al., 2006) (Figure 2.6).
Figure 2.5 Biomechanical effects of periosteal apposition

Increasing periosteal apposition by 10% increases bending strength by ~4 fold (Bouxsein, 2005).

Figure 2.6 Annual rates of change in endocortical resorption, periosteal apposition, net cortical width and section modulus

As endocortical resorption increases, periosteal apposition decreases causing net cortical width to decline in all three groups. The section modulus is an index of bending strength and is increased in pre-menopausal females, maintained in peri-menopausal females and declined in post-menopausal females. Comparison versus zero change *p<0.05, **p<0.01, ***p<0.001 (Szulc et al., 2006).
2.3.1 Abnormalities in bone material composition with ageing

The rate of bone remodelling may affect the material composition of bone and in turn modify its biomechanical properties. Rapid bone remodelling leads to older, highly mineralized bone being replaced with newer, less mineralized bone, which lowers the mean mineralization density of bone (Boivin et al., 2002).

The rate of bone turnover may also effect the isomerization of collagen (Viguet-Carrin et al., 2006). Bone surfaces that are less accessible to remodelling, such as inter-osteonal bone, become highly mineralized and cross-linked with advanced glycation end-products (AGEs), which may enable micro-crack to propagate more readily since the bone is more homogeneously mineralized. All these changes adversely affect the biomechanical properties of bone (Burr, 2003).

2.3.2 Abnormalities in bone microstructure with ageing

Trabecular bone loss

As men and women age, they lose a similar volume of trabecular bone, however the structural basis for the bone loss differ between the sexes. Women lose trabeculae with studies demonstrating reduced trabecular number and increased trabecular separation. Unlike women, men lose trabecular bone by trabecular thinning. These findings have been reported in recent studies using HR-pQCT and earlier studies using histomorphometry on iliac crest biopsies (Aaron et al., 1987; Han et al., 1996; Khosla et al., 2006; Macdonald et al., 2011; Parfitt et al., 1983). There are biomechanical implications for this differential trabecular bone loss with greater loss of bone strength associated with loss of trabeculae rather than trabecular thinning (Silva et al., 1997; van der Linden et al., 2001).

Cortical bone loss

A recent cross sectional study used HR-pQCT to quantify and compare cortical and trabecular bone loss of 122 Caucasian females aged 27 to 98 years at the distal radius (Zebaze et al., 2010). Zebaze et al reported that between 50 to 80 years of age, 68% of bone loss was attributable to cortical and 32% to trabecular bone. Only 16% of bone loss occurred between the ages of 50 to 64 years, while 84% of bone loss occurred thereafter (Figure 2.7). The 68% cortical bone loss was due predominantly to porosity
trabecularizing the cortex adjacent to the marrow (47%) as well as porosity in the compact appearing cortex (21%).

![Image](image_url)

Figure 2.7 Age related bone loss

(A) Cortical and trabecular bone decay at the distal radius stratified according to different age groups. (B) Diagram representing remodelling of cortical and trabecular bone. (C) Bone surface available for remodeling in cortical and trabecular compartments of post-mor tem females in different age groups. SD *p<0.0001 (Zebaze et al., 2010).

The authors highlight the significance of intra-cortical remodelling adjacent to the marrow since this site of remodelling produces cortical remnants that resemble trabeculae (Figure 2.8). Inclusion of the cortical remnants as trabecular bone overestimates trabecular bone volume, which in turn underestimates age related trabecular bone loss as well as underestimating age related increases in cortical porosity.
Figure 2.8 Implications of cortical remnants being erroneously assigned to trabecular bone

(A) Cortical remnants erroneously assigned to trabecular bone underestimate age related trabecular decay and (B) Exclusion of cortical porosity underestimates age related cortical decay (Zebaze et al., 2010).

Similarly, other studies have reported the significance of increased cortical porosity in age-related bone loss. Cross-sectional and longitudinal studies using HR-pQCT have observed increased cortical porosity in the distal radius and/or tibia associated with ageing in both men and women (Kawalilak et al., 2014; Macdonald et al., 2011; Nicks et al., 2012; Shanbhogue et al., 2016).

2.3.3 Sex steroid deficiency

Oestrogen deficiency is accompanied by marked stimulation in bone resorption that is due to the effects of oestrogen on osteoclast lifespan. Furthermore, oestrogen is involved in the regulation of receptor activator of nuclear factor kappa β ligand (RANKL) and osteoprotegerin (OPG) expression, both important in osteoclast differentiation and activity. In vitro and in vivo studies have demonstrated that oestrogen suppresses RANKL but increases OPG production (Eghbali-Fatourechi et al., 2003; Hofbauer et al., 1999; Khosla et al., 2001). Oestrogen deficiency also stimulate the production of cytokines that promotes bone resorption such as
interleukins (IL-1, IL-6), tumour necrosis factor (TNF-α), macrophage colony stimulating factor (m-CSF) and prostaglandin (Pacifici et al., 1991; Syed et al., 2005; Tanaka et al., 1993).

In females, oestrogen deficiency plays a pivotal role in bone decay. During the transition to menopause, serum oestradiol levels drastically fall to a fraction of the pre-menopausal level (Khosla et al., 1997). Serum testosterone levels also fall, although to a milder degree since the ovaries and adrenal glands still continue to produce androgen (Horton et al., 1966). Oestrogen deficiency is associated with increased intensity of bone remodelling as well as imbalance in bone remodelling with less bone deposited after each remodelling cycle.

Although males do not experience an sudden fall in sex steroid levels, total testosterone, ‘free’ bioavailable testosterone and oestradiol levels gradually fall with age due in part to the increase in sex hormone binding globulin (SHBG) (Khosla, 2010). Moreover, it is the oestrogen deficiency and not testosterone deficiency that plays a key role in age related bone decay. Studies have demonstrated positive correlations between free oestradiol levels and aBMD as measured by DXA. The increases in aBMD in younger males and the decline in aBMD in older males appears to be dependent on oestradiol and not testosterone levels (Khosla, 2010). Studies using QCT also corroborate these findings (Khosla, 2010). In addition, clinical studies have suggested that oestrogen regulates bone resorption and both oestrogen and testosterone regulates bone formation (Falahati-Nini et al., 2000).

### 2.3.4 Secondary hyperparathyroidism

Structural decay of bone is further exacerbated during ageing by secondary hyperparathyroidism, which increases bone remodelling (Riggs et al., 1998). Serum PTH levels correlate with histomorphometric indices of bone remodelling (activation frequency, bone resorption rate, bone formation rate) and the calculated rate of bone loss (Kotowicz et al., 1990).

In old age, the factors contributing towards secondary hyperparathyroidism are multi-factorial. Vitamin D deficiency is prevalent in older populations (Lips, 2007). Ageing
causes intestinal malabsorption of calcium resulting in lower levels of serum calcium, which in turn stimulates parathyroid hormone in order to maintain serum calcium levels (Gallagher, 2013). Furthermore, longstanding oestrogen deficiency may cause chronic negative calcium balance since oestrogen facilitates both intestinal calcium absorption and renal tubular calcium reabsorption (Gallagher et al., 1980; Gennari et al., 1990; McKane et al., 1995; Nordin et al., 1991). Other factors contributing to secondary hyperparathyroidism include inadequate dietary calcium intake, use of loop diuretics and impaired renal function (Demontiero et al., 2012; Lips, 2001). Studies have reported that post-menopausal females on either a higher calcium intake of 2400 mg/day or oestrogen therapy appear to have lower levels of serum PTH and bone remodelling indices that are comparable to pre-menopausal females (McKane et al., 1996; McKane et al., 1997; Riggs et al., 2002).

There is a prevailing view that protracted elevations of endogenous PTH is associated with cortical but not trabecular bone loss. However, myself and others using HR-pQCT have demonstrated bone loss in not only cortical but also trabecular bone (Hansen et al., 2010; Vu et al., 2013). Furthermore, my work has demonstrated reduced cortical vBMD due to increased cortical porosity and reduce tissue mineralization density as well as reduced trabecular vBMD. This suggests that trabecular bone previously though to be ‘preserved’ is likely due to inclusion of cortical remnants, which resemble trabeculae.

2.4 Structural basis of fragility fractures across life
Across the lifespan there are periods of peak fracture incidences during growth and later in life with ageing. Distal forearm fractures peak in early adolescence, coinciding with peak growth velocity as well as during the peri-menopausal period in women (Figure 2.9). The incidence of hip and vertebral fractures increase substantially with age in men and women, though this occurs later in life in men. Hence, there may be underlying growth-related, sex-related and age-related abnormalities in bone structure that lead to age specific bone fragility.
Figure 2.9 Incidence of distal radial fracture across the lifespan

(A) Incidence peaking during adolescence in both sexes and during peri-menopause in females, Malmo, Sweden 1953-57. (B) During adolescence the peak incidence coincide with peak growth velocity, Saskatchewan, Canada 1978-85 (Parfitt, 1994).

2.4.1 Fractures in childhood

The peak incidence of childhood fractures occurred during early to mid-adolescence, approximately ~11-12 years old in girls and ~13-14 years old in boys (Hedstrom et al., 2010). Studies suggest that the most common site of fracture in children is the distal radial metaphyseal, comprising up to a third of fractures in children (Hedstrom et al., 2010; Landin, 1983; Rennie et al., 2007; Tiderius et al., 1999). Comparable rates of wrist fractures are also seen in post-menopausal females (Landin, 1983). Many of the metaphyseal fractures in children are thought to be ‘low trauma’ from a fall (Farr et al., 2014; Landin, 1983; Tiderius et al., 1999).

The development of the metaphyseal cortex may make it more prone to underlying bone fragility and increase the risk of fracture during growth. The metaphyseal cortex is formed from epiphyseal derived trabeculae with the spaces between the trabeculae filled in and ‘corticalised’ (Rauch, 2012). Since both cortical and trabecular bone at the radial metaphysis arise from trabecular bone originating from the growth plate, deficits in the metaphyseal trabeculae may cause abnormalities involving both bone compartments in that region. Studies using HR-pQCT in daughter-mother pairs have reported significant correlations in trabecular traits (BV/TV, trabecular thickness,
number and separation) between daughters and their mothers (Figure 2.10). Furthermore, daughters’ trabecular morphology (trabecular BV/TV) highly correlated with mothers’ cortical morphology (cortical thickness) (Wang et al., 2011). Similarly, other studies have shown that trabecular deficits in daughters (lower trabecular number and thickness) were associated with cortical deficits in mothers (higher cortical porosity) (Bala et al., 2015). However, daughter’s cortical morphology did not correlate with mother’s corresponding cortical morphology (Wang et al., 2011).
There were significant correlations in trabecular traits (BV/TV, trabecular thickness, number and separation) between daughters and their mothers and also between daughters’ trabecular morphology (trabecular BV/TV) and their mothers’ cortical morphology (cortical thickness) (Wang et al., 2008).

As already discussed, growth within the skeleton is highly variable. The rate of growth is faster at the distal than proximal end for distal long bones such as the radius. Furthermore, the distal growth plate is responsible for ~90% of the longitudinal growth within the radius, whereas the proximal growth plate contributes ~10% (Pritchett, 1991). Therefore, the discrepancy between linear growth and mineral
mass accrual may become more pronounced by the rapid growth velocity at the distal metaphysis during the pubertal growth spurt.

During growth, a transient decrease in total vBMD at the distal metaphysis of the radius has been observed (Wang et al., 2005; Wang et al., 2010). However, there were sex related differences to underlying bone microstructure to account for this. At the radial metaphysis, boys at Tanner stage III were observed to have bigger total CSA with thinner cortices, lower cortical vBMD and higher trabecular BV/TV relative to boys at Tanner stage I. While girls at Tanner stage III were observed to have bigger total CSA but similar cortical thickness and cortical vBMD as well as similar trabecular BV/TV relative to girls at Tanner stage I (Wang et al., 2010). The authors speculate that the reduced cortical vBMD is likely the result of increased cortical porosity associated with rapid growth. Other studies have corroborated this finding by reporting increased cortical porosity in girls with distal radial fractures (Bala et al., 2015) and in healthy adolescent boys (Nishiyama et al., 2012). Similar results were seen in healthy children during mid to late puberty, which was also accompanied by a decrease in the proportion of load tolerated by cortical bone (indicative of the relative strength of cortical to trabecular bone) as well as the ratio of cortical to trabecular bone volume at the distal radius (Figure 2.11) (Kirmani et al., 2009). These findings coincide with the peak incidence for distal radial metaphyseal fracture, suggesting that transient cortical deficits may partly explain the increase in fragility at the metaphysis during growth.
Figure 2.11 Changes in cortical traits during puberty

(A and B) Proportion of load carried by cortical bone; (C and D) cortical bone volume to trabecular bone volume ratio; and (E and F) cortical porosity index in girls and boys respectively. Children and adolescents were stratified into five groups based on bone age: group I (pre-puberty, 6-8 years), II (early puberty, 9-11 years), III (mid puberty, 12-14), IV (late puberty, 15-17) and V (post-puberty, 18-21). The shaded area represents the likely age range for peak incidence of distal radial fracture. *p<0.05, **p<0.01, ***p<0.001 vs. group 1. †p<0.05, †† p<0.01, †††p<0.001 vs. girls in the corresponding group (Kirmani et al., 2009).

2.4.2 Fractures in adulthood

Distal radial fracture

The incidence of distal radial fracture peaks during the peri-menopausal period from 45 to 60 years of age and decelerating thereafter. Whereas in men, the incidence remains stable across the lifespan. There is a strong predominance of women compared to men with a ratio of 6:1 during the peak incidence (Riggs et al., 2006).

Pre-menopausal women with forearm fractures appear to have predominantly trabecular deficits at the distal radius relative to non-fracture controls. Studies using HR-pQCT demonstrated that these women have lower trabecular density or BV/TV due to reduced trabecular number and thickness (Bala et al., 2015; Rozental et al., 2016).
Cortical porosity was observed to be higher in the inner-transitional zone and not the other cortical compartments (Bala et al., 2015).

Post-menopausal women with forearm fractures appear to have both trabecular as well as cortical deficits compared to women without fractures at the distal radius measured using HR-pQCT. Consistent with this finding, women with fractures have worst measures of bone strength assessed using micro-finite element analysis relative to non-fracture cases (Boutroy et al., 2008; Melton et al., 2007; Riggs et al., 2006). These women also have reduced trabecular vBMD or BV/TV due to lower trabecular number and thickness (Bala et al., 2015; Boutroy et al., 2008; Melton et al., 2010; Melton et al., 2007; Vico et al., 2008). Disruption to the trabecular network was apparent with reduced connectivity density and higher structure model index, which suggests a more rod-like over plate-like trabecular arrangement (Melton et al., 2010). Evidence of cortical bone decay was seen with reduced cortical vBMD due to increased cortical porosity in all cortical compartments in fracture cases compared to controls (Bala et al., 2015; Bala et al., 2014). Though not all studies have reported increased cortical porosity, which may be due to the differences in methods of bone segmentation (Melton et al., 2010).

The underlying structural basis for the preponderance of women over men in developing fragility fractures at the distal radius is established during growth with men developing greater peak bone strength. In addition there are sex-related differences in bone loss during ageing. Cross-sectional studies using HR-pQCT at the distal radius in young adults demonstrate that men have larger bone size with more favourable trabecular morphology compared to women (Khosla et al., 2006; Macdonald et al., 2011) (Figure 2.12). Young men appear to have higher BV/TV due to thicker trabeculae, however, the results on trabecular number have been more inconsistent with some authors suggesting higher trabecular numbers (Macdonald et al., 2011) while others report comparable trabecular numbers to young women (Khosla et al., 2006). Cross-sectional trabecular bone loss in men and women are reportedly similar. However, men experience trabecular thinning, whereas women lose trabecular elements which has worst biomechanical consequences for bone strength which perhaps partly accounts for the greater fragility seen in women at the distal radius (Silva et al., 1997).
Figure 2.12 Age- and sex-related changes in trabecular morphology

(A) Bone volume/tissue volume; (B) trabecular number; (C) trabecular thickness; and (D) trabecular separation at the distal radius for men (solid circle and solid line) and women (open circles and dashed lines) aged 20-97 years (Khosla et al., 2006).

In young adults, men appear to have reduced cortical vBMD due to greater cortical porosity compared to women (Khosla et al., 2006; Macdonald et al., 2011). However, during ageing women experience more cortical bone loss due to greater cortical porosity (Macdonald et al., 2011; Nicks et al., 2012). Longitudinal studies have supported this finding and shown that cortical bone loss begins in mid-life in women and after 75 years of age in men (Riggs et al., 2008). The fall load to bone strength ratio at the distal radius was measured using micro-finite element analysis to estimate fracture risk. It was observed that the load to bone strength ratio in young men was superior and during ageing and more favourable to men compared to women, which the authors postulate may account for the discrepancy in wrist fractures between men and women (Riggs et al., 2006).
**Hip fracture**

The incidence of hip fractures is two times higher in women compared to men and accelerates after 70 years of age and remains high thereafter in both sexes (Figure 2.13) (Riggs et al., 2006).

![Incidence of hip fracture](image)

Figure 2.13 Incidence of hip fracture (Riggs et al., 2006).

Bone fragility at the hip is attributable to cortical bone deficits. Femoral neck biopsy specimens exhibited cortical bone thinning in the inferoanterior to superoposterior region associated with increased cortical porosity in post-menopausal women with intra-capsular hip fractures compared to women without fractures (Bell et al., 1999; Bell et al., 1999) (Figure 2.14). This site specific deficit is commonly impacted during a fall (Lotz et al., 1995). Cortical thinning is also increased at the sub-capital and mid-neck area compared to the trochanteric area (Boyce et al., 1993). The finding of large pores with diameter >385µm, which is thought to be due to the fusion of multiple adjacent remodelling osteons, may contribute to the cortical porosity seen in the fracture cases (Bell et al., 2000; Jordan et al., 2000) (Figure 2.14). In addition, increased cortical porosity has been shown to reduce elasticity and fracture toughness.
in the femoral shaft, which may contribute to hip fragility (Currey et al., 1996; Yeni et al., 1998).

Figure 2.14 Regional cortical deficits associated with hip fractures

(A) Proportion of cortical bone and (B) cortical porosity in different regions of the hip in (male and female) controls and fracture cases (I=inferior; I-A=infero-anterior; A=anterior; S-A=supero-anterior; S=superior; S-P=supero-posterior; P=posterior; I-P=inféro-posterior); and (C) Total canal area in anterior region of the hip stratified according to canal size in fracture cases and controls. "p<0.05 between female and male control groups; "p<0.05 between female controls and fracture cases; "p<0.06 between female controls and fracture cases; "p<0.005 between different regions in female control group (Bell et al., 1999; Bell et al., 1999).
Trabecular bone loss and disruption of trabecular micro-architecture also contributes towards hip fragility. Previous work using femoral neck biopsy specimens in post-menopausal women with intra-capsular hip fractures compared to women with osteoarthritis have shown reduced trabecular bone volume due to trabecular thinning and lower trabecular numbers. In conjunction to these findings, there was also poor trabecular connectivity with reduced number of nodes and a shift towards trabecular rod-like structure. These changes were seen in the inferior and posterior quadrants of the femoral neck (Blain et al., 2008; Boutroy et al., 2011).

The two fold higher incidence of hip fractures in elderly females relative to males is likely to be partly due to greater cortical and trabecular deficits seen in females compared to males. Femoral neck specimens from male and female cadavers aged 57-98 years from a Japanese population were examined. The men and women were grouped into middle (57-68 years), old (72-82 years) and elderly age range (87-98 years). In each of the groups, the women had thinner cortices, greater cortical porosity associated with larger canal diameter compared to men. Unfavourable trabecular morphology was also seen in women compared to men in each of the groups with decreased trabecular bone volume due to thinner trabeculae as well as lower trabecular numbers. Disruption of trabecular micro-architecture and connectivity was also worst in women compared to men in each group with higher structure model index indicating a more rod-like trabecular network and lower connectivity density (Chen et al., 2010).

**Vertebral fractures**

The true incidence of vertebral fractures is difficult to establish since only a third of cases seek assistance due to symptoms and until recently there was no consensus regarding the method used to diagnose vertebral fracture, however, the incidence is about two folds higher in women than men and increases with age in both sexes (Cooper et al., 1992; Schousboe, 2016).

Disruption of trabecular microstructure and connectivity contributes towards fragility in the vertebrae. A study examining iliac crest bone biopsies in post-menopausal women with and without vertebral fractures matched the two groups in terms of age, sex as well as cortical and trabecular bone volume. With bone mass being similar
between the two groups, the only difference found was that in fracture cases trabecular plate density (reflecting trabecular number and connectivity) was reduced, trabecular separation was increased and trabecular plate thickness was increased compared to controls (Figure 2.15) (Kleerekoper et al., 1985). In accordance with this finding, other studies have reported that the fracture risk index (measure of vertebral load to strength ratio) was worst in both men and women with vertebral fractures compared to age matched individuals (Duan et al., 2001).

Figure 2.15 Age related changes in mean trabecular plate density in fracture cases and controls

Post-menopausal women with vertebral fractures (solid circles) and controls (open circles). Solid line depicts regression for healthy post-menopausal women and dash line depicts one standard deviation (SD) of the regression (Kleerekoper et al., 1985).

Cortical bone loss may also have a role in vertebral fragility. Cadavers with and without osteoporosis (clinically and histologically diagnosed) had their complete vertebral column examined. In those with osteoporosis compared to those without significant cortical thinning was found throughout the spine (Ritzel et al., 1997).

The higher incidence of vertebral fractures in women compared to men is due to the smaller bone size and greater trabecular decay with age in women relative to men.
Studies have consistently observed that in young adulthood, cross-sectional area was larger in men than women. In contrast, inconsistent findings have been reported with ageing. Studies using DXA found larger age-related increases in CSA in men compared to women (Duan et al., 2006). While studies examining L2 vertebral body from cadavers found age-related increases in CSA in males but no changes in females (Mosekilde et al., 1990). In contrast, studies using QCT have found similar increases in CSA in both sexes with age (Riggs et al., 2004).

Age- and sex-related changes in vertebral microstructure was assessed using micro-CT and scanning electron microscopy on bone specimens from the fourth lumbar vertebra from men and women aged 57 – 98 years. There was age-related decrease in BV/TV and trabecular number. The decrease in trabecular thickness with age did not reach significance. There were sex-related differences with men having higher trabecular number and better trabecular connectivity in the 72 – 82 year age group (Chen et al., 2008). There are conflicting reports on the changes in trabecular thinning associated with ageing. Ashing and histomorphometry analyses have revealed similar age-related decreases in trabecular bone density and ash density in both sexes due to horizontal trabecular thinning and loss of horizontal trabeculae. However, the loss of horizontal trabeculae was higher in women compared to men from 75 years of age (Mosekilde et al., 1990). Other studies have suggested that age related trabecular thinning affects both horizontal and vertical trabeculae (Bergot et al., 1988). There may be sex-related differences in trabecular bone loss. The study by Bergot et al reported greater trabecular bone loss in women compared to men due to increased vertical and horizontal trabecular thinning as well as loss of vertical trabeculae using quantitative image analysis of L3 vertebrae (Bergot et al., 1988).

**Summary**
Throughout life, bone modelling and remodelling work continuously on the skeleton. During growth bone modelling predominates, changing the size, shape and length of bone. The timing and tempo of growth within the skeleton is highly variable. As such, there are not only differences in the rate of growth of axial and appendicular sites but also in the rate of growth of bone types, regions and surfaces. Once peak bone mass is
attained in adulthood, bone remodelling predominates to maintain skeletal integrity and strength.

After menopause, oestrogen deficiency results in bone loss due to an imbalance between bone resorption and bone formation with less bone deposited after each remodelling cycle. Bone loss is further compounded by the increased activation frequency with many more sites involved in bone remodelling. During ageing, men lose trabecular bone through trabecular thinning, whereas women lose trabecular bone through perforation, which has worst biomechanical implications. Most of the cortical bone loss occurs after 65 years of age and is due predominantly to intra-cortical remodelling, adjacent to the medulla. ‘Trabecularization’ of the cortex causes the pores to fuse and coalesce, which fragments the cortex and resembles trabeculae. The main significance of this is that these cortical remnants may be erroneously included in trabecular bone which then under-estimate the age related trabecular bone loss as well as underestimating the increase in age related cortical porosity.

There are periods of peak fracture incidences across the lifespan – during growth and later in life with ageing. Distal forearm fractures peak in early adolescence, which corresponds to the timing of peak growth velocity, and the peri-menopausal period in women. The incidence of hip and vertebral fractures increase substantially with age in men and women, though this occurs later in life in men. Hence, there may be underlying growth-related, sex-related and age-related abnormalities in bone structure that leads to age specific bone fragility.
Review of the Literature

Secondary Osteoporosis

Chapter Three
Chapter 3: Secondary Osteoporosis

3.1 Definition of secondary osteoporosis

Osteoporosis may be primary or secondary. Primary osteoporosis refers to menopause and/or ageing causing the underlying bone fragility, whereas secondary osteoporosis is caused by illnesses or medication (i.e. factors other than menopause and ageing) (Table 3.1.1).

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Table 3.1.1 Diseases and drugs causing secondary osteoporosis

(Gennari et al., 1998).
3.1.1 Epidemiology of fractures associated with known causes of bone fragility

The prevalence and incidence of secondary osteoporosis varies depending on the patient population studied. Reports have suggested the prevalence of secondary osteoporosis to occur in <70% of males, >50% of premenopausal and perimenopausal females and ~20% of post-menopausal females (Painter et al., 2006). In a young cohort aged 20 to 44 years the incidence of fragility fracture reached 0.2 to 2.3 per 100,000 person-years depending on the underlying disease. Steroid-induced osteoporosis had the highest incidence while other causes included post-menopausal, delayed puberty, idiopathic osteoporosis, anticonvulsant, gastrointestinal conditions, alcoholism and anorexia nervosa. The overall incidence of osteoporosis in this younger population was estimated to be 4.1 per 100,000 person-years (Khosla et al., 1994).

In males, diseases or drugs known to affect bone metabolism account for a significant proportion of cases (50-80%) with fractures (Stein et al., 2003). The common causes in males include steroid-induced, alcoholism and androgen deficiency (Ebeling, 2008). In an older cohort (aged ≥50 years) with fragility fractures, the prevalence of known and newly diagnosed causes of secondary osteoporosis is thought to be ~23% and ~27% respectively (Bours et al., 2011). Common causes in this population include monoclonal proteinaemia, chronic renal failure, primary and secondary hyperparathyroidism, hyperthyroidism and hypogonadism (Bours et al., 2011).

3.2 Diabetes mellitus and bone fragility

3.2.1 Epidemiology of fractures

Type 1 Diabetes Mellitus (T1DM)

Studies in T1DM suggest that fracture risk is elevated across all ages at the hip, vertebral and non-hip, non-vertebral sites in both sexes relative to controls (Vestergaard et al., 2005; Weber et al., 2015).

Hip fractures

Studies have consistently observed an association between T1DM and hip fractures. Two meta-analyses in 2007 included similar studies and produced comparable
findings with a relative risk of 6.3 (95%CI 2.6-15.1) and 6.94 (95%CI 3.25-14.78) being reported respectively for hip fractures in T1DM (Janghorbani et al., 2007; Vestergaard et al., 2005). A more recent meta-analysis included the same studies as Janghorbani et al and yielded a relative risk of 5.76 (95% CI 3.66-9.07) (Fan et al., 2016). A meta-analysis published in 2015 reported a relative risk of 3.78 (95% CI 2.01-6.98) due to the addition of two studies which had not previously been included (Shah et al., 2015).

Two studies from the UK demonstrated increased risk of hip fracture throughout life in T1DM compared to non-diabetic controls. Weber et al used a medical record database to identify ~30,000 T1DM patients aged 0-89 years and 300,000 age- and sex-matched controls and followed them for ~4 years. Hip fracture risk was elevated across the different age groups and in both sexes with hazard ratio ranging from 1.99 to 5.64, however, the hazard ratio for males aged 0-29 years did not reach significance (Weber et al., 2015).

The retrospective cohort study by Hothersall et al used a national Scottish database to identify ~21,000 patients with T1DM aged 20-84 years and 3.66 million non-diabetic individuals. Hip fractures were sourced through a linked hospital admission database. Elevated hip fracture risk were seen in young adulthood through to old age in both sexes with the incidence rate ratio ranging from 1.79 to 8.92, however this did not reach significance in males with age range 50-59 and 80-84 years perhaps due to low fracture rates in these groups. In patients with diabetes compared controls, hip fracture rates were higher and increased with age and were greater in females than males with T1DM, reaching a peak of 15 per 1,000 person-years in males and 36 per 1,000 person-years in females in those aged 80-84 years (Figure 3.2.1) (Hothersall et al., 2014).
Figure 3.2.1 Incidence of hip fracture in patients with and without type 1 diabetes (Hothersall et al., 2014).

The Tromso study has also reported increased hip fracture risk in patients with T1DM, though, the number of T1DM patients were small (52 males and 29 females) and the hip fracture events low (3 in males and 1 in females) which explained the wide confidence intervals reported with the relative risk of 18.43 (95% CI 5.72-59.34) and 9.03 (95% CI 1.25-65.07) for male and female patients with T1DM respectively (Ahmed et al., 2006). A number of other studies also observed elevated hip fracture risk but had similar design flaws (Forsen et al., 1999; Janghorbani et al., 2006; Nicodemus et al., 2001).

Diabetic complications appeared to be associated with increased risk of hip fractures. The retrospective cohort study by Miao et al examined ophthalmic, nephropathic, neurologic and cardiovascular complications and found that complications markedly increased the risk of hip fracture from 20 to 42 fold (Miao et al., 2005). However, there may be selection bias to include patients with more severe disease since these patients needed to be hospitalized for T1DM to be identified and included in the study.
Vertebral fractures

Although the data are limited and the findings are inconsistent, there may be an increase in vertebral fracture risk in patients with T1DM. A recent meta-analysis reported that the pooled relative risk for spinal fracture is 2.88 (95% CI 1.71-4.82), however, only two studies were analyzed (Shah et al., 2015). One of which was a case control study, which reported increased odds ratio for spinal fracture of 2.48 (95% CI 1.33-4.62) in patients with T1DM (Vestergaard, 2007). No information was provided on fracture ascertainment. The other study involved small numbers with 82 patients with T1DM and controls. The presence of morphometric vertebral fracture was evaluated using DXA in conjunction with vertebral fracture assessment software and vertebral fracture was defined using the Genant classification. A greater prevalence of vertebral fracture was seen in patients with T1DM (24.5%) compared to controls (6.1%) (Zhukouskaya et al., 2013). In contrast, a small Canadian cohort study found no increased risk for vertebral ‘deformity’ that was evaluated using spinal x-rays (Hanley et al., 2003).

Non-hip, non-vertebral fractures

The few studies examining non-hip, non-vertebral fracture risk in patients with T1DM have reported inconsistent results. Vestergaard et al reported no increased risk for forearm fractures in patients with T1DM relative to controls (Vestergaard et al., 2005). Similarly, another study observed comparable prevalence of self-reported forearm fracture in patients with and without diabetes (Tuominen et al., 1999). In contrast, a study found a higher prevalence of self-reported upper limb fracture in patients with T1DM (22%) compared to controls (11%) (Neumann et al., 2011). Furthermore, increased risk of proximal humeral fracture has been reported in patients with ‘insulin treated’ diabetes with odds ratio 3.79 (95%CI 1.16-12.36) (Kelsey et al., 1992).

Type 2 Diabetes Mellitus (T2DM)

Although fracture risk is more modest compared to patients with T1DM, the relative risk of fractures is elevated in both sexes for hip, vertebral and non-hip, non-vertebral sites in patients with T2DM.
Hip fractures

Previous and recent meta-analyses have reported modest increases in hip fracture risk in patients with T2DM compared to non-diabetic controls with relative risks estimated at 1.34 to 1.7 (Fan et al., 2016; Janghorbani et al., 2007; Vestergaard, 2007).

In contrast, hip fracture risk estimates for individual studies vary significantly. A large retrospective study by Hothersall et al included ~180,000 patients with T2DM aged 40-84 years in comparison to 3.66 million people without diabetes. In men, there was no increase in hip fracture risk observed overall or when stratified by age group. While in women there was a slight increase in overall hip fracture risk with an incident rate ratio of 1.05 (95% CI 1.01-1.10). Stratification by age yielded no increased risk in the youngest (40-49 years old) and oldest (80-84 years old) age groups with those in between having incidence rate ratio between 1.06 to 1.21 (Figure 3.2.2) (Hothersall et al., 2014).

![Figure 3.2.2 Incidence of hip fracture in patients with and without type 2 diabetes](Hothersall et al., 2014)

A prospective study by Kim et al utilized a national health insurance database to identify ~17,000 patients aged ≥50 years with T2DM and ~34,000 controls and followed them for 6 years. Unlike the previous retrospective study, investigators found increased risk estimates of hip fracture in both sexes and across the age range from 50 to ≥80 years old. However, due to lower patient numbers in the oldest age
group (≥80 year olds) and low hip fracture events, the hip fracture risk estimate in this group did not reach significance (Kim et al., 2016). The hip fracture incidence in this patient population demonstrated an age-related increase in both sexes with peak rates reaching 70 and 48 per 10,000 person years in females and males respectively in those ≥80 years. However, overall the incidence reached 15 and 7 per 10,000 in females and males respectively.

Duration of T2DM may influence risk of hip fracture. The Nurses’ Health Study recruited >8,000 women with T2DM and >100,000 non-diabetic controls aged 34-70 years and were followed up for ~20 years. The reported incidence of hip fracture in women with and without diabetes was 153 and 59 per 100,000 person-years respectively. The risk of hip fracture in patients with T2DM was two fold higher compared to controls. Furthermore, longer duration of diabetes was associated with higher risk. As such, T2DM duration <5 years, 5-11 years and ≥12 years had the following relative risks reported of 1.7 (95%CI 1.2-2.4), 1.8 (95%CI 1.3-2.6) and 3.1 (95% CI 2.3-4.0) respectively (Table 3.2.1) (Janghorbani et al., 2006). Similar findings were also reported in other prospective studies (Koh et al., 2010; Nicodemus et al., 2001).

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of cases</th>
<th>Incidence/100,000 person years</th>
<th>Multivariate-adjusted relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetes</td>
<td>1255</td>
<td>59</td>
<td>1.0</td>
</tr>
<tr>
<td>Type 1 diabetes</td>
<td>18</td>
<td>383</td>
<td>6.4 (3.9 – 10.3)*</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>125</td>
<td>153</td>
<td>2.2 (1.8 – 2.7)*</td>
</tr>
<tr>
<td>Type 2 diabetes &lt;5 years</td>
<td>34</td>
<td>107</td>
<td>1.7 (1.2 – 2.4)*</td>
</tr>
<tr>
<td>Type 2 diabetes 5–11 years</td>
<td>34</td>
<td>127</td>
<td>1.8 (1.3 – 2.6)*</td>
</tr>
<tr>
<td>Type 2 diabetes ≥12 years</td>
<td>57</td>
<td>248</td>
<td>3.1 (2.3 – 4.0)*</td>
</tr>
</tbody>
</table>

Table 3.2.1 Incidence and relative risk of hip fracture stratified by diabetes type and duration of diabetes

Relative risk adjusted for age, BMI, physical activity, menopausal status, oestrogen use, smoking and daily intake of calcium, vitamin D and protein. *p<0.001 (Janghorbani et al., 2006).
Inconsistent results have been seen regarding the effect of insulin therapy on hip fracture risk in patients with T2DM. The Nurses’ Health Study found a higher incidence of hip fractures in patients with T2DM that have used insulin, which was associated with a three-fold increased risk of hip fractures compared to non-diabetic females (Janghorbani et al., 2006). Similar findings were also seen in the Iowa Women’s Health Study (Nicodemus et al., 2001). In contrast, insulin appeared to have no effect on hip fracture risk estimates in other studies (Ivers et al., 2001; Schwartz et al., 2001; Vestergaard et al., 2005).

Although limited data is available, diabetic complications do not appear to be associated with hip fracture risk in patients with T2DM. A large case control study in Denmark with ~124,000 fracture cases and ~373,000 controls evaluated the effect of diabetes and its associated complications on fracture risk. Following adjustments for confounders, no increased hip fracture risk was seen with diabetic macrovascular or microvascular complications (Vestergaard et al., 2009).

Vertebral fractures
A few studies have evaluated vertebral fracture risk in relation to patients with T2DM with their findings being inconsistent. A study used spinal x-ray and the Genant classification to ascertain prevalent vertebral fracture in ~300 individuals with T2DM and ~700 controls and found that T2DM was associated with increased risk of prevalent vertebral fractures with an odds ratio of 1.86 and 4.73 in women and men with T2DM respectively (Yamamoto et al., 2009).

Most studies did not have stringent criteria in vertebral fracture ascertainment with most relying on medical records, radiology reports or self-reporting, however increased vertebral fracture risk associated with T2DM was seen in some (Bonds et al., 2006; Melton et al., 2008) but not all studies (Hanley et al., 2003; Schwartz et al., 2001; Vestergaard et al., 2005).

Non-hip, non-vertebral fractures
Although there is a paucity of data, T2DM may be associated with increased risk of non-hip, non-vertebral fractures. Increased fracture risk was seen at the radius (Oei et
al., 2013; Vestergaard et al., 2005), proximal humerus (Ivers et al., 2001; Melton et al., 2008; Schwartz et al., 2001), ribs (Melton et al., 2008) and foot (Bonds et al., 2006; Melton et al., 2008) in patients with T2DM. However, not all studies have reported increased fracture risk, which may be partly due to low patient numbers (Gerdhem et al., 2005).

The findings on fracture risk, fracture prevalence and incidence vary greatly due to the heterogeneity of the study population. Many studies classified diabetes type based on self-reporting (Hanley et al., 2003; Janghorbani et al., 2006; Meyer et al., 1993; Nicodemus et al., 2001; Seeley et al., 1996). While other studies did not distinguish between type 1 and type 2 diabetes (Chen et al., 2008; Giangregorio et al., 2012; Koh et al., 2010; Lipscombe et al., 2007; Meyer et al., 1993). In addition, some studies did not specify how they defined diabetes (Liao et al., 2014). Many of the studies have small numbers of diabetic patients. Moreover, the fracture event rates were low, resulting in wide confidence intervals. Fracture ascertainment in many studies relied on self-reporting, which will cause under reporting for prevalent vertebral fracture.

3.2.2 Material and structural basis of bone fragility
Abnormalities in the material composition of bone may contribute to bone fragility in patients with diabetes mellitus. Bone material properties contribute substantially to the fracture toughness of bone, that is the ability of bone to withstand damage initiation and propagation. Recent advances have allowed bone material properties to be measured using reference point indentation. Two techniques that have been used include cyclic and impact indentation. The more recently developed impact indentation method allows direct measurements in-vivo and has been used in clinical research. The device calculates the distance that a probe enters the bone for a given force. The deeper the bone penetration, the lower the fracture resistance. The measurement is known as bone material strength index and is the ratio between the probe penetration into bone and penetration into a reference phantom (Herrera et al., 2017). Hence, the tool may be used as a marker of fracture toughness in-vivo (Figure 3.2.3).
Only a few studies have used reference point indentation tools to assess bone material properties in T1DM. Pre-clinical studies have compared wild type mice to mice models of severe early onset T1DM and have observed increased AGEs levels in bone using Raman microspectroscopy associated with reduced bone toughness as well as increased indentation distance using cyclic microindentation in mice models of T1DM (T1DM 9.04 ± 0.77 vs. WT 6.85 ± 0.44 μm, p<0.05) (Rubin et al., 2016). Similar findings were reported using microindentation in middle age men with T1DM compared to men without T1DM. There was ~5% reduction in bone material strength index in men with T1DM compared to those without (Syversen et al., 2017). Reduced tissue mineral density may also contribute to bone fragility in patients with T1DM. Bone mineral content (ash weight, ash density) correlates with biomechanical properties of cortical bone (Currey, 1969; Currey, 1986; Currey, 1999). Both elastic
modulus and strength of cortical bone were positively associated with mineral content. In contrast, energy absorption in cortical bone is reliant on the extent of mineralization. Hence, it may increase if the bone is under-mineralized or decrease if the bone is fully mineralized (Turner, 2002).

A pilot study assessed 16 adults (18-30 years of age) with T1DM diagnosed pre-puberty and controls using HR-pQCT. Cortical tissue mineral density was low at the radius and tibia though cortical vBMD was only reduced at the radius. Unadjusted biomechanical indices measured using micro-finite element analysis was similar in patients and controls, however when reduced cortical tissue mineral density was accounted for there was a significant reduction (Long et al., 2017).

A subset of patients from the histomorphometry study by Armas et al had their bone biopsy specimens further analyzed to evaluate degree of mineralization and collagen cross-linking (enzymatic and non-enzymatic – pentosidine) as well as bone mechanical properties in three groups: patients with T1DM that have fractured (n=5), patients with T1DM that have not had fractures (n=5) and healthy controls (n=5). Collagen cross-linking was measured using high performance liquid chromatography. Pentosidine was found to be higher in trabecular bone in patients with fractures compared to controls. Whereas patients without fractures had similar levels of pentosidine to controls. Measurements of enzymatic cross-links were no different between the three groups of patients. Degree of mineralization was assessed using digitalized microradiography and was observed to be higher in trabecular bone in patients with fractures compared to patients without fractures and controls. Hardness was found to be no different between the groups using microindentation and nanoindentation (Farlay et al., 2016).

Type 2 Diabetes Mellitus
One study that made use of impact microindentation in T2DM clinical research recruited 60 post-menopausal women with and without longstanding T2DM. The diabetic cohort appeared to have significantly reduced bone material strength index (-10.5%) despite comparable bone structure to non-diabetic women measured using HR-pQCT. Furthermore, higher mean glycated haemaglobin over the past 10 years was associated with lower bone material strength index ($r = -0.41$) (Farr et al., 2014).
Similarly, a small study comparing post-menopausal women with longstanding T2DM (n=16) to matched controls (n=19) found ~9% lower bone material strength index in the diabetic cohort which negatively correlated with duration of diabetes (r = -0.68). Cortical vBMD and porosity as well as trabecular micro-architecture evaluated using HR-pQCT in the diabetic cohort were overall comparable to controls. Skin autofluorescence was used as a marker for advanced glycation end products (AGEs) in the skeleton and was found to be increased in patients with diabetes and inversely correlated to bone material strength index (r = -0.65) in the diabetic and not in the control cohort (Furst et al., 2016). A larger study using elderly women (>75 years) with and without T2DM reported higher cortical vBMD and area as well as trabecular bone volume using HR-pQCT associated with reduced bone material strength index in post-menopausal women with T2DM compared to non-diabetic controls (Figures 3.2.4 and 3.2.5) (Nilsson et al., 2017).

![Figure 3.2.4 Trabecular morphology in patients with and without type 2 diabetes](image)

(A) Bone volume fraction, (B) trabecular number, (C) trabecular thickness and (D) trabecular separation. Bars represent mean ± standard error following adjustment for age, BMI, smoking, steroid use, bone active drug use, calcium intake, physical activity. *p<0.05, **p<0.001 (Nilsson et al., 2017).
Figure 3.2.5 Cortical morphology in patients with and without type 2 diabetes

(A) Cortical vBMD, (B) cortical cross-sectional area (CSA) and (C) bone material strength index in individuals with and without type 2 diabetes mellitus (T2DM). Bars represent mean ± standard error following adjustment for age, BMI, smoking, steroid use, bone active drug use, calcium intake, physical activity. *p<0.05, **p<0.001 (Nilsson et al., 2017).

Limited studies have assessed tissue mineralization density in patients with T2DM using HR-pQCT and report conflicting results. Since bone turnover is thought to be low in patients with T2DM, higher tissue mineralization density would be expected. One small study involving post-menopausal African American women with and without T2DM has found reduced cortical tissue mineral density associated with cortical deficits (Yu et al., 2015). In contrast, another small study using a more racially diverse cohort of elderly women with and without T2DM reported no difference in cortical tissue mineral density accompanied with increased cortical porosity (Burghardt et al., 2010). Both studies did not report bone remodelling markers.

Advanced glycation end products

The deficits in bone material properties seen in diabetes may be partly attributable to alterations in collagen cross-linking. Collagen cross-link plays a key role in
determining bone strength. There are two types of cross-links: (1) enzymatic and (2) non-enzymatic (i.e. advanced glycation end products, AGEs). Enzymatic cross-links are needed to maintain bone strength and are closely regulated by enzymes lysyl oxidase and lysine hydroxylase. Diabetes is associated with high levels of homocysteine which can impair enzymatic cross-linking as hyperhomocysteaemia is associated with low levels of vitamin B6, an important co-factor for lysyl oxidase (Saito et al., 2013).

In contrast, non-enzymatic cross-links impair bone strength. The non-enzymatic glycation or oxidation of protein forms AGEs (Figure 3.2.6). Chronic hyperglycaemia and ageing may accelerate this process. Non-enzymatic cross-links are irreversible and are generated at the expense of enzymatic cross-links since both are produced at the same sites. The slow turnover of collagen causes AGEs to accumulate which impairs both bone material properties as well as bone biomechanical properties (Leslie et al., 2012). Diabetic rat models exhibited low vitamin B6 levels, which coincided with reduction in enzymatic cross-links, elevation in formation of AGEs, impairment in bone strength though no difference in aBMD compared to controls (Saito et al., 2006). Studies incubating bone in ribose to promote non-enzymatic cross-linking have found that ribosylated bone specimens had higher AGEs levels as well as reduced post-yield strain and energy compared to controls (Tang et al., 2007; Vashishth et al., 2001).

Reactions between carbohydrate and free amino groups of proteins form a Schiff base which re-assembles to create a more stable molecule leading to the formation of non-enzymatic cross-links between collagen (Saito et al., 2013).

Advanced glycation end products and the interaction with their receptor, RAGE, which is found on osteoblasts, have been demonstrated to impair osteoblast
differentiation, function and attachment to collagen in-vitro (Kume et al., 2005; McCarthy et al., 2004; Sanguineti et al., 2008). However, the influence of AGEs on osteoclast function remains unknown.

Pentosidine is a non-enzymatic cross-link and is used as a marker of AGEs in bone. In animal models and patients with T2DM, pentosidine levels are elevated compared to non-diabetic controls (Okazaki et al., 1997; Saito et al., 2006). A number of studies have observed the association between high pentosidine levels and fractures. Elevated serum pentosidine levels were seen in post-menopausal females with T2DM and vertebral fractures compared to diabetic females without vertebral fractures, which were ascertained by spinal x-ray (Yamamoto et al., 2008). Similar results were seen with urinary pentosidine and clinical fracture incidence in older men and women with T2DM (Schwartz et al., 2009). In addition, patients with T1DM were observed to have increased serum pentosidine levels with prevalent fractures compared to diabetic patients without fractures (Neumann et al., 2014).

*Type 1 Diabetes Mellitus*

A number of studies using peripheral quantitative computed tomography (pQCT) have observed smaller bone size in children and adolescents with T1DM compared to controls at the radius, tibia or both sites. Most studies have not stratified patients according to onset of diabetes in relation to pubertal status.

During growth, there are differences in the rate of axial and appendicular growth as well as differences in the growth of bone types, bone regions and bone surfaces depending on pubertal stage. Hence, depending on the age of onset of diabetes; deficits may occur in the appendicular sites if pre-pubertal onset, axial sites if early pubertal onset or vBMD if late pubertal onset.

A study by Bechtold et al evaluated 88 patients with age of onset of diabetes ranging from 0.7 – 14.6 years and mean disease duration ~6 years. These children were stratified into pubertal status at the time of analysis. At the radius, pre-pubertal (Tanner stage 1) children had low total and cortical cross-sectional areas. While, children in early puberty (Tanner stage 2 and 3) had low total cross-sectional area
only. Adolescents (Tanner stage 4 and 5) had normal bone size. Total vBMD was no different to reference population in all three groups. Early onset of T1DM was associated with smaller bones (Bechtold et al., 2006). Similar findings at the radius using pQCT were seen in another study using adolescents and young adults with pre-pubertal onset of diabetes (Roggen et al., 2013).

Other studies have observed small bone size at the tibia using pQCT and lumbar spine using DXA in adolescents aged 12-18 years with average 4 years duration of diabetes (Moyer-Mileur et al., 2004; Saha et al., 2009). Prospective studies have reported conflicting results with one study indicating normal bone size after 5-6 years follow-up while another study with only a short follow-up of 12 months reported that despite similar growth with height, weight and BMI there was persistence of smaller bone size compared to controls (Bechtold et al., 2007; Moyer-Mileur et al., 2004; Saha et al., 2009).

The findings for trabecular and cortical volumetric density using pQCT in children and adolescents are inconsistent, in part due to the heterogenous patient population and small patient numbers. Results for trabecular and cortical vBMD have been reported as low, high or normal compared to non-diabetic controls (Bechtold et al., 2006; Bechtold et al., 2007; Moyer-Mileur et al., 2004; Roggen et al., 2013; Saha et al., 2009).

Studies in children and adolescents with T1DM using pQCT have suggested reduced estimates of bone strength. Reductions in bone strength indices of 5-17% associated with small bone sizes at both the radius and tibia have been observed in patients compared to controls (Saha et al., 2009). Furthermore, a prospective study suggested that the lower estimated bone strength persisted after 12 months of follow-up (Moyer-Mileur et al., 2004).

Microvascular complications may be a risk factor for bone fragility in patients with T1DM. A study in adults with T1DM used HR-pQCT to evaluate the effects of microvascular complications on bone traits. At the radius, in comparison to controls, middle aged adults with T1DM and microvascular complications exhibited increased total and trabecular cross-sectional areas. These patients also had reduced total,
cortical and trabecular vBMD due to a trend towards fewer and thinner trabeculae and greater trabecular separation with loss of connectivity. The estimated bone strength was comparable between patients and controls, perhaps due to patients having larger bones (Shanbhogue et al., 2015).

Histomorphometry studies in T1DM are scarce and the results are conflicting. The study by Armas et al observed no differences when they examined iliac biopsies using bone histomorphometry (following tetracycline labeling) and micro-CT from patients with uncomplicated, longstanding T1DM and compared them to age and sex matched controls. However, with only 18 patients in each group, the study is likely to have been underpowered to detect differences (Armas et al., 2012). Another study involved 8 patients with diabetes (T1DM n=2, T2DM n=6), the two men with T1DM had longstanding disease (~30 years) with microvascular complications. The study reported a low mean bone formation rate for the combined diabetes cohort relative to premenopausal women who were used for comparison (3.37 ± 3.45 vs. 14.2 ± 6.20 μm³/μm²/year) (Krakauer et al., 1995).

Type 2 Diabetes Mellitus

Individuals with type 2 diabetes mellitus appear to have cortical deficits with preserved or increased trabecular bone relative to non-diabetic controls using HR-pQCT. A study using post-menopausal females (n=19) with and without T2DM found increased cortical porosity and cortical pore volume at the radius and tibia, though significance at the tibia was not reached. However, cortical vBMD and tissue mineral density were comparable to controls. Biomechanical deficits associated with cortical porosity was found to be increased using micro-finite element analysis. In contrast, trabecular vBMD adjacent to the cortex was increased suggesting trabecularization of the cortex (Burghardt et al., 2010). The elevated or normal trabecular vBMD may be related to erroneous segmentation of cortical from trabecular bone with cortical fragments included as ‘trabecular’ bone.

Cortical deficits and preserved or increased trabecular bone were also reported in other studies of patients with T2DM and are likely to reflect errors in segmentation. For example, post-menopausal women with T2DM and fragility fractures had
preserved trabecular bone and reduced cortical vBMD due to increased cortical porosity as well as biomechanical deficits associated with cortical porosity compared to women with T2DM without fractures (Patsch et al., 2013). Patients with T2DM and microvascular complications also had comparable trabecular vBMD and micro-architecture with reduced cortical thickness and cortical vBMD due to increased cortical porosity compared to non-diabetic controls. In contrast, estimated bone strength was no different between patients and controls (Shanbhogue et al., 2016).

African American post-menopausal women with T2DM (n=22) had preserved trabecular bone with reduced cortical vBMD due to increased cortical porosity, and decreased cortical tissue mineral density. Estimated bone strength was comparable between African American women with and without diabetes (Yu et al., 2015). An older diabetic cohort in the UK had preserved trabecular bone accompanied with increased or tendency to increase cortical porosity compared to controls. However, the type of diabetes was not defined in the study (Paccou et al., 2016).

Not all studies have reported cortical deficits and preserved trabecular bone. Some studies did not identify any difference between patients with and without T2DM. This may be due to the small number of participants (Farr et al., 2014; Shu et al., 2012). Other studies observed better bone structure in patients with T2DM (Nilsson et al., 2017). Furthermore, studies using pQCT were unable to assess cortical porosity (Melton et al., 2008; Petit et al., 2010).

### 3.2.3 Abnormalities in bone remodelling

There is evidence to suggest that diabetes mellitus is associated with a state of low bone formation. Recent meta-analyses have reported decreased markers of bone turnover in patients with diabetes (Hygum et al., 2017; Starup-Linde et al., 2014). The pooled analyses combining studies in both T1DM and T2DM reported lower levels of bone formation markers osteocalcin and procollagen type I N-terminal propeptide (PINP) and bone resorption marker C-terminal telopeptide of type I collagen (CTx) compared to non-diabetic controls. Subgroup analysis by diabetes type have found osteocalcin to be substantially lower in T1DM and modestly lower in T2DM patients compared to controls.
Bone formation marker osteocalcin appears to be lower in male and female patients with T1DM throughout most of the lifespan compared to non-diabetic controls. A study involving 104 diabetic and 420 healthy children (aged 5 – 20) and 229 diabetic and 125 healthy adults (aged 21 – 69) observed effects of diabetes, age and sex on osteoblast activity reflected by levels of osteocalcin, skeletal alkaline phosphatase (ALP), total ALP and carboxy-terminal propeptide of type 1 collagen (P1CP). In children with diabetes, osteocalcin levels were lower compared to controls, peaking at 12-14 years of age in both sexes and decreased similarly in both sexes (27-29%) with diabetes during puberty. In adults with diabetes, osteocalcin levels were also lower compared to controls. The levels gradually declined in adulthood (Figure 3.2.7) (Bouillon et al., 1995).
Males (A) and females (B) with type 1 diabetes mellitus (shaded bar) and without (non-shaded bar) (Bouillon et al., 1995).

In children and adults with T1DM, total ALP levels were higher compared to controls, peaking in early to mid-puberty and decreased to adult levels which then stabilized thereafter. In children with diabetes, skeletal ALP levels were lower compared to healthy children and declined to adult levels. Skeletal ALP levels in adult men with diabetes were comparable to controls, levels in adult women with diabetes were marginally higher compared to controls. In contrast, no difference in P1CP levels between individuals with and without diabetes was seen. Higher levels were observed in children compared to adults, peaking earlier in girls (10-12 years) compared to boys (14-16 years) (Bouillon et al., 1995).

Similar results for osteocalcin were found in other studies involving pre-pubertal and pubertal children and adults with T1DM compared to controls (Abd El Dayem et al., 2011; Danielson et al., 2009; Karaguzel et al., 2006; Maggio et al., 2010; Neumann et al., 2011; Shanbhogue et al., 2015). Osteocalcin is negatively correlated to Hba1c and
duration of diabetes (Abd El Dayem et al., 2011; Brandao et al., 2007; Danielson et al., 2009; Maggio et al., 2010; Shanbhogue et al., 2015).

Patients with T2DM have reduced or similar levels of osteocalcin to non-diabetic controls. Younger patients with T2DM (35 – 44 years) have lower osteocalcin, higher total ALP, similar skeletal ALP and P1CP levels to controls. Whereas, older patients with T2DM (45 – 55 years) have comparable osteocalcin, higher total ALP, similar skeletal ALP and P1CP levels compared to controls (Bouillon et al., 1995). While elderly patients (>70 years) with T2DM in nursing homes have lower levels of osteocalcin compared to controls. When patients were stratified by diabetes treatment (diet vs. oral anti-diabetic agents vs. insulin) only patients receiving oral anti-diabetic agents and insulin had lower levels of osteocalcin compared to controls. Furthermore, osteocalcin levels negatively correlated with Hba1c (Dobnig et al., 2006).

The findings are more inconsistent for markers of bone resorption in patients with T1DM and T2DM, which are either reduced or no different to non-diabetic controls in the majority of studies (Starup-Linde, 2013; Starup-Linde et al., 2014). The conflicting results may be due to the heterogeneity between studies in terms of patient characteristics, diabetes duration, glycaemic control, the use of different assays to measure bone turnover and the differences in glucose levels at the time of assessment, which are all potential confounders. Furthermore, not all studies have included renal function nor have blood taken in the fasting state.

**3.2.4 Local and systemic factors influencing bone fragility**

*Adipokines*

The impairment in bone turnover seen in patients with diabetes mellitus may be partly due to abnormalities in adipokine production.

Adiponectin is secreted exclusively by adipocytes. Adiponectin appears to stimulate osteoblastogenesis and suppress osteoclastogenesis (Williams et al., 2009). Reduce levels of adiponectin was seen in individuals with T2DM. Furthermore, a positive relationship has also been observed between adiponectin and aBMD at the distal
radius and not at other sites (Tamura et al., 2007). Though this finding has not been observed in other studies (Lenchik et al., 2003; Napoli et al., 2010).

Sclerostin
The association between low bone formation and diabetes mellitus may in part be attributable to increased sclerostin levels. Sclerostin is produced by osteocytes and inhibits bone formation via the Wnt/β-catenin signaling pathway. A recent meta-analysis has reported high levels of sclerostin in patients with T1DM and T2DM compared to non-diabetic individuals, though the number of studies was small (Hygum et al., 2017). Suppression of sclerostin by PTH in healthy individuals was not observed in patients with T1DM nor T2DM. Moreover, there was a trend for a positive correlation between sclerostin and PTH in both T1DM and T2DM patients (Gennari et al., 2012).

High levels of sclerostin associated with low β-catenin levels in post-menopausal women with T2DM compared to controls have been reported (Figure 3.2.8) (Gaudio et al., 2012). Elevated sclerostin levels were also found in patients with T2DM and vertebral fractures (Yamamoto et al., 2013). Post-menopausal women with T2DM and prevalent fractures exhibited lower femoral neck vBMD associated with thinner cortices using QCT and elevated serum sclerostin levels compared to controls (Heilmeier et al., 2015). These findings are suggestive that sclerostin may play a role in reducing bone formation in diabetes.
Figure 3.2.8 Correlation between serum sclerostin and β-catenin in patients with (A) and without type 2 diabetes (B) (Gaudio et al., 2012).

**Insulin**

Evidence suggests that insulin has anabolic effects on bone. A large number of insulin receptors are found on the cell surface (Pun et al., 1989). Insulin deficiency may negatively impact bone formation, material composition of bone and bone strength. Insulin deficient rodents exhibited impaired fracture healing, deficits in the biomechanical properties of the fracture callus, decreased cell proliferation and decreased collagen content in the callus (Funk et al., 2000; Macey et al., 1989; Spanheimer, 1992). Short-term spontaneously diabetic rats (3-4 weeks) had reduced histological indices of bone formation (osteoblast surface, osteoid surface and bone mineral apposition rate) associated with lower serum osteocalcin levels compared to healthy rodents. Though no difference was seen with bone volume in the tibia and the lumbar vertebrae or biomechanical properties between rodents with and without diabetes (Verhaeghe et al., 1989).

Long-term spontaneously diabetic rats (>12 weeks) had reduced histological parameters of bone formation as well as reduced serum osteocalcin levels associated with diminished bone volume and impaired strength related properties relative to controls (Verhaeghe et al., 1990; Verhaeghe et al., 1990). Other long-term (~12 months) diabetic models appear to have deficits in bone mineralization associated with impairments in perfection of hydroxyapatite crystal, calcium to phosphate
composition of ash and ash content compared to non-diabetic animals. Corroborative evidence for the anabolic effects of insulin was observed when insulin administration in insulin deficient models appear to preserve the histomorphometric, biomechanical and biochemical bone formation indices relative to controls (Figure 3.2.9) (Hou et al., 1993; Thrailkill et al., 2005). Furthermore, in-vitro studies have shown that insulin was able to stimulate osteoblast proliferation, collagen production and alkaline phosphatase activity (Canalis, 1983; Hashizume et al., 1993; Thomas et al., 1996).

![Figure 3.2.9 Impaired bone formation in diabetes model](image)

(A) Radiographic and (B) histological measurement of new bone formation during distraction osteogenesis in non-diabetic, vehicle treated diabetic and insulin treated diabetic models (Thrailkill et al., 2005).

However, there is some evidence to suggest that reduced insulin signaling alone may not explain the bone loss and low bone formation seen in T1DM. A study used a mouse model with knockout of insulin receptor rescued by human insulin receptor transgene expression in the brain, liver and pancreas but not the bone, which caused the mice to be euglycaemic and allowed researchers to study the impact of reduced...
insulin signaling in bone without the confounding effects of hyperglycaemia. Compared to wild-type mice, knockout mice were observed to have similar trabecular vBMD and micro-architecture as well as similar cortical vBMD and thickness. In addition, bone formation and bone resorption markers were comparable in both groups. Increased insulin as well as IGF-1 receptor in bones were seen which suggests that signaling through insulin receptor or IGF-1 receptor is needed for optimal bone development (Irwin et al., 2006).

Hyperinsulinaemia and insulin resistance predominates the early phase of T2DM. Clinical studies have observed a positive relationship between insulin levels and aBMD (Haffner et al., 1993; Stolk et al., 1996). Furthermore, a negative association was seen between insulin sensitivity index (measured using oral glucose tolerance test) and aBMD, independent of BMI (Abrahamsen et al., 2000). Animal models have observed a negative influence of insulin resistance on bone through abnormalities in insulin signaling in osteoblasts (Fulzele et al., 2010; Lee et al., 2004).

Longstanding T2DM is characterized by relative insulin deficiency, hyperglycaemia and ageing associated with development of advanced glycation end products which have already been discussed.

Hyperglycaemia

Hyperglycaemia impairs osteoblast differentiation, proliferation and function. A number of different mechanisms are thought to be involved including inhibition of genes involved with osteoblast differentiation, suppression of Wnt/β-catenin signaling and stimulating peroxisome proliferator activated receptor γ (PPARγ) (Gopalakrishnan et al., 2006; Hie et al., 2011; McCabe, 2007; Merlotti et al., 2010).

Clinical, animal and in-vitro studies have suggested that bone formation may be impaired by hyperglycaemia. A number of clinical studies have reported a negative association between bone formation markers and glucose level or Hba1c. Animal studies have shown that acute as well as chronic hyperglycaemia inhibit the expression of genes involved with osteoblast differentiation in models of diabetes (McCabe, 2007; Merlotti et al., 2010). In-vitro studies have demonstrated that
hyperglycaemia impairs the differentiation of bone marrow stem cells into osteoblasts, bone marrow stem cell proliferation, alkaline phosphatase activity, collagen matrix deposition and mineralized collagen nodule formation. However, insulin administration partly reversed some of the deficits (Gopalakrishnan et al., 2006).

Chronic hyperglycaemia stimulates peroxisome proliferator activated receptor γ (PPARγ) activation. PPARγ is a transcription factor that is involved in adipogenesis and promotes adipogenesis over osteoblastogenesis in bone marrow mesenchymal stem cells. In-vivo studies have observed elevated bone marrow PPARγ2 activity and higher bone marrow adiposity in T1DM models (Diascro et al., 1998; McCabe, 2007). However, it remains uncertain as to whether marrow adiposity actually causes skeletal deficits in T1DM since suppressing PPARγ led to reduced bone marrow adiposity and lipid levels but did not preclude bone decay in mouse models (McCabe, 2007).

3.2.5 Effect of treatment on bone fragility
There is accumulating evidence to suggest that diabetic therapies can affect bone metabolism and influence fracture risk.

Insulin
Although insulin has anabolic effects on bone, some studies have suggested that insulin therapy may be an indicator of disease severity in patients with T2DM, while other studies have found insulin was associated with frequent falls and elevated fracture risk (Ivers et al., 2001; Melton et al., 2008; Schwartz et al., 2001). Furthermore, falls in this group of patients may be due to hypoglycaemia (Lipscombe et al., 2007; Melton et al., 2008).

Metformin
Metformin may work through 5’ adenosine monophosphate activated protein kinase (AMP-activated protein kinase) activation. The AMP-activated protein kinase is expressed in osteoblasts and osteoclasts. Metformin may induce mesenchymal stem cells to differentiate into osteoblast through increase expression of osteoblast transcription factor Runx2 and activation of AMP-activated protein kinase.
Metformin administration appears to promote osteoblastogenesis as demonstrated with increased ALP activity, type 1 collagen synthesis, osteocalcin expression and mineral deposition of mesenchymal stem cells (Figure 3.2.12). Studies assessing the association between different diabetic treatment options and fracture risk indicated that metformin may have a favourable or neutral effect on bones (Borges et al., 2011; Kahn et al., 2006; Monami et al., 2008; Vestergaard et al., 2005).

Figure 3.2.10 Effect of metformin on bone marrow stem cell differentiation.

Bone marrow stem cells from control or metformin treated animals were incubated in osteogenic medium for 15-21 days following which alkaline phosphatase activity (A), type 1 collagen (B), osteocalcin expression (C) and mineralization nodules were measured. Data expressed as mean ± SEM. *p<0.05 (Molinauerto et al., 2010).

Sulphonureas

There are limited studies available on the effects of sulphonureas on bone. Sulphonureas have either a favourable or neutral impact on bones. Reports have linked sulphonureas to reduction in all fractures, hip fractures and vertebral fractures or no effect on fracture (Vestergaard et al., 2005) (Kanazawa et al., 2010; Monami et al., 2008).
**Thiazolidinediones**

Thiazolidinediones are peroxisome proliferator-activated receptor (PPAR-\(\gamma\)) agonists. PPAR-\(\gamma\) is expressed in a number of different cells. In bones, PPAR-\(\gamma\) is a nuclear transcription factor that promotes adipogenesis over osteoblastogenesis in mesenchymal stem cells (Zhu et al., 2014). Whereas, the findings on the effects of PPAR-\(\gamma\) on osteoclastogenesis are inconsistent (Mbalaviele et al., 2000; Okazaki et al., 1999).

Thiazolidinediones are associated with bone decay and increased fragility, which in part may be due to impaired bone formation (Grey et al., 2007; Kawai et al., 2010; Loke et al., 2009; Schwartz, 2008; Schwartz, 2009). A recent meta-analysis analyzed ~24,500 participants and ~900 fracture cases in 22 randomised controlled trials and reported a doubling of fracture risk with thiazolidinediones in female but not in male diabetic patients. The fracture risk was similar for rosiglitazone or pioglitazone (Zhu et al., 2014).

**Glucagon-like peptide (GLP-1) agonists and dipeptidyl peptidase (DPP-4) inhibitors**

Animal studies suggest that incretins may have an anabolic effect on bones (Kim et al., 2013; Nuche-Berenguer et al., 2009). A recent meta-analysis evaluating ~11,000 participants in 16 randomised controlled trials investigated the relationship between fractures and GLP-1 analogues compared to placebo or other therapies. Overall, the findings suggest that there is no increased fracture risk with GLP-1 analogue. However, the results were inconsistent when individual GLP-1 analogues (liraglutide and exenatide) were examined separately.

Liraglutide was associated with a lower risk of fractures (OR 0.38, 95% CI 0.17 – 0.87). Whereas exenatide was associated with higher risk (OR 2.09, 95% CI 1.03 – 4.21) compared to placebo or active therapies. The primary outcome was not fractures in the individual studies and fractures were analyzed as an adverse event. The studies had short follow-up to appropriately assess fracture outcome. The fracture events were low (20 in the intervention vs. 23 in the comparator group). Moreover, individuals in the exenatide cohort exhibited a trend towards weight gain, higher HbA1c and hypoglycaemia, which may influence fracture risk. Falls were no different.
between the two GLP-1 analogues, however not all studies reported on falls (Su et al., 2015).

Another meta-analysis examining ~12,000 participants on DPP-4 inhibitors and ~9000 participants on placebo or active treatment in 28 randomised controlled trials reported lower fracture risk associated with DPP-4 use in comparison to placebo or active treatment (OR 0.6, 95% CI 0.37 – 0.99). Similar limitations to the previous meta-analysis were observed with fractures reported as adverse events not primary end-points, studies were of short duration and fracture event rates were low (Monami et al., 2011).

Sodium-glucose co-transporter type 2 inhibitors
Sodium-glucose co-transporter type 2 (SGLT2) is found in the proximal tubule and facilitates re-absorption of ~90% of the filtered glucose load (Figure 3.2.13) (Jabbour, 2014). SGLT2 inhibitors induce urinary glucose excretion (Clar et al., 2012). In rodent models high doses (>150mg/kg/day) of dapagliflozin was associated with increased trabecular bone formation on histomorphometry, increased soft tissue mineralization, elevated aBMD and increased strength estimates (Tirmenstein et al., 2013).

Figure 3.2.11 Renal handling of glucose.
The majority of glucose is reabsorbed in the proximal tubule by sodium glucose co-transporter 2 (Jabbour, 2014).

In a randomized controlled trial of human subjects during 50 weeks and 102 weeks (extension) evaluating skeletal outcomes with dapagliflozin, no difference in bone remodelling markers, aBMD and fracture incidence were found compared to placebo (Bolinder et al., 2014). Whereas another randomized controlled trial assessing glycaemic control as the primary end-point included patients with T2DM and moderate renal failure reported increased fractures in the dapagliflozin group (13 fractures) compared to placebo (0 fracture) during 104 weeks of follow-up. Five fractures occurred on the 5mg dose and 8 fractures on the 10mg dose, suggesting a possible dose dependent relationship (Kohan et al., 2014). The findings communicated to a US Food and Drug Administration advisory committee, indicated that canagliflozin was associated with 35% greater fracture risk which mainly involved fractures of the upper limb and vertebrae (Shanbhogue et al., 2016).

**Summary**
The risk of hip, vertebral and non-hip, non-vertebral fractures is elevated in individuals with type 1 diabetes mellitus and type 2 diabetes mellitus. As T1DM commonly occurs during growth, children with T1DM appear to have smaller bone size compared to their peers. Whereas, adults with T2DM have cortical deficits, namely increased cortical porosity with preserved or increased trabecular bone. Deficits in bone material properties are evident using reference point indentation, which may be attributable to increased formation of advanced glycation end products. Furthermore, both biochemical and histomorphometric indices have indicated that diabetes is associated with a state of low bone formation. In diabetes, the mechanisms contributing to bone fragility are complex and not completely understood but a number of local and systemic factors have been implicated which include: adipokine dysregulation, hyperglycaemia, and abnormal levels of insulin and sclerostin. Additional factors may include anti-diabetic treatment causing hypoglycaemia and falls as well as having direct effects on bone metabolism.
3.3 Primary hyperparathyroidism and bone fragility

3.3.1 Epidemiology of fractures in primary hyperparathyroidism

Patients with primary hyperparathyroidism have increased fracture risk. Since this condition commonly affects post-menopausal females, increasing age and female sex appears to be strongly associated with fracture risk (Khosla et al., 1999). Some studies have suggested increased fracture risk occurs up to 10 years prior to the diagnosis (Melton et al., 1992; Vestergaard et al., 2003). While other studies have found the fracture risk was increased prior to but not after surgical treatment (Vestergaard et al., 2000).

Hip fractures

Although studies are scarce, there does not appear to be increased hip fracture risk in patients with primary hyperparathyroidism. One study evaluated hip fracture outcome specifically. The prospective study by Larsson et al involved 1373 females with mean age 63 years and 551 males with mean age 54 years. These patients were identified through an electronic register following hospital admission for primary hyperparathyroidism and followed up for a mean 17 years. In females, 67 hip fractures were reported which yielded a relative risk of 0.93 (95% CI 0.72 – 1.19). Whereas, in males only 11 fractures were seen which resulted in a relative risk of 1.39 (95% CI 0.69 – 2.50), however, the 95% CI for this estimate includes unity (Larsson et al., 1993). Despite this being one of the largest study assessing fracture risk in primary hyperparathyroidism, there still may be insufficient power to detect increased fracture risk.

Similarly, other studies have not observed elevated hip or proximal femoral fracture risk in patients with primary hyperparathyroidism (Kenny et al., 1995; Khosla et al., 1999; Melton et al., 1992; Vestergaard et al., 2000; Vestergaard et al., 2003). Many of these studies also assessed fracture risk at multiple other sites.

Vertebral fractures

Increased vertebral fracture risk has been reported in patients with primary hyperparathyroidism. Most studies have used spinal x-rays to detect morphometric
vertebral fractures (Dauphine et al., 1975; De Geronimo et al., 2006; Kaji et al., 2005; Kochersberger et al., 1987; Wilson et al., 1988). However, one study used lateral scans of the vertebrae acquired using DXA and a software program provided by the manufacturer (vertebral fracture assessment) to identify vertebral fractures using the Genant method. The accuracy, sensitivity and specificity of this technique reported by the authors were 92%, 82% and 97% respectively compared to x-ray. Using this method, a greater prevalence of vertebral fractures was observed in post-menopausal women with primary hyperparathyroidism compared to aged-matched controls (24.6% vs. 4.0%). Subgroup analysis revealed that the prevalence was higher in symptomatic than asymptomatic patients, though this did not reach significance. The prevalence was also greater in those that met the criteria for surgical treatment among both symptomatic and asymptomatic patients compared to those that did not meet the criteria (Figure 3.3.1) (Vignali et al., 2009).

Figure 3.3.1 Prevalence of morphometric vertebral fractures

(A) Symptomatic and asymptomatic patients with primary hyperparathyroidism and controls; (B) asymptomatic patient were categorized as to whether they fulfilled the criteria for surgery proposed by the 2002 workshop on asymptomatic primary hyperparathyroidism as well as controls (Vignali et al., 2009).
Not all studies have reported increased prevalence of morphometric vertebral fracture in patients with primary hyperparathyroidism compared to healthy individuals (Kaji et al., 2005; Wilson et al., 1988). The inconsistent results may be due to several factors. Firstly, primary hyperparathyroidism is a heterogeneous condition with differing disease severity. Wilson et al included only patients with mild, asymptomatic disease. While Kaji et al did not differentiate between patients with asymptomatic and symptomatic disease. Unlike other work, these studies also included patients that were non-Caucasian. Finally, the radiological criteria used to define vertebral fractures were not consistent between studies.

In contrast to studies that have used radiological methods to diagnosed morphometric vertebral fractures, other studies have relied on patient recall, medical records or radiology reports to identify vertebral fractures, which may underestimate the true prevalence since it is well known that only a small proportion of vertebral fractures come to medical attention (Kenny et al., 1995; Melton et al., 1992; Vestergaard et al., 2003).

Non-hip, non-vertebral fractures
Although there is paucity in data, non-hip, non-vertebral fracture risk may be elevated in patients with primary hyperparathyroidism. Much of the data is derived from Vestergaard et al in Denmark and Khosla et al in Rochester, Minnesota (U.S.A). These investigators take advantage of their well integrated medical databases to identify patients with primary hyperparathyroidism and ascertain fracture at multiple skeletal sites. Vestergaard et al observed increased forearm, lower leg and any fracture prevalence prior to but not after surgery (Vestergaard et al., 2000). While Khosla et al reported increased prevalence in distal forearm, rib, pelvis and any fracture following the diagnosis of primary hyperparathyroidism (Figure 3.3.2) (Khosla et al., 1999).
3.3.2 Increased bone turnover in primary hyperparathyroidism

Protracted elevation in endogenous PTH is associated with increased bone turnover. Accordingly, biochemical markers of bone remodelling are either elevated or in the high normal range in patients with untreated primary hyperparathyroidism (Guo et al., 1996; Silverberg et al., 1995).

Many studies have shown increased histomorphometric indices of bone resorption and bone formation. Furthermore, enhanced bone turnover is evident on trabecular, endocortical and intra-cortical surfaces. This finding has been highlighted by work from a Danish group which has featured prominently in this area of research (Christiansen et al., 1992). Their study comprised 69 patients with surgically proven primary hyperparathyroidism with median age 58 years (range 17 – 79). Thirty age- and sex-matched living and autopsy cases with median age 55 years (range 23 – 79) were used to compare static variables. However, a much younger control group (n=20) was used to compare dynamic variables with median age 25 years (range 19 – 56). In the trabecular compartment, greater activation frequency, eroded, osteoid and mineralizing surfaces, were reported, however, mineral apposition rate and bone

Figure 3.3.2 Fracture risk in primary hyperparathyroidism

Standardized incidence ratio and 95% CI in fractures at multiple skeletal sites in patients with primary hyperparathyroidism (Khosla et al., 1999).
formation rate were no different in patients with primary hyperparathyroidism compared to controls.

Using the same cohort, the Danish group has also reported increased remodelling in the cortical compartment. On the endocortical surface, increased eroded surface, osteoid surface and bone formation rate was found. Whereas, mineralizing surface and mineral appositional rate were no different. Greater cortical porosity was also seen which correlated with intra-cortical remodelling (Brockstedt et al., 1995; Christiansen et al., 1993). Since the control group used for dynamic variables was smaller in number, considerably younger and not matched to menopausal status this raises some concern with the validity of the data for dynamic parameters.

Histomorphometric evidence of increased bone remodelling in primary hyperparathyroidism is also supported by other studies (Charhon et al., 1982; Delling, 1987; Delmas et al., 1986; Dempster et al., 1999; van Doorn et al., 1993).

3.2.3 Structural basis of bone fragility in primary hyperparathyroidism

Despite evidence of increased bone remodelling in both trabecular and cortical compartments, longstanding PTH excess is held to be associated with cortical but not trabecular bone loss. This view has been prevalent in studies using bone densitometry as well as histomorphometry.

Trabecular bone is thought to be preserved or increased in patients with primary hyperparathyroidism compared to controls. Using histomorphometry, the Danish investigators have observed similar trabecular bone volume, inter-trabecular distance, marrow star volume and mean trabecular plate density in patients with primary hyperparathyroidism compared to controls (Christiansen et al., 1992). While other studies have reported increased trabecular bone volume due to greater trabecular number, thicker trabeculae and/or preserved trabecular connectivity (Figure 3.3.3) (Parisien et al., 1995; Parisien et al., 1992; Parisien et al., 1990; Silverberg et al., 1989; Uchiyama et al., 1999; Vogel et al., 1995). Furthermore, wall width has been invariable reported as normal or increased (Christiansen et al., 1992; Dempster et al., 1999; Uchiyama et al., 1999; Vogel et al., 1995).
Figure 3.3.3 Trabecular structure derived from histomorphometry in patients with primary hyperparathyroidism and controls


In contrast, chronic PTH excess is thought to be detrimental to cortical bone. The Danish investigators have reported reduction in cortical width, which was associated with increased cortical porosity (Brockstedt et al., 1995; Christiansen et al., 1993). Other studies have confirmed finding reduced cortical width and/or increased cortical porosity (Parisien et al., 1995; Parisien et al., 1990; Silverberg et al., 1989; Uchiyama et al., 1999).

In view of the increased bone remodelling that occurs on all three endosteal surfaces, it is difficult to explain the discrepant histomorphometry findings in the trabecular and cortical compartments. Moreover, the three components of the endosteal surfaces are contiguous. Therefore, an alternate view is presented in my work as well as work from others (Charopoulos et al., 2006; Chen et al., 2003; Hansen et al., 2010; Vu et al., 2013).
We, as well as others, proposed that PTH excess causes structural deterioration in both cortical and trabecular bone. Our study involved 43 patients with untreated primary hyperparathyroidism with age range 30 – 84 years and 47 age- and sex-matched controls. Images were acquired using high resolution peripheral quantitative computed tomography and a non-thresholding base algorithm was used analyze bone morphology. At the tibia and radius, cortical area and vBMD were reduced due to increased cortical porosity and lower tissue mineralization density. In addition, medullary area was increased and trabecular vBMD reduced due to low trabecular number, though this did not reach significance (Vu et al., 2013). Similar results were found in other studies using either peripheral quantitative tomography or high resolution pQCT (Charopoulos et al., 2006; Hansen et al., 2010).

These findings suggest that chronic PTH excess is detrimental to both trabecular and cortical bone compartments because high bone turnover increases the surface extent of remodelling and worsens the negative bone multicellular unit balance (Parfitt, 1983). Remodelling upon trabeculae thins and perforates them. Whereas, remodelling on endocortical surface thins the cortex and enlarges the marrow space. Remodelling on the intra-cortical surfaces adjacent to the marrow causes cavitation where pores form which coalesce, worsening porosity and thinning the cortex from ‘within’. This process is referred to as trabecularisation and produces cortical remnants that resemble trabeculae. Thereby, the preserved or increased trabecular bone mass is an artifact produced by inclusion of cortical remnants which resemble trabeculae (Zebaze et al., 2010).

### 3.3.4 Material basis of bone fragility in primary hyperparathyroidism

Studies have emerged revealing the abnormalities in material composition of bone in patients with primary hyperparathyroidism. The studies have highlighted the effects of high bone turnover on mineral and collagen properties.

Bone mineralization density distribution (BMDD) in patients with primary hyperparathyroidism was investigated using quantitative backscattered electron imaging. Roschger et al used iliac crest biopsy specimens from 51 patients with mild
primary hyperparathyroidism with age range 26 – 74 years. The results from patients with primary hyperparathyroidism were compared to normative data established by the investigators (Roschger et al., 2003). The cohort of patients with primary hyperparathyroidism exhibited modest reduction in mean mineralization density, greater heterogeneity of mineralization, increased BMDD variable reflecting primary mineralization and high inter-individual variability. Furthermore, the histomorphometric bone formation index, mineralizing perimeter, negatively correlated with mean mineralization density and positively with the extent of heterogeneity of mineralization as well as with the BMDD variable reflecting primary mineralization (Roschger et al., 2007).

My work supports these findings and demonstrated a left shift in tissue mineralization density distribution in patients with untreated primary hyperparathyroidism compared to controls, indicating lower mean bone mineralization density (Vu et al., 2013).

The same investigators also used Fourier transform infrared imaging on their cohort of patients with mild primary hyperparathyroidism to characterize collagen properties. The results of patients with primary hyperparathyroidism were compared to reference data compiled by the authors (Paschalis et al., 2003). The patients had decreased collagen maturity as indicated by the lower non-reducible (pyridinium) to reducible (dehydrodihydroxylsinonorleucine) collagen cross-link ratio. Moreover, the collagen cross-link ratio negatively correlated with histomorphometric indices of bone formation (bone formation rate, mineralizing surface, osteoid surface) and bone resorption (eroded surface) (Figure 3.3.4) (Zoehrer et al., 2008).
Figure 3.3.4 Correlations between collagen cross-link and histomorphometric indices of bone formation and bone resorption

Negative correlations between pyridinium to dehydrodihydroxylysinoevidence of bone formation and bone resorption

3.3.5 Effect of treatment in primary hyperparathyroidism

Improvements in bone turnover, bone structure and bone material properties appeared following surgical cure for primary hyperparathyroidism.

Bone turnover

Biochemical markers of bone turnover decline and normalize with some studies reporting that this occurred within 6 months following surgery (Christiansen et al., 1999). Similarly, histomorphometric indices of bone turnover improved following parathyroidectomy. Paired comparisons (n=19) before and after surgery (3 years), in trabecular bone, demonstrated reductions in eroded surface, osteoid surface, mineralizing surface, bone formation rate and activation frequency (Steiniche et al., 2008).
2000). The investigators also assessed bone remodelling in the cortical compartment following surgery. Only 9 patients with primary hyperparathyroidism were followed up prior to and 6 – 12 months after surgery. Reductions in the surface extent of remodelling, mineralizing surface, activation frequency and cortical porosity were observed following surgical treatment. However, mineral apposition rate was unchanged. No controls were prospectively followed up (Brockstedt et al., 1995).

**Bone structure**
Following parathyroidectomy, there may be partial reversibility in cortical deficits, whereas trabecular deficits are either maintained or improved. The Danish cohort of 19 patients with primary hyperparathyroidism exhibited increments in relative cortical width and reduction in cortical porosity 3 years following surgery. No changes were seen in trabecular bone volume or structure (Steiniche et al., 2000).

Studies using non-invasive imaging have confirmed the histomorphometric findings for cortical bone. Peripheral quantitative computed tomography was used to scan the radius of 20 post-menopausal females with primary hyperparathyroidism (pre- and post-surgery) and 30 age- and sex-matched controls followed up for 1 year. Twelve months after surgery, patients with primary hyperparathyroidism exhibited increased total vBMD, cortical vBMD, cortical area and cortical thickness (Figure 3.3.5). This was associated with increased estimates of bone strength. Unlike patients, healthy controls had decreased total vBMD, cortical vBMD, area and thickness after 12 months of follow up. No trabecular parameters were reported (Kaji et al., 2008).
Figure 3.3.5 Effect of surgical treatment on cortical morphology in patients with primary hyperparathyroidism

Percentage change from baseline (i.e. pre-parathyroidectomy) in individual patients with primary hyperparathyroidism for (A) total vBMD (Tt BMD), (B) cortical vBMD (Ct BMD), (C) cortical area (Ct Ar) and (D) cortical thickness (Ct Th). Values are expressed as mean ± SD of the percent change from baseline to 1 year (Kaji et al., 2005).

A prospective study used high resolution peripheral quantitative computed tomography on 27 patients with primary hyperparathyroidism and 31 controls and were followed them for 1 year. In patients total vBMD, cortical vBMD, cortical thickness and cortical porosity were maintained. Whereas, controls exhibited reductions in total vBMD, cortical vBMD and thickness with maintenance of cortical porosity. Patients with primary hyperparathyroidism had improvements in trabecular bone volume and structure at the radius but not tibia. In contrast, trabecular bone volume and structure were maintained in controls. Estimated bone strength using finite element analysis showed improvements after surgery in patients. Whereas,
estimated bone strength decreased or remained unchanged in controls (Hansen et al., 2012).

Although my work was cross-sectional, HR-pQCT was used on untreated and treated patients with primary hyperparathyroidism. Treated patients had higher cortical area and cortical vBMD and lower cortical porosity compared to untreated patients, though this did not reach significance. Tissue mineralization density was no different between treated and untreated patients. Trabecular morphology was also similar between the two groups.

Material composition
Following surgical cure in patients with primary hyperparathyroidism, there appears to be improvements the mineral and collagen properties. One component of the study by Roschger et al investigated the effects of parathyroidectomy on bone mineralization density using quantitative backscattered electron imaging. Paired comparison in two patients before and 3 years after surgery were performed. In addition, 7 successfully treated patients were compared with untreated patients. In both cohorts of patients that received surgical treatment, there were increases in mean bone mineralization density, diminished heterogeneity in mineralization and reduction in primary mineralization (Roschger et al., 2007)

My work has similarly demonstrated a right shift in tissue mineralization density distribution in patients with treated primary hyperparathyroidism relative to untreated patients, indicating increased mean bone mineralization density towards healthy control range (Vu et al., 2013).

The previous investigators also used Fourier transform infrared imaging to examine the effects of surgical treatment on collagen cross-link. In patients with primary hyperparathyroidism, parathyroidectomy resulted in higher collagen cross-link ratio, which was comparable to a reference population (Figure 3.3.6) (Zoehrer et al., 2008).
Figure 3.3.6 Effect of surgical treatment on collagen cross-link in patients with primary hyperparathyroidism

Comparisons between normal controls (NL), patients with untreated (PHPT) and treated primary hyperparathyroidism (PostPTX) in collagen cross-link ratio (pyridinium to dehydrodihydroxylysinoonorleucine) derived from Fourier transform infrared imaging. Values expressed as mean ± SD (Zoehrer et al., 2008).

**Summary**

Untreated primary hyperparathyroidism is characterized by high bone turnover, increased histomorphometric indices of bone resorption and bone formation on all three endosteal bone surfaces. Despite this, studies using histomorphometry have reported preservation or increased trabecular bone associated with cortical bone loss. However, studies using non-invasive three-dimensional imaging suggest that trabecular as well as cortical bone are lost. This is more in line with high bone remodelling seen in trabecular, endocortical and intra-cortical bone compartments. As such, the enhanced bone remodelling on the intra-cortical surfaces adjacent to the marrow causes trabecularization and produces cortical remnants that resemble trabeculae. Inclusion of these cortical remnants as trabeculae overestimates trabecular bone mass. In addition, abnormalities in material composition may also contribute to the increased fracture risk in patients with primary hyperparathyroidism. However, surgical treatment for this condition appears to reduce bone turnover, improve bone structure as well as bone material composition.
3.4 Skeletal abnormalities in hypoparathyroidism

3.4.1 Epidemiology of fractures in hypoparathyroidism

There is uncertainty as to whether the skeletal abnormalities (encompassing abnormal bone turnover, bone structure and material composition) seen in patients with hypoparathyroidism cause bone fragility as fracture outcome studies are sparse and their findings inconsistent.

A small study reported increased morphometric vertebral fracture prevalence in a post-surgical hypoparathyroid cohort compared to age-, weight- and height-matched controls (10 of 16 vs. 2 of 17 respectively). The patient inclusion criteria were more stringent – patients with a history of thyroid cancer or hyperthyroidism were precluded and all participants were post-menopausal females. Fracture ascertainment was performed using lateral spine radiographs and assessed by 2 radiologists who were blinded to the diagnosis. Although there was higher fracture prevalence, aBMD assessed using DXA was similar at lumbar spine and total hip with deficits at the 1/3 radius in hypoparathyroid patients compared to controls (Mendonca et al., 2013).

Another study reported no difference in risk of vertebral fractures or fractures at any other site in hypoparathyroid patients compared to controls. Furthermore, the risk of upper limb fractures appeared to be lower (Figure 3.4.1). The study used the Danish National Patient Registry and regional prescription databases to identify a large cohort (n=688) with post-surgical hypoparathyroidism due to non-malignant causes and age- and sex-matched controls (n=2064). Fractures were confirmed through review of hospital records. (Underbjerg et al., 2014).
Figure 3.4.1 Fracture risk in secondary hypoparathyroidism

The relative risk of fractures at different sites in post-surgical hypoparathyroid patients compared to controls (Underbjerg et al., 2014).

In contrast, a separate registry study by the same authors reported increased risk of upper limb fractures with no difference in risk of fractures at other sites and overall in a large cohort (n=180) with non-surgical hypoparathyroidism most commonly due to idiopathic hypoparathyroidism compared to age- and sex-matched controls (n=540) (Underbjerg et al., 2015).

A different study involving a large cohort with idiopathic hypoparathyroidism (n=104) reported increased morphometric vertebral fractures compared to controls (n=64). Fracture ascertainment was performed using lateral spine radiographs and assessed through a computer software program and analyzed by one radiologist who was blinded to the diagnosis (Chawla et al., 2017).

Since hypoparathyroidism is a relatively rare condition, recruiting large patient numbers for a prospective study with fracture outcome is challenging. Hence, small patient numbers and retrospective study design may contribute towards the inconsistent findings in the literature. Hypoparathyroidism is caused by different underlying conditions. Hyperthyroidism, primary hyperparathyroidism and thyroid cancer may require neck surgery, thereby increasing the risk of post-surgical
hypoparathyroidism but may also cause negative effects on bone. Although there are no studies comparing post-surgical hypoparathyroidism and non-surgical hypoparathyroidism, it is worth considering that the two conditions may be dissimilar as non-surgical hypoparathyroidism is likely to influence skeletal growth. This may partly explain the discrepant findings of the two registry studies.

3.4.2 Impaired bone turnover in hypoparathyroidism

Parathyroid hormone (PTH) plays a key role in bone metabolism through increasing renal reabsorption of calcium, renal excretion of phosphate, synthesis of 1,25(OH)₂D₃ to augment intestinal absorption of calcium, enhancing bone turnover and mobilizing the vast calcium stores from the skeleton into the circulation.

Longstanding parathyroid hormone (PTH) deficiency is associated with significant suppression in bone turnover. In patients with chronic hypoparathyroidism receiving oral calcium and vitamin D therapy, biochemical indices of bone turnover are generally suppressed or in the lower half of the normal range compared to healthy age- and sex-matched individuals (Rubin et al., 2011; Sikjaer et al., 2011; Winer et al., 1998).

More convincing evidence of the low bone turnover state in hypoparathyroidism is found in two studies using double-tetracycline labeling of iliac crest bone biopsy specimens. The study by Langdahl et al comprised 12 patients 36 – 70 years of age with 9 diagnosed with post-surgical hypoparathyroidism and 3 with idiopathic hypoparathyroidism for 2 – 53 years duration. Histomorphometric indices indicating reduced bone resorption and bone formation were observed in hypoparathyroid patients compared to controls. There was a trend towards decreased bone resorption surfaces, a longer resorptive period (80 vs. 26 days), diminished resorption depth (41.7 vs. 55.3 μm) and lower resorption rate (0.9 vs. 3.8 μm/day).

In addition, there were reductions in bone formation surface by 58% and bone formation rate by 80%. The quiescent period was prolonged (7.6 vs. 1.7 years) and the activation frequency was reduced (0.13 vs. 0.60 per year) compared to healthy controls. The BMU balance was modestly positive compared to controls, though this
did not reach significance (+0.96 vs. -4.4 μm) (Figure 3.4.2) (Langdahl et al., 1996).

Figure 3.4.2 Effect of PTH deficiency on bone remodelling

Total resorption period is prolong (80 vs. 26 days p<0.001) and a trend towards increased formation period (179 vs. 86 days) in patients with hypoparathyroidism (A) compared to controls (B) (Langdahl et al., 1996).
There is reduced bone remodeling upon trabecular, endocortical and intra-cortical bone surfaces in hypoparathyroid patients. Rubin and colleagues evaluated bone remodelling on all three endosteal surfaces in 33 patients 25 – 68 years of age with 18 diagnosed with post-surgical hypoparathyroidism, 13 with autoimmune hypoparathyroidism and 2 with DiGeorge syndrome for 3 – 45 years duration. In all three bone compartments, osteoid width and osteoid surface were diminished. In addition, bone formation rate was reduced with the greatest reduction on the trabecular surface. Although the eroded surface was similar, the bone resorption rate was reduced in hypoparathyroid patients compared to controls (Rubin et al., 2008).

As shown by the histomorphometric studies, conventional treatment with oral calcium and vitamin D therapy does not alleviate the impairment in bone turnover. Whereas recombinant human PTH(1-34) and PTH(1-84) both increased bone turnover markers which remained elevated for 3 years and 6 years respectively during follow-up (Rubin et al., 2016; Winer et al., 2003). Histomorphometric remodelling indices further corroborate these findings (Rubin et al., 2011). Since recombinant human PTH is injected subcutaneously, the recent development of an oral agent with PTH-like actions would simplify therapy for patients. Furthermore, the orally active PTH type 1 receptor agonist has been shown to be effective in animal models with hypoparathyroidism. It is currently being studied in phase 1 clinical trials in humans (Tamura et al., 2016).

3.4.3 Structural basis of skeletal abnormalities in hypoparathyroidism
Analyses using histomorphometry have observed greater trabecular bone mass due largely to increased trabecular width associated with preserved trabecular micro-architecture in hypoparathyroid patients. The study by Langdahl et al showed a trend towards higher trabecular bone volume (20.7 vs. 17.7%) compared to controls. However results for trabecular thickness, trabecular star volume and marrow star volume was similar between individuals with deficient and replete levels of PTH. Cortical morphology was not reported. (Langdahl et al., 1996). The larger study by Rubin et al reported higher trabecular bone volume and trabecular width associated with similar trabecular number and trabecular separation compared to healthy individuals. In addition, cortical width was higher and cortical porosity lower in
hypoparathyroid subjects, though neither reached significance (Figure 3.4.3). The duration of PTH deficiency was found to be positively associated with trabecular width ($r = 0.41$, $p = 0.02$) and cortical width ($r = 0.40$, $p = 0.02$) (Rubin et al., 2008).

Figure 3.4.3 Effect of PTH deficiency on cortical and trabecular morphology derived from histomorphometry

(Bilezikian et al., 2011).

Studies using micro-computed tomography have reported increased trabecular bone volume due to superior trabecular micro-architecture and connectivity in hypoparathyroid patients. Iliac crest biopsy specimens evaluated in the study by Rubin et al were also analysed using micro-computed tomography. Increased trabecular bone volume due to higher trabecular number, greater trabecular thickness
and reduced trabecular separation. This was associated with higher connectivity density and lower structure model index, which favours a plate-like over rod-like trabecular arrangement. Trabecular micro-architecture parameters derived from micro-computed tomography highly correlated with parameters derived from histomorphometry (Figure 3.4.4) (Rubin et al., 2010).

![Figure 3.4.4 Correlations between µCT and histomorphometry parameters in hypoparathyroid patients (Rubin et al., 2010).](image.png)

Data derived using high resolution quantitative computed tomography have shown increased cortical vBMD due to low cortical porosity in hypoparathyroid patients. Post-surgical hypoparathyroidism and idiopathic hypoparathyroidism comprised the majority of patients who were stratified by sex and age [pre-menopausal females <40 years (n=18); peri-menopausal females 40-54 years (n=17); post-menopausal females >55 years (n=13); males <50 years (n=7); and males >50 years (n=5)] and compared to a healthy, much younger cohort aged 20 – 29 years from the Calgary Canadian
Multi-centre Osteoporosis study. However, cortical morphology in post-menopausal hypoparathyroid females was compared to healthy post-menopausal females from the Calgary Canadian Multi-centre Osteoporosis study.

The major findings were increased cortical vBMD at the radius and tibia due to low or tendency towards low cortical porosity in all groups of hypoparathyroid women and young men, although bone mineralization density was not measured. Cortical thickness and cortical area were not increased but were similar or low. Trabecular vBMD was similar or low compared to controls. Bone strength was assessed using finite element analysis and found to be no different to controls (Figure 3.4.5) (Cusano et al., 2016).

There are a several factors to be considered when interpreting these findings. Firstly, the control group used for comparison was significantly younger which may reduce the likelihood of detecting increased cortical and trabecular morphology in the older hypoparathyroid cohort. There was also no justification given for the use of this control group. Secondly, the method used to segment cortical and trabecular bone and quantify cortical porosity may not be accurate. It is possible that the cortical vBMD may be overestimated and that some thickened trabeculae that is adjacent to the cortex and may appear ‘corticalized’ due to the reduce bone turnover and included as cortical bone which may then underestimates medullary area and trabecular vBMD.
Figure 3.4.5 Cortical and trabecular morphology derived from HR-pQCT and finite element analysis in patients with hypoparathyroidism

Tt.Ar/ht = total area/height; Tt.BMD = total vBMD; BV/TV = trabecular bone volume fraction; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Ar/ht = cortical area/height; Ct.BMD = cortical vBMD; Ct.Po = cortical porosity; Ct.Th = cortical thickness. Comparison made to controls *p<0.05 and radius versus tibia ]+ p<0.05 (Cusano et al., 2016).

3.4.4 Material basis of skeletal abnormalities in hypoparathyroidism

The skeletal abnormalities observed in chronic PTH deficiency may to some extent be due to anomalies in the material composition of bone. As such, marked reductions in bone remodelling have been well documented in chronic PTH deficiency, which may cause detrimental effects on the mineral and collagen components of the bone matrix. As this is an emerging area of research, there are very few studies available.

Bone mineral quantity, distribution and properties in hypoparathyroid patients were examined using quantitative backscattered electron imaging as well as Raman and Fourier transform infrared imaging. Two studies from Paschalis et al used iliac crest bone specimens from 19 hypoparathyroid patients. The first study reported increased
inter-individual variation in bone mineralization density distribution as well as similar mean mineralization density compared to reference values (Paschalis et al., 2009).

The second study reported lower mineral crystal maturity/crystallinity compared to healthy pre- and post-menopausal females with untreated low bone turnover osteoporosis. Low and similar mineral to matrix ratio was observed compared to healthy pre-menopausal women and post-menopausal females with untreated low bone turnover osteoporosis respectively (Paschalis et al., 2012). Both control populations were not well characterized.

A more recent study by the same authors included 30 hypoparathyroid patients treated with one or two years of PTH(1-84). Quantitative backscattered electron imaging was used to measure bone mineralization density distribution variables. At baseline, both one year and two year PTH(1-84) treatment groups demonstrated increased bone mineralization density distribution parameters in trabecular bone but not cortical bone (Figure 3.4.6) (Misof et al., 2016).

The collagen characteristics in hypoparathyroid patients were also evaluated using Raman and Fourier transform infrared imaging. The main findings from the two studies by Paschalis et al suggests that collagen maturity is increased as indicated by high pyridinoline/divalent collagen cross-link ratio. Unlike the bone mineralization results, these findings are consistent with the low bone turnover state associated with hypoparathyroidism.
Figure 3.4.6 Effect of PTH deficiency on bone mineralization density distribution parameters

Patients with hypoparathyroidism at baseline (white bars), one year PTH(1-84) or two year PTH(1-84) treatment groups (grey bars). Comparisons made to baseline (***p<0.001, **p<0.01, *p<0.05), BMDD parameter reference values (**p<0.001, o<p<0.01, °p<0.05) or two year versus one year PTH(1-84) treatment (#p<0.05). Grey bars indicate inter-quartile range of BMDD reference values. CaMean = mean calcium concentration of bone area; CaPeak = peak of histogram; CaWidth = heterogeneity of mineralization; CaLow = proportion of lowly mineralized bone; CaHigh = proportion of highly mineralized bone (Misof et al., 2016).

Summary
The skeletal abnormalities observed in patients with hypoparathyroidism is associated with suppression in bone turnover. Double-tetracycline labeling of iliac crest bone biopsy specimens reveal reduced histomorphometric indices of resorption and formation on all three endosteal bone surfaces (trabecular, endocortical and intracortical). Analyses using different modalities such as histomorphometry, micro-
computed tomography and high resolution peripheral quantitative computed tomography provide insight into the structural basis for the increased trabecular and cortical bone mass. Although favourable trabecular micro-architecture and trabecular network connectivity as well as increased cortical thickness and reduced cortical porosity has been observed, this has not consistently translated into increased bone strength. One possible explanation may be the coexistent abnormalities in the mineral and collagen component of the matrix. However, it remains uncertain as to whether the skeletal abnormalities observed in patients with hypoparathyroidism causes increased fracture risk as studies are sparse and their findings have been inconsistent.
Methodology

Chapter Four
Chapter 4: Methodology

The detection of bone fragility has evolved. Early on bone fragility was crudely detected on radiographs as osteopenia or fractures, which are advanced features of bone decay. Later on, densitometry was developed to try and detect individuals at risk before fracture and before severe irreversible bone microstructure deterioration occurred. However, DXA is confined to measuring areal bone mineral density and is associated with a number of limitations. DXA is not a sensitive method at detecting bone fragility as most fractures occur above the threshold used to define osteoporosis (i.e. T score <2.5). Bone microstructural deterioration is the hallmark of bone fragility but this is not captured by DXA. Recent advances have enabled bone microstructure to be measured in vivo using high resolution peripheral quantitative computed tomography (HR-pQCT). Advances have also occurred in software analyzing images acquired using HR-pQCT.

4.1 Dual energy x-ray absorptiometry

Dual energy x-ray absorptiometry (DXA) has been widely used in clinical practice and research because it is universally available, simple to use, has low radiation exposure and has been well validated. An x-ray tube provides a source of photon energy, which becomes attenuated after passing through bone and soft tissue. DXA measures the tissue absorption of dual (i.e. low and high) energy x-ray spectrum. Areal BMD (aBMD, g/cm²) is measured directly while bone mineral content (BMC, g) is derived from multiplying the area of the region of interest (ROI) and aBMD.

The DXA system used in my studies is the Prodigy, GE Lunar (Madison, WI, USA). The anteroposterior spine is measured with the region of interest covering L1 – L4 inclusive of vertebral body (which predominantly is comprised of trabecular bone) and posterior processes (which predominantly is comprised of cortical bone) (Figure 4.1). Ageing may lead to falsely high aBMD of spine as artifacts such as vertebral osteophytes and aortic calcification can influence readings. The CV for aBMD of anteroposterior spine was 1.1–1.4%.

The Left femur is scanned in my studies with the region of interest covering the femoral neck, wards’s triangle, trochanter and total hip (Figure 4.1). Only the aBMD
of the femoral neck and total hip are included in my studies. The CV for aBMD of femoral neck and total hip was 2.2–2.6%

![Image of DXA images](image_url)

Figure 4.1 DXA images

(A) Anteroposterior spine with region of interest covering L1 – L4 and (B) left femur with region of interest covering femoral neck, ward’s, trochanter and total hip.

The non-dominant forearm is measured in my studies with the region of interest covering the ultra-distal, 33% and total radius. Only the aBMD of the total radius is included in my studies.

There are a number of limitations to using DXA. The technology provides two-dimensional measurements and does not account for bone size. As such, DXA can overestimate aBMD in bigger bones, as bones that are wider are typically thicker. Furthermore, DXA is unable to discriminate between cortical and trabecular bone. Although DXA has high specificity for fracture prediction, its sensitivity is low since more than 50% of females and more than 70% of males that fracture have either osteopenia or ‘normal’ aBMD (Ebeling et al., 2013; Siris et al., 2004). Therefore, the threshold (i.e. T score of ≤ 2.5) used to defined osteoporosis overlooks many patients that fracture. Moreover, DXA fails to capture bone microstructural deterioration, which is one of the hallmarks of bone fragility.
4.2 Image acquisition using high resolution peripheral quantitative computed tomography

Recent advances have led to the development of high resolution peripheral quantitative computed tomography (HR-pQCT; Xtreme CT, Scanco Medical AG, Bsserdorf, Switzerland) which is able to measure bone microstructure in vivo with high resolution, good precision and low radiation. The technology uses a two-dimensional detector array in combination with a 0.08mm point focus x-ray tube, which facilitates the acquirement of a stack of parallel CT slices with voxel size of 82µm (Laib et al., 1997). The machine setting is as follows: effective energy of 60kVp, x-ray tube current of 0.9mA and matrix size of 1536 x 1536. During scanning, the non-dominant upper limb and lower limb of the study participant is immobilized in a carbon fiber shell.

An anteroposterior scout view is used to determine the region of interest and to set the reference line manually in the mid-point of the endplate of the radius and tibia (Figure 4.2). The initial CT slice in the radius and tibia is set at 9.5mm and 22.5mm proximal to the reference line respectively. At each scanned site, 110 CT slices are acquired and used to recreate a 9.02mm three-dimensional image. The radiation dose is less than 3µSv per measurement (Boutroy et al., 2005).

Figure 4.2 Scout views used in HR-pQCT

(A) Radius (left) and (B) tibia (left) to produce three-dimensional images of the radius and tibia (right) respectively (Cheung et al., 2013).
**Image analysis**

Following acquisition, the images are processed using the manufacturer’s standard analysis protocol to calculate a range of parameters relating to bone geometry and structure. The periosteal surface is segmented from the surrounding soft tissue through a semi-automated contouring around the bone perimeter and edge finding algorithm. Threshold based algorithm is used to segment cortical and trabecular bone in the volume of interest (Figure 4.3). A gaussian filter is used to digitally subtract trabecular bone while preserving cortical bone. Then thresholding is used to binarize the image and identify cortical bone from adjacent soft tissue. For cortical bone the threshold is set at the manufacturer’s standard protocol. The corresponding mineral density threshold is calculated by using the density calibration at the time of scanning and is about one third of the apparent cortical density. A Laplace-Hamming filter and thresholding is used to identify and binarize the trabecular (Davis et al., 2007; Kazakia et al., 2008; Laib et al., 1998).

![Figure 4.3 Segmentation of bone using a threshold based algorithm](image)

Separation of (A) distal radius into (B) cortical bone and (C) trabecular bone by a standard analysis protocol used by HR-pQCT (MacNeil et al., 2007).
Parameters for cortical morphology

Cross-sectional area (CSA, mm$^2$) of the total bone and cortex are determined as the average of 110 slices. Trabecular area is the area of the medullary cavity, which is calculated through the subtraction of cortical area from total CSA. Cortical thickness is derived from dividing the average cortical volume by the outer bone surface. The volumetric bone mineral densities (vBMD, mg HA/cm$^3$) in the medullary compartment, cortical compartment and total bone are determined as the mean mineral density within the medullary, cortical and entire bone respectively.

Parameters for trabecular morphology

Trabecular bone volume/tissue volume (BV/TV, %) is derived from trabecular vBMD divided by 1200 mg HA/cm$^3$, which reflects bone that is fully mineralized. Trabecular number (Tb.N, mm$^{-1}$) is determined as the inverse of the average distance between the ridges, which are the ‘center points’ of trabeculae. (Laib et al., 1998; Laib et al., 1997). The ridges are derived from the original three dimensional gray scale images. The distances of the ridges are then evaluated by the methods developed from distance transformation (Hildebrand et al., 1997). Trabecular thickness (Tb.Th, µm) and trabecular separation (Tb.Sp, µm) are derived from trabecular bone volume/tissue volume and trabecular number using standard histomorphometry techniques (Parfitt et al., 1983).

For density measurements, the co-efficient of variation (CV) ranged from 0.6 – 0.9% and for structural parameters the CV is 1.4 – 7.4% (Evans et al., 2007). There appears to be reasonable accuracy between HR-pQCT and µCT for the assessment of bone morphology ($R^2 = 0.59 – 0.96$) (MacNeil et al., 2007).

There are a number of limitations to HR-pQCT. Unlike DXA, HR-pQCT is not readily available and its use in Australia is restricted to research. There are no large databases to provide reference ranges. Furthermore, measurement sites are difficult to duplicate. The variability in the positioning of the region of interest due to differences in forearm length needs taken into account when investigating age related differences, sexual dimorphism or racial differences as there is heterogeneity in bone geometry and bone morphology within a small region of bone (Ghasem-Zadeh et al., 2017).
4.3 Image analysis using StrAx

Images acquired using HR-pQCT are analysed with new software (StrAx1.0, University of Melbourne, Melbourne, Australia) for the primary hyperparathyroidism and hypoparathyroidism data.

*Segmentation of bone into compact-appearing cortex, transitional zone and trabecular compartment*

The software processes the 40 most proximal image slices out of 110 as they contain thicker cortices to allow accurate assessment of cortical porosity. The method uses ~3600 radial attenuation profile curves around each slice to segregate bone from soft tissue background into compact-appearing cortex, outer transitional zone (OTZ, which is adjacent to the cortex), inner transitional zone (ITZ, which is adjacent to the medulla) and trabecular compartment. Essentially StrAx segments (separates) bone into cortical-transitional-trabecular which may lessen the erroneous inclusion of cortical remnants into the trabecular compartment (which can overestimate trabecular vBMD) and improve the accuracy of cortical and trabecular bone segmentation. (Zebaze et al., 2013). In contrast, other threshold independent algorithm only separate bone into cortical and trabecular compartments (Hangartner, 2007; Treece et al., 2010; Valentinitsch et al., 2012; Yang et al., 2004).

*Measuring cortical porosity*

StrAx excludes voxels with attenuation between 80-100% of the maximum attenuation produced by 1200 mg HA/cc (i.e. fully mineralized bone) when quantifying porosity because these voxels contain younger bone at various stages of secondary mineralization responsible for the heterogeneity in mineralization that could be mistakenly interpreted as porosity. These voxels are unlikely to contain Haversian canals because few are <25 µm diameter. Porosity is quantified by estimating the void fraction of each of the remaining voxels.

To do so, the mineralized bone matrix volume of each voxel is quantified using an interpolation function derived from two referents (i) the attenuation of voxels containing fully mineralized bone matrix (equivalent to that produced by
1200mgHA/cc) are assigned a value of 100% and (ii) voxels that are empty with an attenuation equivalent to background are assigned a value of 0%. The volume fraction of a voxel that is void (also referred to as porosity) is 100% minus the mineralized bone matrix fraction. The porosity of the bone compartment is the mean void volume fraction of all composite voxels within the compartment, which vary in their proportions of void and mineralized bone matrix. This feature enables the analysis of porosity below and above the HR-pQCT resolution (Zebaze et al., 2013).

Measuring tissue mineralization density
To quantify tissue mineralization density, only voxels with attenuation values between 80 and 100% of the attenuation produced by 1200mgHA/cc are used. The difference between the attenuation produced by voxels containing only mineralized bone and the attenuation produced by background represents the attenuation produced by the mineral within the bone matrix. Tissue mineral density is the ratio of the attenuation produced by the mineral in the bone matrix to the attenuation produced by 1200mgHA/cc. Frequency distribution curves depicting the composition of the cortex according to voxel content (empty, mineralized bone matrix only, varying proportions of each) are calculated (Zebaze et al., 2013).

Precision and accuracy
The reproducibility (expressed as root mean square coefficient of variation, RMSCV) using StrAx ranged from 1.88 – 3.98% for segmentation of compartments and 0.54 – 2.92% for quantifying cortical porosity. There appears to be reasonably high accuracy between StrAx and µCT for segmenting compartments ($R^2 = 0.95 – 0.99$) and quantifying porosity ($R^2 = 0.87 – 0.98$) (Figure 4.4) (Zebaze et al., 2013).
Figure 4.4 Correlations between HR-pQCT and µCT

(A) Total cross sectional area, (B) compact-appearing cortex, (C) transitional zone and (D) medullary area (Zebaze et al., 2013).

4.4 Study design

The studies presented are all cross-sectional and conducted at a tertiary referral centre (Austin Health, Melbourne, Australia).

Participants

Patients with type 1 diabetes, type 2 diabetes, primary hyperparathyroidism and hypoparathyroidism are recruited through specialist clinics in the Endocrinology Department at Austin Health or private consulting rooms. All controls are recruited from the community through flyers or word of mouth as part of ongoing research in our department.

Patient data and HR-pQCT images from Columbia University (New York, U.S.A) are used in the primary hyperparathyroidism and hypoparathyroidism studies as part of a joint collaboration with Professor Bilezikian. These patients are part of the ongoing research from Columbia University.
At the study visit anthropometry is assessed, a questionnaire is administered, relevant biochemistry results collected, areal bone mineral density measured using DXA and bone structure measured using HR-pQCT.

**Anthropometry**
Standing height is evaluated with a Holtain stadiometer and documented to the nearest 0.1cm. Body weight is assessed using electronic scale and documented to the nearest 0.1kg.

**Questionnaire**
A questionnaire is utilized to collate data concerning demographics, reproductive history, medical history including details of fractures and medications.

**Biochemical analyses**
HbA1c is measured using turbidimetric inhibition immunoassay on Integra 800, Roche Diagnostics, Germany. Bone turnover markers procollagen type I N-terminal propeptide (PINP) and C-terminal telopeptide of type I collagen (CTx) as well as PTH (1-84) and 25(OH) vitamin D are analysed using electro-chemiluminescence immunoassay on Cobas 8000, Roche Diagnostics, Germany. Serum calcium and phosphate are analysed by a spectrophotometric immunoassay using NM-BAPTA and ammonium molybdate on Cobas 8000, Roche Diagnostics, Germany.

**Consent**
*Type 1 and Type 2 diabetes mellitus study*
The study protocols are approved by the Austin Health Human Research Ethics Committee and participants gave written informed consent.

*Primary hyperparathyroidism and Hypoparathyroidism study*
The study protocols are approved by the Austin Health Human Research Ethics Committee and at Columbia University Medical Centre Institutional Review Board (New York, U.S.A) to assist with recruiting patients with primary hyperparathyroidism and hypoparathyroidism.
Results

Bone Microstructure and Material Composition in Endocrine Disorders

Chapter Five

Chapter 5.3 has been published
Chapter 5: Bone Microstructure and Material Composition in Endocrine Disorders

5.1 Type 1 diabetes mellitus

5.1.1 Summary
To examine the underlying bone structural changes associated with skeletal fragility in type 1 diabetes mellitus, I studied the bone morphology at the distal radius and tibia in 49 patients (30 male, 19 female) with T1DM, mean ages 49 and 38 years respectively, and compared them to appropriate age- and sex-matched controls using high resolution peripheral quantitative computed tomography.

In male patients at the distal radius, total cross-sectional area, cortical area and medullary area were no different to controls. Cortical vBMD and compact cortical porosity were similar in male patients and controls. Trabecular vBMD did not differ. Whereas trabecular thickness was increased (11.1%, p=0.04) offsetting lower trabecular number (9.0%, p=0.04) and greater trabecular separation (6.8%, p=0.08). At the distal tibia, male patients had similar total cross-sectional area, cortical area and medullary area. Cortical vBMD was 2.7% lower (p=0.10) due to 6.8% greater (p=0.12) compact cortical porosity, neither of which reached significance. Trabecular vBMD, thickness and separation did not differ, while trabecular number tended to be lower (7.4%, p=0.09). Female patients had similar bone morphology to controls at both skeletal sites. Results were similar following adjustments for height, weight and BMI for both sexes.

In male, not female, patients, longer duration of T1DM was associated with lower cortical vBMD at the radius (r=-0.31, p=0.10), and tibia (r=-0.40, p<0.05) as well as higher compact cortical porosity at the radius (r=0.31, p=0.09) and tibia (r=0.42, p<0.05). Areal BMD at the spine and hip did not differ in either sex relative to controls. Male patients had lower procollagen type I N-terminal propeptide (PINP, 43.9 ± 18.3 vs. 67.4 ± 31.9 µg/L; p<0.05) and lower C-terminal telopeptide of type I collagen (CTx, 389.0 ± 179.1 vs. 548.8 ± 291.5 ng/L; p=0.14). Female patients also had lower PINP (42.7 ± 14.0 vs. 78.3 ± 43.4 µg/L; p=0.07). Whereas CTx was no different in female patients.
Thus, there were no significant differences in bone structure between patients with diabetes and controls. Therefore, abnormalities in microstructure do not explain bone fragility in patients with T1DM.

5.1.2 Introduction

The structural basis underlying increased fracture rates in patients with type 1 diabetes mellitus (T1DM) is poorly defined. Studies have reported elevated fracture risk across all ages in male and female patients with T1DM at the hip, vertebral and non-hip, non-vertebral sites relative to healthy controls (Vestergaard et al., 2005; Weber et al., 2015). In particular, meta-analyses have reported relative risk of ~6 for hip fractures in patients with T1DM (Fan et al., 2016; Janghorbani et al., 2007; Vestergaard, 2007). The markedly elevated fracture risk observed in these patients makes it an important public health issue.

The disproportionately high hip fracture risk seen in patients with T1DM is out of keeping with the findings of areal BMD, which is only modestly reduced or normal (Vestergaard, 2007). Hence, densitometry is of limited use in this patient population and more advance technology such as HR-pQCT is needed to study structural changes that may lead to bone fragility in this condition. As such, few studies have made use of this technology in patients with T1DM. Thus far one study has used HR-pQCT to examine the alterations in bone structure in patients with T1DM with and without microvascular complications in comparison to controls. The study suggested that microvascular complications in patients with T1DM is associated with both cortical and trabecular deficits (Shanbhogue et al., 2015).

The mechanisms underlying bone fragility in T1DM are not fully elucidated. Reduced bone formation has been proposed to play a role. Animal models of T1DM have shown reduced expression of transcription factors that regulate osteoblast differentiation (Lu et al., 2003). Long-term spontaneously diabetic rats (>12 weeks) had reduced histological parameters of bone formation (osteoblast surface, osteoid surface and bone mineral apposition rate) as well as reduced serum osteocalcin levels associated with diminished bone volume and impaired strength related properties.
relative to controls (Verhaeghe et al., 1990; Verhaeghe et al., 1990). Reduced bone formation was also seen in diabetic rodents during tibial distraction osteogenesis and was accompanied with lower levels of serum osteocalcin (Thrailkill et al., 2005).

The purpose of this study was to investigate changes in bone structure in type 1 diabetes mellitus. In view of low bone formation observed in T1DM, I hypothesis that this condition is associated with lower total cross-sectional area and cortical area, higher medullary area, cortical porosity and tissue mineralization density and lower trabecular thickness compared to controls.

5.1.3 Materials and Methods

Subjects
Forty-nine patients with T1DM were recruited from a diabetes clinic at Austin Health. The diagnosis of T1DM was based on positive antibodies to glutamic acid decarboxylase (GAD), islet antigen 2 (IA-2) and/or insulin, low to undetectable c-peptide levels, requirement for insulin therapy since diagnosis and clinical records. The healthy participants for the two T1DM cohorts were recruited from the community as part of ongoing research in the department. Participants did not have any other diseases or drugs known to affect bone.

Image acquisition and analysis
Refer to chapter 4. Images acquired using high resolution peripheral quantitative computed tomography were further analyzed for cortical porosity. The cortical porosity was derived using equation: Density based compact cortex porosity = [1 – (cortical vBMD/1200)]*100

Biochemical analyses
HbA1c was measured using turbidimetric inhibition immunoassay on Integra 800, Roche Diagnostics, Germany. Bone turnover markers procollagen type I N-terminal propeptide (PINP) and C-terminal telopeptide of type I collagen (CTx) as well as 25(OH) vitamin D were analysed using electro-chemiluminescence immunoassay on Cobas 8000, Roche Diagnostics, Germany.
Statistical analysis

All statistical analyses were conducted using SPSS version 23.0 (SPSS Inc, Chicago, USA). Comparisons of bone structural parameters between patients with T1DM and controls were performed using student t tests and adjusted for height, weight and BMI. The results were expressed as mean ± standard deviation for patient characteristics and mean ± standard error of the mean for bone variables. Linear correlation between duration of diabetes and bone variables were calculated and expressed using Pearson’s correlation coefficient. A p value of <0.05 was considered to be statistically significant.

5.1.4 Results

As shown in table 5.1.1 the mean age, height, weight and body mass index for T1DM patients and controls were similar. In male patients with diabetes, procollagen type I N-terminal propeptide levels (PINP, p=0.04) as well as C-terminal telopeptide of type I collagen levels (CTx, p=0.14) were lower. In female patients, PINP levels (p=0.07) were lower, whereas CTx levels (p=NS) were no different to controls.
### Table 5.1.1 Baseline characteristics of patients with type 1 diabetes mellitus

<table>
<thead>
<tr>
<th></th>
<th>All T1DM (n=49)</th>
<th>All Controls (n=53)</th>
<th>Male T1DM (n=30)</th>
<th>Male controls (n=31)</th>
<th>Female T1DM (n=19)</th>
<th>Female controls (n=22)</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.1 ± 15.3</td>
<td>44.1 ± 15.2</td>
<td>49.3 ± 14.7</td>
<td>48.5 ± 15.1</td>
<td>38.4 ± 13.9</td>
<td>37.9 ± 13.2</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73 ± 0.09</td>
<td>1.71 ± 0.10</td>
<td>1.77 ± 0.08</td>
<td>1.77 ± 0.08</td>
<td>1.66 ± 0.07</td>
<td>1.63 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.6 ± 17.1</td>
<td>76.2 ± 16.4</td>
<td>85.1 ± 15.2</td>
<td>84.2 ± 14.6</td>
<td>73.3 ± 17.7</td>
<td>65.6 ± 12.2</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.0 ± 5.5</td>
<td>26.0 ± 4.7</td>
<td>27.1 ± 4.4</td>
<td>27.1 ± 5.0</td>
<td>26.9 ± 7.0</td>
<td>24.5 ± 3.9</td>
<td></td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>63.0 ± 25.4</td>
<td>77.7 ± 36.9</td>
<td>61.0 ± 25.1</td>
<td>78.6 ± 28.9</td>
<td>66.4 ± 26.3</td>
<td>74.3 ± 68.3</td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.3 ± 8.5</td>
<td>5.6 ± 0.3</td>
<td>7.7 ± 2.0</td>
<td>5.6 ± 0.3</td>
<td>11.5 ± 12.6</td>
<td>5.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>PINP (µg/L)</td>
<td>43.5 ± 16.4</td>
<td>69.7 ± 33.1</td>
<td>43.9 ± 18.3*</td>
<td>67.4 ± 31.9</td>
<td>42.7 ± 14.0</td>
<td>78.3 ± 43.4</td>
<td>F &lt; 59, M &lt; 76</td>
</tr>
<tr>
<td>CTx (ng/L)</td>
<td>367.4 ± 173.6</td>
<td>520.4 ± 276.9</td>
<td>389.0 ± 179.1</td>
<td>548.8 ± 291.5</td>
<td>337.8 ± 173.0</td>
<td>416.0 ± 229.6</td>
<td>F &lt; 570, M &lt; 580</td>
</tr>
<tr>
<td>Spine BMD (g/cm²)</td>
<td>1.20 ± 0.20</td>
<td>1.21 ± 0.19</td>
<td>1.25 ± 0.22</td>
<td>1.27 ± 0.20</td>
<td>1.11 ± 0.11</td>
<td>1.14 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>Femoral neck BMD (g/cm²)</td>
<td>0.97 ± 0.13</td>
<td>1.02 ± 0.16</td>
<td>0.98 ± 0.15</td>
<td>1.04 ± 0.19</td>
<td>0.95 ± 0.08</td>
<td>0.99 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Total hip BMD (g/cm²)</td>
<td>1.02 ± 0.15</td>
<td>1.04 ± 0.16</td>
<td>1.06 ± 0.17</td>
<td>1.08 ± 0.18</td>
<td>0.95 ± 0.08</td>
<td>0.99 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Duration of T1DM (years)</td>
<td>20.4 ± 12.4</td>
<td>21.4 ± 13.9</td>
<td></td>
<td></td>
<td>18.7 ± 9.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 in comparison to control group.

Type 1 diabetes mellitus (T1DM), body mass index (BMI), bone mineral density (BMD), procollagen type I N-terminal propeptide (PINP), C-terminal telopeptide of type I collagen (CTx). Values expressed as mean ± SD.
Distal radius

Male patients had similar total cross-sectional area, medullary area, cortical area and cortical thickness as controls. Cortical vBMD and porosity were no different. Trabecular vBMD was similar due to thicker trabeculae (11.1%, p=0.04) offsetting lower trabecular number (9.0%, p=0.04) and greater trabecular separation (6.8%, p=0.08), which did not reach significance (Table 5.1.2). Female patients with T1DM had similar cortical and trabecular bone morphology as controls.

Distal tibia

Male patients had similar total cross-sectional area, medullary area, cortical area and cortical thickness to controls. Cortical vBMD was 2.7% lower (p=0.10) due to 6.8% greater compact cortical porosity (p=0.12), neither of which reached statistical significance. Although trabecular vBMD, thickness and separation did not differ, trabecular number tended to be lower (7.4%, p=0.09) than controls (Table 5.1.2). Female patients had similar cortical and trabecular bone traits to controls. When height, weight and BMI were adjusted for, the findings were similar (data not shown).

Effect of disease duration

In male, but not female, patients there was an inverse relationship between duration of diabetes and cortical vBMD at both the radius (r=-0.31, p=0.10) and tibia (r=-0.40, p<0.05). There was a positive correlation between duration of diabetes and compact cortical porosity at both the radius (r=0.31, p=0.09) and tibia (r=0.42, p<0.05) (Figure 5.1.1).

There was no association between duration of diabetes and cortical area or cortical thickness. In addition there was no relationship between HbA1c and bone turnover markers (PINP and CTx) with cortical or trabecular parameters.
<table>
<thead>
<tr>
<th></th>
<th>All T1DM (n=49)</th>
<th>All Controls (n=53)</th>
<th>Male T1DM (n=30)</th>
<th>Male controls (n=31)</th>
<th>Female T1DM (n=19)</th>
<th>Female controls (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trabecular separation (mm)</strong></td>
<td>258.4 ± 10.8</td>
<td>285.1 ± 13.6</td>
<td>284.7 ± 13.0</td>
<td>285.0 ± 13.6</td>
<td>284.8 ± 13.0</td>
<td>285.0 ± 13.0</td>
</tr>
<tr>
<td><strong>Trabecular thickness (mm)</strong></td>
<td>248.0 ± 10.1</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
</tr>
<tr>
<td><strong>Trabecular number (mm)</strong></td>
<td>380.7 ± 13.6</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
</tr>
<tr>
<td><strong>Cortical thickness (mm)</strong></td>
<td>285.1 ± 13.6</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
<td>335.8 ± 10.4</td>
</tr>
<tr>
<td><strong>Cortical vBMD (mgHA/cm²)</strong></td>
<td>285.4 ± 11.9</td>
<td>881.8 ± 11.8</td>
<td>881.8 ± 11.8</td>
<td>881.8 ± 11.8</td>
<td>881.8 ± 11.8</td>
<td>881.8 ± 11.8</td>
</tr>
<tr>
<td><strong>Compact cortical porosity (%)</strong></td>
<td>225.8 ± 0.9</td>
<td>225.8 ± 0.9</td>
<td>225.8 ± 0.9</td>
<td>225.8 ± 0.9</td>
<td>225.8 ± 0.9</td>
<td>225.8 ± 0.9</td>
</tr>
<tr>
<td><strong>Cortical vBMD (mgHA/cm²)</strong></td>
<td>192.7 ± 6.8</td>
<td>192.0 ± 7.1</td>
<td>192.0 ± 7.1</td>
<td>192.0 ± 7.1</td>
<td>192.0 ± 7.1</td>
<td>192.0 ± 7.1</td>
</tr>
<tr>
<td><strong>Bone volume/total volume (%)</strong></td>
<td>16.1 ± 0.6</td>
<td>16.3 ± 0.6</td>
<td>16.3 ± 0.6</td>
<td>16.3 ± 0.6</td>
<td>16.3 ± 0.6</td>
<td>16.3 ± 0.6</td>
</tr>
<tr>
<td><strong>Trabecular number (mm²)</strong></td>
<td>1.86 ± 0.04</td>
<td>1.81 ± 0.06</td>
<td>1.81 ± 0.06</td>
<td>1.81 ± 0.06</td>
<td>1.81 ± 0.06</td>
<td>1.81 ± 0.06</td>
</tr>
<tr>
<td><strong>Trabecular thickness (mm)</strong></td>
<td>0.44 ± 0.01</td>
<td>0.47 ± 0.02</td>
<td>0.47 ± 0.02</td>
<td>0.47 ± 0.02</td>
<td>0.47 ± 0.02</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td><strong>Trabecular separation (mm)</strong></td>
<td>0.16 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
</tbody>
</table>

Table 5.1.2 Bone microstructure in patients with type 1 diabetes and controls

Values expressed as mean ± SEM. *p<0.05 compared to controls.
Figure 5.1.1 Correlations between cortical parameters and duration of type 1 diabetes

Cortical vBMD and compact cortical porosity in male (open dots, dashed line) and female (filled dots, solid line) patients with type 1 diabetes mellitus plotted against disease duration at the distal radius and tibia.

5.1.5 Discussion
I report that patients with type 1 diabetes had no significant deficits in cortical and trabecular bone relative to controls. Although male patients had lower cortical vBMD and higher cortical porosity at the distal tibia, neither measurement reached
significance. These results are consistent with areal BMD in the lumbar spine and femur, which was no different in patients and controls. Patients with diabetes had a tendency towards lower bone formation and bone resorption markers.

**Bone microstructure**

Studies investigating changes in bone structure in adults with T1DM using HR-pQCT are lacking. So far, one study has made use of HR-pQCT to investigate alterations in bone morphology and bone strength in patients with type 1 diabetes (n=55) in comparison to age- and sex-matched controls. Unlike my study, male and female patients were grouped together and patients were stratified according to the presence or absence of microvascular complications. Within the diabetic and control groups, post-menopausal women were included. The study reported no differences in bone structure between patients without microvascular complications and controls.

However, in comparison to patients without microvascular complications, patients with microvascular complications had predominantly trabecular deficits. When compared to controls, patients with microvascular complications exhibited both cortical and trabecular deficits at the radius with larger total cross-sectional area and trabecular area; lower total vBMD was due to lower trabecular vBMD and cortical vBMD associated with thinner cortices. At the tibia, patients with microvascular complications had lower total vBMD and trabecular vBMD in comparison to controls. No differences in bone strength were found using finite element analysis. The findings in this study suggest that microvascular complications may be a risk factor for bone fragility in T1DM (Shanbhogue et al., 2015).

No underlying structural differences were seen in female patients with T1DM may be due to the smaller patient numbers causing insufficient power to detect changes. Furthermore, females were younger when compared to male patients with T1DM. Although fracture risk is elevated for both men and women with diabetes, some studies using DXA have suggested that men with T1DM may be at higher risk of bone decay (Hamilton et al., 2009; Hamilton et al., 2012; Rakic et al., 2006). A small longitudinal study followed up 17 men and 9 women with T1DM for 5 years and reported that aBMD at the femoral neck but not the spine nor forearm decreased significantly in male patients only. However, there was no control group, patient
numbers were small and baseline patient characteristics were not analyzed by sex (Hamilton et al., 2012).

Studies using histomorphometry in T1DM are scarce. One such study reported no differences observed in bone structure using histomorphometry and micro-CT from bone specimens from patients with uncomplicated, longstanding T1DM (mean age ~31 years) and age- and sex-matched controls. However, the study is likely to have been underpowered to detect differences with only 18 patients in each group. Furthermore these patients with diabetes were otherwise well with no known complications (Armas et al., 2012).

**Material properties**

Anomalies in the material properties of bone may offer a possible explanation for the underlying bone fragility in diabetes since my work and others have found bone structure in patients with T1DM to be no different to healthy controls. This is why a subset of patients from the histomorphometry study by Armas et al had their bone biopsy specimens further analyzed to assess certain aspects of material composition such as degree of mineralization and collagen cross-linking (enzymatic and non-enzymatic – pentosidine) as well as bone mechanical properties in three groups: patients with T1DM that have fractured (n=5), patients with T1DM that have not had fractures (n=5) and healthy controls (n=5). Collagen cross-linking was measured using high performance liquid chromatography. Pentosidine was found to be higher in trabecular but not cortical bone in patients with fractures compared to controls. Whereas patients without fractures had similar levels of pentosidine to controls. Measurements of enzymatic cross-links were no different between the three groups of patients.

Degree of mineralization was assessed using digitalized microradiography and was observed to be higher in trabecular but not cortical bone in patients with fractures compared to patients without fractures and controls. Hardness was found to be no different between the groups using microindentation and nanoindentation. Although statistical significance was not achieved in any of the histological indices of bone turnover, there was a trend indicating low bone formation with lower osteoid surface,
mineralizing surface and bone formation rate as well as lower activation frequency in patients with fractures compared to controls. These results suggest that diabetes is associated with low bone turnover (Farlay et al., 2016).

Similar findings of increased AGES in bones in rodent models of T1DM have been reported. Mouse models of severe early onset T1DM have shown increased AGES levels in bone using Raman microspectroscopy associated with reduced bone toughness as well as increased indentation distance using cyclic microindentation when compared to wild type mice (T1DM 9.04 ± 0.77 vs. WT 6.85 ± 0.44 µm, p<0.05) (Rubin et al., 2016).

**Bone turnover**

Although I found the bone formation and bone resorption markers to be low in my patients relative to controls, most did not reach significance. As such, a large cross-sectional study found that bone formation marker, osteocalcin, was lower in both male and female patients (age range 5 – 69 years) with T1DM throughout most of life. Recent meta-analyses support these findings and report osteocalcin to be lower in patients with T1DM compared to controls (Hygum et al., 2017; Starup-Linde et al., 2014). The findings for markers of bone resorption in patients with T1DM are more inconsistent, with either reduced or no different values relative to non-diabetic controls (Starup-Linde, 2013; Starup-Linde et al., 2014).

**Limitations**

There are a number of limitations to this study. Firstly, the study is cross-sectional so causality between T1DM and changes in bone structure cannot be inferred. The patient sample sizes are small, especially in female patients, which may cause insufficient power to detect changes. Furthermore, information in patients with longstanding T1DM regarding precise pubertal status at time of diagnosis is lacking. Throughout childhood and adolescence, there are growth related differences in the rate of axial and appendicular growth as well as differences in the growth of bone types, bone regions and bone surfaces depending on pubertal stage of development. Hence deficits may occur in the appendicular sites if pre-pubertal onset, axial sites if early pubertal onset or affect vBMD if late pubertal onset of T1DM.
Another limitation is the technique used to analyze bone structure. Threshold based analysis algorithms are unable to accurately identify the endocortical surface, especially when intra-cortical remodelling in the inner cortex is present. In addition, accurate measurement of cortical porosity is difficult, especially when the image resolution of high resolution peripheral quantitative computed tomography is at best 95 – 130µm while >80% of intra-cortical pores are <100µm in diameter. Hence, this results in underestimation of cortical porosity (Zebaze et al., 2013).

In conclusion, patients with type 1 diabetes mellitus may have underlying bone fragility, however, I was not able to detect any significant bone micro-structural deficits. Future studies will be done re-examining the effects of diabetes using the StrAx algorithm and further work is needed to determine whether abnormalities in the material properties of bone may account for the increased fracture risk seen in T1DM.
5.2 Type 2 diabetes mellitus

5.2.1 Summary

To investigate the structural basis underlying the increased fracture risk in patients with type 2 diabetes mellitus (T2DM), I studied 55 patients with T2DM (28 females and 27 males, mean ages 63 and 62 years respectively) and 57 age- and sex-matched controls using high resolution peripheral quantitative computed tomography.

At the distal radius, relative to controls, males with diabetes had 10.4% smaller total cross sectional area (p = 0.06) and 14.2% smaller medullary area (p<0.05). Cortices were 12.7% thicker, not significantly so (p = 0.08). Cortical vBMD and compact cortical porosity did not differ from controls. Trabecular vBMD was 24.8% higher due to 28.6% thicker trabeculae in males (both p<0.05).

Females with diabetes had similar total cross-sectional area and medullary area with a 24.4% larger cortical area due to thicker cortices (24.1%) (both p<0.05). Cortical vBMD and compact cortical porosity did not differ from controls. Trabecular vBMD was 26.1% greater due to there being 12.2% more trabeculae, 13.3% thicker trabeculae, and 13.6% lower trabecular separation (all p<0.05). Similar results were found at the distal tibia. Following adjustment for height, weight and BMI, some of the results became attenuated. In females, longer duration of T2DM was associated with smaller cortical area, lower cortical vBMD and higher cortical porosity.

Male and female patients had 10.0% and 14.3% higher lumbar spine aBMD and 13.4% and 12.5% higher total hip aBMD respectively (all p<0.05). Patients also had lower bone formation markers (PINP, male 27.8 ± 7.1 vs. 70.8 ± 4.6 µg/L and female 42.2 ± 11.3 vs. 51.2 ± 7.8 µg/L; both p<0.05) and bone resorption markers (CTx, male 237.7 ± 93.6 ng/L vs. 577.8 ± 41.1 and female 372.4 ± 50.6 vs. 548.2 ± 76.8 ng/L; both p<0.05)
Thus, cortical bone microstructure was no different from controls while trabecular bone microstructure was better maintained. Therefore, it is unlikely that the higher fracture risk in patients with diabetes is explained by deterioration in microstructure.

5.2.2 Introduction

Although type 2 diabetes is associated with characteristics that would be beneficial for the skeleton such as higher areal BMD as well as elevated body weight and body mass index, yet fracture risk is elevated. Patients with T2DM have higher fracture risk at the hip, vertebral and non-hip, non-vertebral sites (Janghorbani et al., 2007; Vestergaard, 2007). The risk of fracture is higher at a given femoral neck BMD T score and age or for a given FRAX score in individuals with diabetes (Giangregorio et al., 2012; Schwartz et al., 2011).

While fracture risk is more modestly increased compared to patients with type 1 diabetes mellitus (T1DM), the prevalence of T2DM is far greater. In Australia, the prevalence of T2DM in adults is ~1 million people in 2014-15, which has trebled since 1989-90 (Australian Institute of Health and Welfare, 2017). With this diabetes epidemic, the issue of fragility fractures in diabetes becomes a public health issue.

There are many mechanisms contributing to bone fragility in diabetes. As bone structure is an important determinant of bone strength, abnormalities in skeletal structure have been documented for diabetes. Studies using bone histomorphometry to assess bone structure in patients T2DM are lacking but studies using HR-pQCT have reported variable skeletal phenotypes. Many studies have reported cortical deficits with preserved or increased trabecular bone (Burghardt et al., 2010; Patsch et al., 2013; Shanbhogue et al., 2016; Yu et al., 2015). While other studies have reported no difference in bone structure relative to controls (Farr et al., 2014; Shu et al., 2012). One study reported better maintained bone structure in patients with T2DM compared to controls (Nilsson et al., 2017).

Evidence suggesting low bone turnover in type 2 diabetes appears to be consistent with the finding that bone structure in patients with T2DM is preserved and/or better
maintained compared to controls. Recent meta-analyses have analyzed bone remodelling markers in patients with type 1 and type 2 diabetes mellitus. (Hygum et al., 2017; Starup-Linde et al., 2014). The pooled analyses combining studies in both T1DM and T2DM reported lower levels of bone formation markers osteocalcin and procollagen type I N-terminal propeptide (PINP) and bone resorption marker C-terminal telopeptide of type I collagen (CTx) compared to controls. Sub-group analysis by diabetes type found osteocalcin to be borderline lower in T2DM patients. Sub-group analysis for bone resorption markers were not analyzed. Individual study findings are more inconsistent for markers of bone resorption which have been reported as either increased, reduced or no different to controls (Starup-Linde, 2013; Starup-Linde et al., 2014).

The aim of the study was to examine bone microstructure in patients with type 2 diabetes mellitus. In light of low bone turnover seen in patients with T2DM, I hypothesize that this illness is associated with higher cortical area and cortical density due to lower cortical porosity as well as lower medullary area associated with better preserved trabecular morphology compared to controls.

5.2.3 Materials and Methods

Subjects
Fifty-five patients diagnosed with type 2 diabetes mellitus were recruited from a diabetes outpatient clinic at Austin Health. The diagnosis of type 2 diabetes was based on elevated fasting plasma glucose levels, HbA1c and/or oral glucose tolerance test; use of oral hypoglycaemic agents; clinical records and negative anti-glutamic acid decarboxylase and anti-islet antigen 2 antibodies. Healthy volunteers were recruited from the community as part of ongoing research in the department. No participants have other diseases or drug therapy known to affect bone.

Image acquisition and analysis
Refer to chapter 4. Images acquired using high resolution peripheral quantitative computed tomography were further analyzed for cortical porosity. The cortical porosity was derived using equation: Density based compact cortex porosity = \( [1 - (\text{cortical vBMD/1200})] \times 100 \)
Biochemical analyses

HbA1c was measured using turbidimetric inhibition immunoassay on Integra 800, Roche Diagnostics, Germany. Bone turnover markers procollagen type I N-terminal propeptide (PINP) and C-terminal telopeptide of type I collagen (CTx) as well as 25(OH) vitamin D were analysed using electro-chemiluminescence immunoassay on Cobas 8000, Roche Diagnostics, Germany.

Statistical analysis

All statistical analyses were conducted using SPSS version 23.0 (SPSS Inc, Chicago, USA). Comparisons of bone structural parameters between patients with T2DM and controls were performed using two sample student t tests. Multivariate analyses were used to make adjustments for differences in height, weight and BMI between cases and controls. The results were expressed as mean ± standard deviations for patient characteristics and mean ± standard error of the mean for bone variables. Linear correlation between duration of diabetes and bone variables were calculated and expressed using Pearson’s correlation coefficient. A p value of <0.05 was considered to be statistically significant.

5.2.4 Results

As shown in Table 5.2.1 patients with type 2 diabetes (T2DM) were matched with controls by age and sex. Patients with diabetes were heavier and had significantly greater body mass index (BMI). Procollagen type I N-terminal propeptide (PINP) and C-terminal telopeptide of type I collagen were lower and areal BMD at the spine and hip were significantly higher in patients with diabetes than controls.
<table>
<thead>
<tr>
<th></th>
<th>All T2DM n=55</th>
<th>All Controls n=57</th>
<th>Male T2DM n=27</th>
<th>Male Controls n=27</th>
<th>Female T2DM n=28</th>
<th>Female Controls n=30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.4 ± 10.4</td>
<td>61.8 ± 10.6</td>
<td>61.4 ± 10.6</td>
<td>61.1 ± 10.7</td>
<td>63.4 ± 10.2</td>
<td>62.4 ± 10.7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.66 ± 0.09</td>
<td>1.67 ± 0.11</td>
<td>1.72 ± 0.08</td>
<td>1.76 ± 0.08</td>
<td>1.59 ± 0.06</td>
<td>1.59 ± 0.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.7 ± 15.2*</td>
<td>75.0 ± 16.8</td>
<td>91.4 ± 12.5*</td>
<td>85.0 ± 13.7</td>
<td>78.2 ± 2.8*</td>
<td>66.4 ± 14.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.6 ± 4.1*</td>
<td>26.8 ± 4.6</td>
<td>30.7 ± 3.1*</td>
<td>27.5 ± 3.9</td>
<td>30.7 ± 0.9*</td>
<td>26.2 ±0.9</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>24</td>
<td>25</td>
<td></td>
<td></td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>61.8 ± 25.5</td>
<td>51.3 ± 10.1</td>
<td>54.5 ± 26.9</td>
<td>51.3 ± 12.2</td>
<td>69.5 ± 22.1*</td>
<td>51.3 ± 7.2</td>
</tr>
<tr>
<td>Hb₉ (%)</td>
<td>8.1 ± 1.2*</td>
<td>5.1 ± 0.1</td>
<td>8.0 ± 1.3*</td>
<td>5.0 ± 0.2</td>
<td>8.2 ± 1.3*</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>PINP (μg/L)</td>
<td>36.8 ± 12.1*</td>
<td>62.3 ± 10.6</td>
<td>27.8 ± 7.1*</td>
<td>70.8 ± 4.6</td>
<td>42.2 ± 11.3*</td>
<td>51.2 ± 7.8</td>
</tr>
<tr>
<td>CTx (ng/L)</td>
<td>321.9 ± 175.7*</td>
<td>564 ± 85.5</td>
<td>237.7 ± 93.6*</td>
<td>577.8 ± 41.1</td>
<td>372.4 ± 50.6*</td>
<td>548.2 ± 76.8</td>
</tr>
<tr>
<td>Spine BMD (g/cm²)</td>
<td>1.26 ± 0.21*</td>
<td>1.12 ± 0.22</td>
<td>1.32 ± 0.22*</td>
<td>1.20 ± 0.23</td>
<td>1.20 ± 0.19*</td>
<td>1.05 ± 0.18</td>
</tr>
<tr>
<td>Femoral neck BMD</td>
<td>0.97 ± 0.16</td>
<td>0.88 ± 0.16</td>
<td>1.01 ± 0.17</td>
<td>0.92 ± 0.18</td>
<td>0.92 ± 0.03</td>
<td>0.85 ± 0.03</td>
</tr>
<tr>
<td>Total hip BMD</td>
<td>1.05 ± 0.16*</td>
<td>0.92 ± 0.17</td>
<td>1.10 ± 0.15*</td>
<td>0.97 ± 0.18</td>
<td>0.99 ± 0.15*</td>
<td>0.88 ± 0.15</td>
</tr>
<tr>
<td>Duration of T2DM (years)</td>
<td>13.9 ± 8.1</td>
<td>12.0 ± 7.9</td>
<td></td>
<td></td>
<td>15.7 ± 7.9</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.2.1 Baseline patient characteristics

Values expressed as mean ± SD. *p<0.05 compared to controls.
Type 2 diabetes mellitus (T2DM), body mass index (BMI), bone mineral density (BMD), procollagen type I N-terminal propeptide (PINP), C-terminal telopeptide of type I collagen (CTx).
**Bone microstructure**

At the distal radius, male patients with T2DM, had smaller (10.4%, p=0.06) total cross-sectional and smaller (14.2%, p<0.05) medullary area both before and after adjustment for height, weight and BMI. Cortical area did not differ though cortical thickness was 12.7% higher (p=0.08). Total vBMD was 18.5% higher because of 24.8% higher trabecular vBMD due to 28.6% thicker trabeculae (all p<0.05). However, trabecular number and separation did not differ. Cortical vBMD and compact cortical porosity were similar to controls (Table 5.2.2).

Females with T2DM had similar total cross-sectional area and medullary area but larger cortical area (24.4%) associated with thicker cortices (24.1%, both p<0.05) than controls. Following adjustment for height, weight and BMI, results for total cross-sectional area, medullary area, cortical area (11.6%, p=0.14) and cortical thickness (13.1%, p=0.12) did not differ from controls. Total vBMD was 20.5% higher because trabecular vBMD was 26.1% greater due to 12.2% more trabeculae, 13.3% thicker trabeculae and 13.6% lower trabecular separation (all p<0.05). Following adjustment, results for trabecular vBMD was similar, however, differences in trabecular number (6.6%, p=0.23), thickness (7.8%, p=0.09) and separation (7.5%, p=0.26) relative to controls did not reach significance. Cortical vBMD and compact cortical porosity did not differ from controls. The findings for female and male patients with T2DM at the distal tibia were similar. (Table 5.2.2).

**Effect of disease duration**

Female patients had an inverse relationship between disease duration and distal radial cortical area (r=-0.54) and cortical vBMD (r=-0.66). Longer duration of disease was associated with higher compact cortical porosity (r=0.66) (all p<0.05) (Figure 5.2.1).

At the distal tibia, in male and female patients, there were weak or non-significant correlations between disease duration and cortical area (r=-0.26, p=0.20 and r=-0.31, p=0.10 respectively) and cortical vBMD (r=-0.33, p=0.10 and r=-0.54, p<0.05 respectively). Longer duration of disease was associated with higher compact cortical porosity (r=0.33, p=0.09 and r=0.55, p<0.05 respectively), which was not statistically significant in males (Figure 5.2.1). There were no significant associations between HbA1c and bone remodelling markers and bone structural parameters.
<table>
<thead>
<tr>
<th></th>
<th>Male T2DM</th>
<th>Male Controls</th>
<th>Male T2DM</th>
<th>Male Controls</th>
<th>Female T2DM</th>
<th>Female Controls</th>
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<tr>
<td></td>
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<td>(adjusted)</td>
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<td>(unadjusted)</td>
<td>(adjusted)</td>
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<tr>
<td><strong>Radius</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Total cross-sectional area (mm²)</td>
<td>349.0 ± 13.3</td>
<td>389.3 ± 16.6</td>
<td>351.9 ± 12.3</td>
<td>384.5 ± 12.8</td>
<td>258.4 ± 7.1</td>
<td>250.4 ± 8.7</td>
<td>256.4 ± 6.9</td>
<td>252.3 ± 6.6</td>
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<tr>
<td>Cortical area (mm²)</td>
<td>71.2 ± 2.7</td>
<td>67.5 ± 3.2</td>
<td>71.4 ± 3.2</td>
<td>67.9 ± 3.3</td>
<td>48.5 ± 2.1*</td>
<td>39.0 ± 2.3</td>
<td>46.1 ± 2.2</td>
<td>41.3 ± 2.1</td>
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<tr>
<td>Medullary area (mm²)</td>
<td>268.3 ± 13.7*</td>
<td>312.7 ± 15.6</td>
<td>270.8 ± 12.0*</td>
<td>307.9 ± 12.6</td>
<td>201.1 ± 7.3</td>
<td>201.8 ± 7.8</td>
<td>201.2 ± 6.7</td>
<td>201.7 ± 6.5</td>
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<tr>
<td>Total vBMD (mgHA/cm³)</td>
<td>360.4 ± 12.7*</td>
<td>304.2 ± 10.4</td>
<td>359.3 ± 12.0*</td>
<td>307.4 ± 12.5</td>
<td>328.0 ± 12.2*</td>
<td>272.1 ± 9.6</td>
<td>316.4 ± 10.9*</td>
<td>282.9 ± 10.5</td>
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<tr>
<td>Cortical vBMD (mgHA/cm³)</td>
<td>846.4 ± 12.0</td>
<td>843.1 ± 12.3</td>
<td>846.1 ± 12.9</td>
<td>844.8 ± 13.4</td>
<td>830.0 ± 13.3</td>
<td>801.6 ± 12.7</td>
<td>822.6 ± 14.0</td>
<td>808.5 ± 13.4</td>
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<tr>
<td>Cortical thickness (mm)</td>
<td>0.89 ± 0.04</td>
<td>0.79 ± 0.04</td>
<td>0.89 ± 0.04</td>
<td>0.80 ± 0.04</td>
<td>0.72 ± 0.04*</td>
<td>0.58 ± 0.03</td>
<td>0.69 ± 0.04</td>
<td>0.61 ± 0.03</td>
</tr>
<tr>
<td>Compact cortical porosity (%)</td>
<td>25.5 ± 0.9</td>
<td>25.5 ± 0.9</td>
<td>25.5 ± 1.0</td>
<td>25.4 ± 1.0</td>
<td>26.7 ± 1.0</td>
<td>28.2 ± 1.0</td>
<td>27.1 ± 1.1</td>
<td>27.9 ± 1.0</td>
</tr>
<tr>
<td>Trabecular vBMD (mgHA/cm³)</td>
<td>205.3 ± 8.9*</td>
<td>164.5 ± 6.5</td>
<td>204.1 ± 8.3*</td>
<td>166.3 ± 8.7</td>
<td>177.7 ± 7.7*</td>
<td>140.9 ± 6.9</td>
<td>170.8 ± 7.5*</td>
<td>147.3 ± 7.2</td>
</tr>
<tr>
<td>Bone volume/total volume (%)</td>
<td>17.1 ± 0.7*</td>
<td>13.7 ± 0.5</td>
<td>17.0 ± 0.7*</td>
<td>13.9 ± 0.7</td>
<td>14.8 ± 0.6*</td>
<td>11.7 ± 0.5</td>
<td>14.2 ± 0.6*</td>
<td>12.3 ± 0.6</td>
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<tr>
<td>Trabecular number (mm⁻¹)</td>
<td>1.91 ± 0.06</td>
<td>1.97 ± 0.06</td>
<td>1.89 ± 0.06</td>
<td>2.01 ± 0.06</td>
<td>1.75 ± 0.06*</td>
<td>1.56 ± 0.06</td>
<td>1.70 ± 0.06</td>
<td>1.66 ± 0.06</td>
</tr>
<tr>
<td>Trabecular thickness (mm)</td>
<td>0.090 ± 0.002*</td>
<td>0.070 ± 0.002</td>
<td>0.090 ± 0.003*</td>
<td>0.069 ± 0.003</td>
<td>0.085 ± 0.003*</td>
<td>0.075 ± 0.002</td>
<td>0.083 ± 0.003</td>
<td>0.077 ± 0.003</td>
</tr>
<tr>
<td>Trabecular separation (mm)</td>
<td>0.45 ± 0.02</td>
<td>0.45 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>0.44 ± 0.02</td>
<td>0.51 ± 0.02*</td>
<td>0.59 ± 0.03</td>
<td>0.53 ± 0.03</td>
<td>0.57 ± 0.03</td>
</tr>
<tr>
<td><strong>Tibia</strong></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total cross-sectional area (mm²)</td>
<td>862.4 ± 36.5</td>
<td>927.0 ± 33.9</td>
<td>877.3 ± 24.7</td>
<td>908.2 ± 25.2</td>
<td>651.7 ± 15</td>
<td>663.7 ± 18.8</td>
<td>641.5 ± 14.2</td>
<td>673.2 ± 13.7</td>
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<td>Cortical area (mm²)</td>
<td>144.8 ± 6.7</td>
<td>128.6 ± 5.3</td>
<td>142.9 ± 6.3</td>
<td>130.2 ± 6.5</td>
<td>100.2 ± 4.4</td>
<td>88.1 ± 5.2</td>
<td>96.6 ± 4.9</td>
<td>91.6 ± 4.8</td>
</tr>
<tr>
<td>Medullary area (mm²)</td>
<td>704.3 ± 38.3</td>
<td>790.2 ± 33.5</td>
<td>720.6 ± 27.2</td>
<td>770.4 ± 27.8</td>
<td>537.4 ± 16.2</td>
<td>561.0 ± 18.2</td>
<td>531.0 ± 15.4</td>
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<tr>
<td>Total vBMD (mgHA/cm³)</td>
<td>323.4 ± 11.1*</td>
<td>277.2 ± 8.5</td>
<td>320.7 ± 10.7*</td>
<td>279.2 ± 10.9</td>
<td>300.1 ± 9.8*</td>
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<td>838.1 ± 12.8</td>
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<td>833.8 ± 12.9</td>
<td>816.4 ± 15.0</td>
<td>789.5 ± 12.2</td>
<td>813.5 ± 14.7</td>
<td>792.2 ± 14.2</td>
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<tr>
<td>Cortical thickness (mm)</td>
<td>1.27 ± 0.07*</td>
<td>1.07 ± 0.05</td>
<td>1.24 ± 0.06</td>
<td>1.10 ± 0.06</td>
<td>1.01 ± 0.05</td>
<td>0.87 ± 0.05</td>
<td>0.98 ± 0.05</td>
<td>0.89 ± 0.05</td>
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<td>Compact cortical porosity (%)</td>
<td>28.7 ± 1.0</td>
<td>28.6 ± 1.0</td>
<td>28.9 ± 1.1</td>
<td>28.5 ± 1.2</td>
<td>30.4 ± 1.2</td>
<td>32.5 ± 1.0</td>
<td>30.5 ± 1.2</td>
<td>32.3 ± 1.2</td>
</tr>
<tr>
<td>Trabecular vBMD (mgHA/cm³)</td>
<td>198.2 ± 8.2*</td>
<td>174.7 ± 7.01</td>
<td>199.5 ± 8.0*</td>
<td>172.3 ± 8.2</td>
<td>184.8 ± 6.9*</td>
<td>161.6 ± 7.6</td>
<td>174.9 ± 7.1</td>
<td>166.6 ± 6.9</td>
</tr>
<tr>
<td>Bone volume/total volume (%)</td>
<td>16.5 ± 0.7*</td>
<td>14.6 ± 0.6</td>
<td>16.6 ± 0.7*</td>
<td>14.4 ± 0.7</td>
<td>15.4 ± 0.6*</td>
<td>13.5 ± 0.6</td>
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<td>Trabecular number (mm⁻¹)</td>
<td>1.82 ± 0.09</td>
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<td>1.81 ± 0.09</td>
<td>2.02 ± 0.09</td>
<td>1.67 ± 0.07</td>
<td>1.66 ± 0.08</td>
<td>1.60 ± 0.07</td>
<td>1.72 ± 0.07</td>
</tr>
<tr>
<td>Trabecular thickness (mm)</td>
<td>0.095 ± 0.004*</td>
<td>0.074 ± 0.003</td>
<td>0.095 ± 0.004*</td>
<td>0.073 ± 0.004</td>
<td>0.094 ± 0.003*</td>
<td>0.083 ± 0.003</td>
<td>0.095 ± 0.003*</td>
<td>0.082 ± 0.003</td>
</tr>
<tr>
<td>Trabecular separation (mm)</td>
<td>0.47 ± 0.02</td>
<td>0.45 ± 0.02</td>
<td>0.53 ± 0.06</td>
<td>0.46 ± 0.06</td>
<td>0.54 ± 0.03</td>
<td>0.58 ± 0.05</td>
<td>0.56 ± 0.04</td>
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</tr>
</tbody>
</table>

Table 5.2.2 Bone microstructure in patients with type 2 diabetes and controls

Values expressed as mean ± SEM. *p<0.05 compared to controls. Adjusted for height, weight and body mass index.
Figure 5.2.1 Correlations between cortical parameters and duration of type 2 diabetes

Cortical area, cortical vBMD and compact cortical porosity in female (filled dots, solid line) and male (open dots, dashed line) patients with type 2 diabetes mellitus plotted against duration of disease at the distal radius and tibia.
5.2.5 Discussion

In this cohort of patients with type 2 diabetes mellitus I report that at the distal radius and distal tibia, cortical morphology was no different whereas trabecular morphology was better maintained than controls. In male patients with diabetes, cortical area, vBMD and porosity were no different. Medullary area was smaller and trabecular vBMD was higher due to thicker trabeculae. In female patients, whilst cortical area was larger, cortical vBMD and porosity were no different from controls. Medullary area was no different, whereas trabecular vBMD was higher due to more trabeculae, thicker trabeculae and lower trabecular separation. These results are consistent with higher aBMD in the spine and hip as well as lower bone remodelling markers observed in diabetic patients.

Bone microstructure

In contrast to my findings, several studies have reported cortical deficits associated with preserved or increased trabecular bone in patients with T2DM. These observations have been reported in various cohorts with T2DM, including post-menopausal women, post-menopausal women with fractures, African American post-menopausal women and patients with microvascular complications (Burghardt et al., 2010; Patsch et al., 2013; Shanbhogue et al., 2016; Yu et al., 2015).

In these studies, increased cortical porosity accounted for most of the cortical deficits. Most of the cohorts were composed entirely of older post-menopausal women. Evidence in healthy women has suggested that most of the bone loss occurs after 65 years of age with much of it being cortical (Zebaze et al., 2010). Therefore, older post-menopausal women are more likely to have cortical deficits. Another interpretation of the findings may be that intra-cortical remodelling adjacent to the medulla produces trabecularization of the cortex. This results in cortical remnants that resemble trabeculae and erroneously included in the medullary compartment.

Consistent with my findings, a large study using post-menopausal women with T2DM has also reported larger cortical area, higher cortical vBMD and reduced cortical porosity along with greater trabecular bone volume fraction due to more trabeculae and lower trabecular separation in cases compared to controls (Nilsson et al., 2017). Other studies have reported no changes to cortical as well as trabecular bone microstructure in cases compared to
controls, which does not contradict my findings of preserved cortical bone and better maintained trabecular bone (Farr et al., 2014; Shu et al., 2012).

Despite maintained or better bone structure in some patients with T2DM, they do not appear to derive any additional benefit with bone strength. Studies using micro-finite element analysis have generally demonstrated equivalent estimates of bone strength in individuals with and without T2DM (Burghardt et al., 2010; Patsch et al., 2013; Shanbhogue et al., 2016; Yu et al., 2015). Therefore, other factors may be responsible for the excess skeletal fragility in diabetes.

**Material properties**

Abnormalities in material composition may compromise bone strength and account for the higher fracture rate reported in many studies. Two studies have assessed tissue mineralization density in patients with T2DM and report discrepant results. A study involving post-menopausal African American women with (n=22) and without (n=78) T2DM has found reduced cortical tissue mineral density associated with increased cortical porosity and lower cortical vBMD as well as comparable trabecular micro-architecture (Yu et al., 2015). In contrast, another study using a more racially diverse cohort of elderly women with (n=19) and without (n=19) T2DM reported no difference in cortical tissue mineral density and cortical vBMD accompanied with increased cortical porosity (Burghardt et al., 2010). Although neither study measured remodelling markers, T2DM is associated with low bone remodelling so higher, not lower, tissue mineralization density would be expected.

Several studies involving post-menopausal women with longstanding T2DM have assessed fracture toughness using impact micro-indentation, which is a reference point indentation technique. Although these women appear to have maintained bone microstructure to controls as assessed by HR-pQCT, the bone material strength index, which is the outcome measured by micro-indentation was lower in patients compared to controls (Farr et al., 2014; Furst et al., 2016; Nilsson et al., 2017).

Advanced glycation end-products (AGEs) may play a role in impaired material properties in patients with T2DM. A small study used skin autofluorescence to measure AGEs as well as micro-indentation in post-menopausal women with (n=16) and without T2DM. Similar to other studies, the bone material strength index was reduced in individuals with diabetes.
Higher levels of skin autofluorescence were seen in patients. In addition, skin autofluorescence correlated inversely with bone material strength index for the diabetes cohort ($r=-0.65$, $p=0.006$) (Furst et al., 2016).

**Bone turnover**

Histomorphometry studies confirm the findings of lower bone remodelling in patients with T2DM. The study by Krakauer et al has reported reduced histological indices of bone turnover in patients with diabetes. However the diabetes cohort comprised 8 patients in total (2 with T1DM and 6 with T2DM) and included men and women as well as African Americans and Caucasians. These patients were inappropriately compared to pre-menopausal females. No histological bone structure parameters were reported. (Krakauer et al., 1995). Similar results were found in another study demonstrating lower osteoid volume and osteoblast numbers in a small cohort of patients with T2DM (n=26) compared to controls (n=20) (Leite Duarte et al., 1996). Furthermore, reduced bone turnover has been observed in all three bone compartments: trabecular, endocortical and intra-cortical. The low bone turnover state associated with diabetes may help explain the preserved bone microstructure seen in a subset of patients with T2DM.

**Limitations**

One limitation of the study is the relatively small sample size and heterogeneity in patient characteristics in each group, which may restrict the ability to adequately demonstrate bone structural differences between individuals with and without diabetes. Since this is a cross-sectional observational study, causality cannot be proven. Another limitation is the method used to analyze bone microstructure. Threshold based analysis techniques are unable to accurately identify the endocortical margin, especially when intra-cortical remodelling in the inner cortex takes place. Furthermore, accurate quantification of cortical porosity is difficult when the image resolution of high resolution peripheral quantitative computed tomography is at best 95 – 130µm while >80% of intra-cortical pores are <100µm in diameter. Hence, this results in underestimation of cortical porosity (Zebaze et al., 2013).

In conclusion, patients with type 2 diabetes may have bone fragility predisposing to fractures but my study and many others failed to identify microstructural abnormalities responsible for any bone fragility. I propose that it is more likely that abnormalities in material composition
may contribute to bone fragility. Future studies will be done re-examining the effects of diabetes using the StrAx algorithm.
5.3 Primary hyperparathyroidism

New Insights into the Effects of Primary Hyperparathyroidism on the Cortical and Trabecular Compartments of Bone

Short title: Primary Hyperparathyroidism and Bone Microarchitecture

Thuy D.T. Vu
Xiao Fang Wang
Qingju Wang
Natalie E. Cusano
Dinaz Irani
Barbara C. Silva
Ali Ghasem-Zadeh
Julia Udesky
Megan E. Romano
Roger Zebaze
George Jerums
Stephanie Boutroy
John P. Bilezikian
Ego Seeman

Department of Medicine and Endocrinology, Austin Health, University of Melbourne, Heidelberg, Australia; Department of Medicine and Division of Endocrinology, Metabolic Bone Diseases Unit, College of Physicians and Surgeons, Columbia University, New York, USA.

All authors contributed substantially to this work

Correspondence:

E Seeman
Department of Endocrinology, level 2, Centaur Building, Austin Health
300 Waterdale Road Heidelberg West, Victoria 3081, Australia
Phone: +61394965489 Fax: +61394963365 Email: egos@unimelb.edu.au

Disclosures:

Authors state that they have no conflicts of interest

142
Abstract

In primary hyperparathyroidism (PHPT), protracted elevation of serum parathyroid hormone (PTH) is held to be associated with cortical, but not trabecular, bone loss. However, an alternative explanation for the apparent preservation of trabecular bone is fragmentation of the cortex by intracortical remodeling. The cortical fragments resemble trabeculae and so may be erroneously included in the quantification of ‘trabecular’ bone density.

To test this hypothesis, we compared bone microarchitecture in 43 patients with untreated PHPT (mean 62.9 years, range 31-84) with 47 healthy age-matched controls and 25 patients with surgically treated PHPT (63.6 years, 30-82). Images of the distal radius and tibia were acquired using high-resolution peripheral quantitative CT and analysed using StrAx1.0, a new software program that quantifies bone morphology in-vivo. Results were expressed as the mean number of standardized deviations (SD) from the age-specific mean (Z scores, mean ± SEM).

In subjects with PHPT, total tibial cortical area was reduced (-0.26 ± 0.08SD; p=0.002). Cortical volumetric bone mineral density (vBMD) was reduced (-0.29 ± 0.06SD; p<0.001) due to higher cortical porosity (0.32 ± 0.06SD; p<0.001) and lower tissue mineralization density (-0.21 ± 0.06SD; p=0.002). Medullary area was increased (0.26 ± 0.08SD; p=0.002) and trabecular vBMD was reduced (-0.14 ± 0.04SD; p<0.001).

In subjects who had undergone successful parathyroidectomy, cortical (-0.18 ± 0.10; NS) and medullary areas (0.18 ± 0.10; NS) did not differ from controls. Cortical vBMD was reduced (-0.15 ± 0.05SD; p=0.003) due to high porosity (0.15 ± 0.05SD; p=0.006), values numerically
lower than in untreated PHPT. Tissue mineralization density (-0.26 ± 0.04SD; p<0.001) and trabecular vBMD were also low (-0.16 ± 0.04SD, p<0.001). The results were similar in the distal radius.

In PHPT, chronically elevated endogenous PTH does not spare trabecular bone; it causes bone loss and microarchitectural deterioration in both cortical and trabecular compartments of bone.

**Key words:** bone microarchitecture, cortical porosity, high-resolution peripheral quantitative computed tomography, parathyroid hormone excess, tissue mineralization density
1.0 Introduction

Bone remodeling is surface dependent; it is initiated upon the three (intracortical, endocortical and trabecular) components of a bone’s inner (endosteal) envelope. During young adulthood remodeling is balanced; the volume of old or damaged bone matrix excavated beneath the surface is replaced with an equal volume of new bone matrix (Hattner et al., 1965). During advancing age, remodeling becomes unbalanced; less bone is deposited than was removed so that each time a remodeling event occurs, focal structural deterioration ensues (Seeman et al., 2006).

After menopause and in diseases such as primary hyperparathyroidism (PHPT), structural deterioration accelerates because the surface extent of remodeling increases and the negative bone multi-cellular unit (BMU) balance worsens (Parfitt, 1983). Remodeling upon trabecular surfaces thins and removes them. Remodeling upon endocortical surfaces thins the cortex and enlarges the medullary cavity. Intra-cortical remodeling enlarges haversian canals focally producing intracortical porosity. Intense intracortical remodeling in cortex adjacent to the medullary canal cavitates it, haversian canals coalesce worsening the porosity, thinning the cortex from ‘within’ producing cortical remnants that look like trabeculae (‘trabecularization’) (Zebaze et al., 2010). Intracortical remodeling and porosity account for 70% of total bone lost with age (Zebaze et al., 2010).

Parathyroid hormone (PTH) excess in PHPT produces cortical bone loss but is purported to preserve bone at trabecular sites (Bilezikian, 2003; Dempster et al., 2007; Dempster et al., 1999; Parfitt, 1983; Parisien et al., 1990; Silverberg et al., 2010; Silverberg et al., 1989; Zebaze et al., 2010). This notion is based on finding normal or increased so-called
‘trabecular’ bone volumetric bone mineral density (vBMD) when assessed by noninvasive imaging and normal volume/total volume (BV/TV) using histomorphometry (Bilezikian, 2003; Dempster et al., 2007; Dempster et al., 1999; Parfitt, 1983; Parisien et al., 1990; Silverberg et al., 2010; Silverberg et al., 1989; Zebaze et al., 2010).

These disparate findings in the cortical and trabecular compartments are unusual because the three, endocortical, trabecular and intracortical, components of the endosteal (inner) envelope are contiguous; they are connected forming one inner (endosteal) surface ‘enveloping’ the mineralized bone matrix volume. Remodeling with loss of bone due to the negative bone balance is generally similar on each of these three surface (Han et al., 1996).

Thus, an alternative interpretation is that the normal or increased trabecular vBMD in PHPT is an artifact produced by inclusion of the cortical remnants because they resemble true trabeculae, namely those of end plate origin (Zebaze et al., 2010). Cortical remnants are unlikely to confer normal bone strength because ‘trabecularized’ bone is chaotic and lacks the cancellous architectural design of trabecular bone (Zebaze et al., 2010). This view would also reconcile the increased vertebral fracture risk in PHPT, an anomalous observation if endogenous PTH preserved or increased trabecular bone volume in PHPT (Bilezikian, 2003).

We hypothesized that endogenous PTH in PHPT produces both cortical and trabecular bone loss. In addition, we hypothesized that the cortical remnants are erroneously called “trabeculae” and are included in the analysis of the trabecular density in the medullary compartment. We also hypothesized that there are residual deficits in both cortical and trabecular bone following parathyroidectomy. To test these hypotheses, we analyzed images
of the distal radius and tibia acquired by high-resolution peripheral quantitative CT (HR pQCT), using StrAx1.0, a new software program that quantifies bone morphology in-vivo.

2.0 Materials and Methods

2.1 Patient cohort
Patients with untreated and surgically treated PHPT were recruited from Austin Hospital (Melbourne, Australia) and Columbia University Medical Center (New York, USA). We studied 43 untreated PHPT, 25 treated PHPT, and 47 age-matched healthy controls. The healthy participants, previously published were recruited from the community (Zebaze et al., 2010). The mean duration post-parathyroidectomy was 4.3 ± 4.8 years. The study was approved by the Human Research Ethics Committee of Austin Health and the Institutional Review Board of Columbia University Medical Center. All subjects gave written, informed consent.
Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Untreated PHPT (n = 43)</th>
<th>Treated PHPT (n = 25)</th>
<th>Controls (n = 47)</th>
<th>Reference ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.9 ± 13.8</td>
<td>63.6 ± 13.6</td>
<td>61.1 ± 14.9</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.63 ± 0.11</td>
<td>1.64 ± 0.09</td>
<td>1.66 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.9 ± 18.0</td>
<td>75.3 ± 16.7</td>
<td>74.4 ± 13.5</td>
<td></td>
</tr>
<tr>
<td>Female:Male</td>
<td>31:12</td>
<td>17:8</td>
<td>31:16</td>
<td></td>
</tr>
<tr>
<td>Caucasian:Hispanic:African American</td>
<td>41:1:1</td>
<td>24:1:0</td>
<td>47:0</td>
<td></td>
</tr>
<tr>
<td>Melbourne:New York patients</td>
<td>31:12</td>
<td>15:10</td>
<td>47:0</td>
<td></td>
</tr>
<tr>
<td>Time since diagnosis (years)</td>
<td>1.9 ± 2.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time since surgery (years)</td>
<td>5.3 ± 5.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected serum calcium (mmol/L)</td>
<td>2.66 ± 0.16$^a$</td>
<td>2.41 ± 0.20$^a$</td>
<td>2.42 ± 0.11$^b$</td>
<td>2.10 – 2.60</td>
</tr>
<tr>
<td>Serum phosphate (mmol/L)</td>
<td>1.02 ± 0.21</td>
<td>1.10 ± 0.16</td>
<td>1.24 ± 0.20</td>
<td>0.60 – 1.40</td>
</tr>
<tr>
<td>Serum PTH (pmol/L)</td>
<td>13.0 ± 5.7$^a$</td>
<td>6.4 ± 4.3$^b$</td>
<td>4.1 ± 0.9$^b$</td>
<td>1.6 – 6.9</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>58.9 ± 13.8</td>
<td>55.4 ± 18.1</td>
<td>54.5 ± 23.3</td>
<td>&gt;75$^c$</td>
</tr>
<tr>
<td>% on cholecalciferol supplementation</td>
<td>48</td>
<td>47</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>% on calcium supplementation</td>
<td>0</td>
<td>26</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD

$^a$Untreated PHPT vs. Treated PHPT p <0.05

$^b$Untreated PHPT vs. Control p <0.05

$^c$Represents the desirable level of 25(OH) vitamin D at our institution

2.2 Image acquisition & analysis

High-resolution peripheral quantitative CT (HR-pQCT, XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland) was used to quantify morphology at the non-dominant distal radius and tibia (Laib et al., 1998). Cortical and trabecular cross sectional area, vBMD and microarchitecture were measured with an isotropically resolved voxel size of 82 µm. The CV for vBMD was 0.6–1.4% and for microarchitectural indices was 0.9–4.4%. Daily quality control was carried out by scanning a phantom containing rods of hydroxyapatite (QRM
Moehrendorf, Germany). Radiation exposure was ~3 $\mu$Sv per measurement. The manufacturer’s imaging protocol was followed. Trabecular number, thickness and separation were analysed using Scanco Medical AG software. The coefficient of variation ranged 2-4% for microstructure at both institutes.

Images acquired using HR-pQCT were analysed with new software (StrAx1.0, University of Melbourne, Melbourne, Australia). This uses a non-threshold image segmentation algorithm to quantify bone morphology (Zebaze et al., 2012; Zebaze et al., 2012). From the original images, bone is segmented from soft tissue and color-coded according to the void content of each voxel, then segmented into its compact-appearing cortex, outer transitional zone (OTZ) adjacent to the cortex, inner transitional zone (ITZ) adjacent to the medulla and trabecular bone. The total cortex consists of the compact-appearing cortex and transitional zone. Frequency distribution curves depicting the composition of the cortex according to voxel content (empty, mineralized bone matrix only, varying proportions of each) are calculated. Bone compartments, cortical morphology and trabecular vBMD were derived using this method (Figure 1 & table 2). The CV for these analyses were 1-2% (Zebaze et al., 2012; Zebaze et al., 2012). Total cortical area, the compact-appearing cortical area, OTZ and ITZ and medullary area were expressed as a percentage of the total CSA to adjust for bone size. Tissue mineralization density was determined by the attenuation produced by voxels whose volume was occupied by bone matrix and no void volume.
Figure 1. Segmentation and quantification of HR-pQCT images by StAx1.0. The acquired grey scale image (A) is analysed using StAx1.0 which separates the whole bone from the soft tissue background and the bone into its compact-appearing cortical, transitional (consisting of inner and outer zones) and trabecular compartments (B, C). Color-coding is based on the proportion of mineralized matrix and void in the voxels.

2.3 Biochemical analyses

Serum intact PTH and 25(OH) vitamin D were measured using an electro-chemiluminescence immunoassay (Roche Modular E170, Switzerland). Serum calcium and phosphate were analysed by automated techniques (unicel DXC 800, Beckman Coulter Inc, USA).
2.4 Statistical analyses

Measurements were expressed as mean Z scores (± SEM), the number of standardised deviations (SD) from age-, sex- height- and weight- adjusted mean of zero based on the linear regression in 47 healthy controls. T-tests assessed whether the Z scores differed from zero. Group comparisons were made using ANOVA. The contribution of cortical porosity and tissue mineralization density to the variance in cortical vBMD was determined using stepwise regression analysis. A frequency distribution curve of mineralized bone matrix content of compact-appearing cortex was plotted for the three groups. A p value of <0.05 was considered to be statistically significant. Analyses were conducted using SPSS version 20 software (SPSS Inc, Chicago, USA).

3.0 Results

3.1 Untreated PHPT vs. controls

As shown in Figure 2 and Table 2, for the distal tibia, relative to controls, untreated patients with PHPT had reduced total cortical (compact-appearing plus transitional zone) area (-0.26 ± 0.08 SD, p = 0.002) and increased medullary area (0.26 ± 0.08 SD, p = 0.002). The compact-appearing cortical area and outer transitional zone area were each reduced (-0.31 ± 0.08 SD, p <0.001 and -0.43 ± 0.15 SD, p = 0.007 respectively). The inner transitional zone area was not reduced.

Cortical vBMD was reduced (-0.29 ± 0.06 SD, p <0.001) due to (i) increased cortical porosity in the compact-appearing (0.13 ± 0.04 SD, p = 0.003) and in the transitional zones (OTZ, 0.16 ± 0.05 SD, p = 0.002 and ITZ, 0.32 ± 0.04 SD, p <0.001) and (ii) reduced mineralization density (-0.21 ± 0.06 SD, p = 0.002) of the surrounding bone matrix. Of the reduction in cortical vBMD, 97.5% was attributable to the increased cortical porosity with only 2.0%
attributable to lower tissue mineralization density. Cortical vBMD and cortical porosity correlated inversely with $r = 0.975$ before and $0.983$ after adjustment for tissue mineralization (both $p <0.001$). The void bone matrix mineralization distribution curve was shifted left relative to controls (Figure 4). Trabecular vBMD was reduced $(-0.14 \pm 0.04$ SD, $p <0.001$). Trabecular separation was increased $(0.07 \pm 0.03$ SD, $p = 0.030$) but trabecular number $(-0.06 \pm 0.05$ SD; $p = 0.250$) and the trabecular thickness $(0.10 \pm 0.06$ SD; $p = 0.110$) did not differ from controls.

3.2 Treated PHPT vs. controls

In successfully treated patients relative to controls, total cortical area was not reduced and medullary area was not increased. Compact-appearing cortex was reduced $(-0.22 \pm 0.09$ SD; $p = 0.03)$. The outer and inner transition zones were no different from controls. Cortical vBMD was reduced $(-0.15 \pm 0.05$ SD; $p = 0.003$). Overall, total cortical porosity was increased due to increased ITZ porosity, while porosity in compact-appearing cortex and OTZ were no different relative to controls. Tissue mineralization density distribution was shifted to the left relative to controls.

3.3 Treated PHPT vs. untreated PHPT

Total cortical area and compact-appearing cortex were larger than untreated PHPT patients by 3% and 10% respectively, though not significantly so. The outer transition zone area was greater than the untreated PHPT patients by 15%. The inner transition zone area was no different. Cortical vBMD was greater than the untreated PHPT patients by 8% ($p = 0.075$). Total cortical porosity was increased relative to controls but was 9% less than the untreated PHPT patients ($p=0.058$). In all three compartments, cortical porosity was increased but to a lesser extent relative to untreated PHPT patients (compact-appearing region by -22%; outer
zone porosity by -16%, and inner zone porosity by -2%). Tissue mineralization density was low to the same extent as in the untreated group (p = NS) (Figure 2 and Table 2). Relative to controls, trabecular vBMD was reduced (p <0.001). There was also reduced trabecular number (-0.19 ± 0.05 SD; p <0.001) but not thickness (0.09 ± 0.08 SD; NS). Trabecular separation was increased (0.24 ± 0.09 SD; p <0.010). In the group that underwent parathyroid surgery, trabecular vBMD, number, thickness and separation was no different than in the untreated group. Results for the distal radius were attenuated but showed a similar trend to that of the distal tibia.
Table 2. Bone morphology expressed as Z scores (mean ± SEM)

<table>
<thead>
<tr>
<th>Bone compartments</th>
<th>Untreated PHPT</th>
<th>Treated PHPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tibia</td>
<td>Radius</td>
</tr>
<tr>
<td>Total cortical area</td>
<td>-0.26 ± 0.08β</td>
<td>-0.04 ± 0.05</td>
</tr>
<tr>
<td>CC area</td>
<td>-0.31 ± 0.08γ</td>
<td>-0.03 ± 0.05</td>
</tr>
<tr>
<td>OTZ area</td>
<td>-0.43 ± 0.15β</td>
<td>0.02 ± 0.05</td>
</tr>
<tr>
<td>ITZ area</td>
<td>0.03 ± 0.04</td>
<td>-0.08 ± 0.08</td>
</tr>
<tr>
<td>Medullary area</td>
<td>0.26 ± 0.08β</td>
<td>0.04 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Tibia</td>
<td>Radius</td>
</tr>
<tr>
<td>Total cortical self</td>
<td>-0.18 ± 0.10</td>
<td>-0.11 ± 0.06</td>
</tr>
<tr>
<td>CC area</td>
<td>-0.22 ± 0.09α</td>
<td>-0.10 ± 0.07</td>
</tr>
<tr>
<td>OTZ area</td>
<td>-0.07 ± 0.09</td>
<td>0.02 ± 0.06</td>
</tr>
<tr>
<td>ITZ area</td>
<td>-0.03 ± 0.06</td>
<td>-0.06 ± 0.07</td>
</tr>
<tr>
<td>Medullary area</td>
<td>0.18 ± 0.10</td>
<td>0.11 ± 0.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cortical Morphology</th>
<th>Untreated PHPT</th>
<th>Treated PHPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cortical vBMD</td>
<td>-0.29 ± 0.06γ</td>
<td>-0.10 ± 0.05α</td>
</tr>
<tr>
<td>CC porosity</td>
<td>0.13 ± 0.04β</td>
<td>0.06 ± 0.06</td>
</tr>
<tr>
<td>OTZ porosity</td>
<td>0.16 ± 0.05β</td>
<td>0.14 ± 0.06α</td>
</tr>
<tr>
<td>ITZ porosity</td>
<td>0.32 ± 0.04γ</td>
<td>0.20 ± 0.06α</td>
</tr>
<tr>
<td>Tissue Mineralization Density</td>
<td>-0.21 ± 0.06β</td>
<td>-0.22 ± 0.05γ</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trabecular Morphology</th>
<th>Untreated PHPT</th>
<th>Treated PHPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabecular vBMD</td>
<td>-0.14 ± 0.04γ</td>
<td>-0.09 ± 0.06</td>
</tr>
<tr>
<td>Trabecular number^</td>
<td>-0.06 ± 0.05</td>
<td>-0.12 ± 0.09</td>
</tr>
<tr>
<td>Trabecular thickness^</td>
<td>0.10 ± 0.06</td>
<td>-0.43 ± 0.17α</td>
</tr>
<tr>
<td>Trabecular separation^</td>
<td>0.07 ± 0.03α</td>
<td>0.30 ± 0.15</td>
</tr>
</tbody>
</table>

Area is expressed as a proportion of the total bone cross-sectional area
CC (compact-appearing cortex)
OTZ (outer transitional zone)
ITZ (inner transitional zone)
α p value <0.05 compared to zero
β p value <0.01 compared to zero
γ p value <0.001 compared to zero
^ parameter derived from Scanco Medical AG software
Figure 2. Distal tibia (A, C) and radius (B, D) bone morphology expressed as Z scores adjusted for height and weight (mean ± SEM). To adjust for bone size, morphology is expressed as the proportion of the total bone cross-sectional area. Total cortical area, compact-appearing cortical (CC) area, outer transitional zone (OTZ), inner transitional zone (ITZ) and medullary area are shown in patients with untreated primary hyperparathyroidism (PHPT) and surgically treated patients. Cortical morphology with total cortical volumetric bone mineral density (vBMD) determined by total cortical porosity (comprising CC; OTZ, ITZ) and tissue mineralization density (mineralization). *p <0.05 compared to zero.
Figure 3. Distal tibia (A) and radius (B) trabecular (trab) morphology (vBMD, number, thickness and separation). *p <0.05 compared to zero.

Figure 4. Frequency or void-bone matrix distribution curve of voxels within the compact-appearing cortex of the distal tibia (A) and distal radius (B). Voxel on the left contain only void volume (0%), more void than mineralized bone matrix volume, more mineralised bone matrix than void volumes, and only mineralized bone matrix volume on the right (100%). Relative to controls, untreated PHPT had a left shift. Relative to untreated PHPT, treated PHPT had a right shift. These relative shifts were similar in both distal tibia and radius.
4.0 Discussion

We report that endogenous PTH excess as seen in PHPT is deleterious to both cortical and trabecular bone in the distal tibia. Specifically, (i) cortical bone area was reduced due to remodeling upon the intracortical surface eroding the cortex from ‘within’ producing porosity and upon the endocortical surface eroding it ‘outwards’ enlarging the medullary cavity, (ii) mean tissue mineralization density of the surrounding compact-appearing cortical bone matrix was reduced, (iii) there was a left shift of the void-matrix mineralization density distribution curve relative to controls due to a increased cortical porosity which reduced cortical vBMD. (iv) Trabecular vBMD was reduced. (v) Patients with surgically corrected PHPT had residual but somewhat less severe deficits in several traits. The results were similar but attenuated at the distal radius.

4.1 Cortical deficits

These results, and those using peripheral quantitative computed tomography (Charopoulos et al., 2006; Chen et al., 2003) and high resolution pQCT (Hansen et al., 2010), suggest that endogenous PTH excess results in structural deterioration of both cortical and trabecular bone and that these deficits may be partly reversible (Hansen et al., 2012). Patients with PHPT had reduced cortical bone area for two reasons. First, intracortical remodeling removed more bone than it replaced leaving void (porosity). Second, endocortical resorption eroded the cortex enlarging the medullary area. While endocortical resorption is commonly regarded as a main mechanism responsible for cortical thinning in PHPT, the main mechanism responsible is intracortical remodeling which thins the cortex from the ‘inside’ by cavitating the inner compact cortex (Zebaze et al., 2010). The transitional zone area increases at the price of compact appearing cortex as a result of intense remodeling in this region.
During remodeling, ~95% of the bone removed is replaced in patients with advancing age. This newly deposited (younger) bone matrix has a lower tissue mineralization density than the bone matrix removed so that there is a shift in the tissue mineralization density distribution to the left as more void is produced and an increasing proportion of the newly formed osteons have a lower tissue mineral density. Nevertheless, as primary mineralization occurs rapidly and achieves ~95% of full mineralization of collagen, little of the deficit in cortical vBMD was due to reduced tissue mineralization density; most of the deficit was the result of increased intracortical porosity.

4.2 Trabecular deficits

Intracortical remodeling in the inner cortex produces cortical remnants which resemble true trabeculae (i.e. of growth plate origin). These ‘trabeculae’ derived from cortical bone are usually included in quantification of trabecular vBMD. Most measurements of trabecular bone are not able to distinguish cortical remnants and true trabeculae and therefore show increased or normal trabecular bone volume in PHPT. When these cortical remnants were excluded from the analysis, trabecular vBMD was reduced due primarily to an increase in trabecular separation.

4.3 Increased bone remodeling intensity causes bone deficits

Increased bone formation rate determined using dynamic histomorphometry is reported in PHPT (Bilezikian, 2003; Delling, 1987; Dempster et al., 2007; Dempster et al., 1999; Parfitt, 1983; Parisien et al., 1995; Parisien et al., 1990; Silverberg et al., 2010; Silverberg et al., 1989; van Doorn et al., 1993; Zebaze et al., 2010; Zebaze et al., 2012; Zebaze et al., 2012). This measurement is the product of the surface extent of bone formation (reflecting activation
frequency or the intensity of bone remodeling) and the mineral apposition rate (MAR). Remodeling intensity and the degree of negativity of the BMU balance vary from surface to surface, but there is no evidence that remodeling events produce a net positive balance upon trabecular surfaces (resulting in trabecular bone gain) and a net negative balance upon the endocortical and intracortical surfaces (resulting in cortical bone loss). The increased in bone forming surfaces upon trabeculae in PHPT is the result of high surface extent of remodeling – there are more BMUs per unit surface area. This will not preserve trabecular bone volume unless the negative BMU balance is corrected. It will not increase trabecular bone volume unless the volume of bone deposited is greater than the volume removed by each of the increased numbers of BMUs. Evidence of increased mean wall thickness or MAR is not compelling. Thus, there is little convincing evidence to support the notion that PTH preserves or increases trabecular bone volume.

4.4 Residual bone deficits post-parathyroidectomy

In many of the parameters measured in the post-parathyroidectomy group, deficits were still present but less so than in untreated patients. The residual deficits suggest that the effects of PTH excess in PHPT are not entirely reversible because the negative BMU balance irreversibly removes complete trabeculae that cannot be reconstructed and produces large coalescent pores that cannot be refilled during the bone formation phase of bone remodeling because of the negative bone balance.

A cautionary note is needed because as this was not a prospective study; preoperative morphology was not known. The extent to which these changes can be shown to be reversible will require prospective studies with long follow-up after parathyroidectomy. Another
limitation is that all the controls were recruited in Melbourne, however there were no differences between the Melbourne and New York cases.

5.0 Conclusion

In conclusion, contrary to prevailing notions, PTH excess in PHPT is deleterious both to cortical and trabecular compartments of bone. Some data on fracture risk in PHPT, albeit limited, may be explained by these observations. The normal or high trabecular density reported in several studies is likely to be the result of inclusion of cortical remnants in the medullary compartment. It is likely that these cortical remnants do not function as competent trabecular elements.

Acknowledgement

The authors wish to thank Ms Karey Cheong for her assistance. Some of this work was supported, in part, by NIH grant DK32333.
References


[17] Hansen S, Beck Jensen JE, Rasmussen L, Hauge EM, Brixen K. Effects on bone geometry, density, and microarchitecture in the distal radius but not the tibia in


5.4 Hypoparathyroidism

5.4.1 Summary
To examine the underlying abnormalities in bone structure and material composition in patients with parathyroid hormone (PTH) deficiency, I investigated 14 patients with primary hypoparathyroidism and 24 patients with secondary hypoparathyroidism (13 males and 27 females with mean age of ~50 years for either sex) and 49 age- and sex-matched controls. Images were obtained using high resolution peripheral quantitative computed tomography and analysed using non-threshold based methods that has been published (Zebaze et al., 2013).

At the distal radius, relative to controls, males with hypoparathyroidism had larger total cortical area and compact cortical area by 10.0% and 18.7% respectively (both p<0.05). Total cortical vBMD was 11.3% (p=0.12) higher due to 11.1% (p=0.12) lower total cortical porosity and 2.0% (p=0.11) greater matrix mineral density, although significance was not reached for all. Trabecular vBMD was 50.5% (p=0.10) higher without achieving statistical significance.

Female patients with hypoparathyroidism had similar bone areas to controls. Total cortical vBMD was 4.2% (p=0.19) higher due to 4.6% (p=0.19) lower total cortical porosity and 1.2% (p<0.05) greater matrix mineral density, though significance was not consistently achieved. Trabecular vBMD was 26.1% (p=0.13) higher, without reaching significance, due to 16.2% (p<0.05) more trabeculae.

In patients with primary hypoparathyroidism, relative to controls, total cortical, compact cortical and OTZ area were larger by 15.3% (p<0.01), 24.5% (p<0.01) and 11.5% (p=0.06) respectively, although statistical significance was not achieved in some instances. Cortical and trabecular morphology were no different in patients and controls.

In patients with secondary hypoparathyroidism, relative to controls, bone cross-sectional areas were comparable. Total cortical vBMD was 7.7% higher due to 7.6% lower total cortical porosity and 1.8% greater matrix mineral density (all p<0.05). Porosities in the compact cortex, OTZ and ITZ were lower by 6.6% (p=0.15), 6.8% (p=0.09) and 5.4%
Trabecular vBMD was 45.6% (p<0.05) higher in patients than controls due to 84.2% (p=0.12) thicker trabeculae and 18.9% (p=0.06) better trabecular connectivity, without reaching significance in some instances.

At the distal tibia, male patients had similar bone areas to controls. Total cortical vBMD was 11.7% (p=0.16) higher due to 9.5% (p=0.16) lower total cortical porosity and 1.5% (p=0.15) higher matrix mineral density, although significance was not reached for all. Trabecular vBMD tended to be higher (35.5%, p=0.19) without reaching significance.

Female patients had 6.0% and 11.9% greater total cortical area and compact cortical area respectively (both p<0.05) compared to controls. Total cortical vBMD was 6.6% (p=0.13) higher because of 5.6% (p=0.13) lower total cortical porosity and 1.5% (p<0.05) greater matrix mineral density, although significance was not reached for all. Trabecular vBMD was 31.3% (p=0.11) higher due to 9.6% (p=0.19) more trabeculae, though neither reached significance.

The findings in the distal tibia for primary hypoparathyroid and secondary hypoparathyroid patients were similar to the distal radius.

At the distal radius and tibia in female, but not male patients, longer disease duration was associated with greater total cortical vBMD (r=0.35, p=0.07 and r=0.52, p<0.01 respectively), greater medullary vBMD (r=0.64, p<0.01 and r=0.59, p<0.01 respectively), higher matrix mineral density (r=0.54, p<0.01 and r=0.41, p<0.05 respectively) and lower total cortical porosity (r=−0.34, p=0.09 and r=−0.52, p<0.01 respectively).

The void bone matrix mineralization distribution curve was shifted to the right relative to controls at both the distal radius and tibia.

Thus, PTH deficiency due to secondary rather than primary hypoparathyroidism was associated with better maintained cortical and trabecular bone as well as higher matrix mineral density relative to controls. Similar results were found when the data was analysed by sex, though significance was not achieved in all instances. Furthermore, longer disease...
duration was associated with better maintained bone morphology. More prospective studies are needed to confirm these finding.

5.4.2 Introduction
The effects of PTH deficiency on fracture risk remain uncertain. Despite reports of increased areal BMD and better maintained bone structure, fracture outcome studies have produced inconsistent results. Two studies have reported increased morphometric vertebral fracture prevalence relative to controls in a post-surgical hypoparathyroid as well as idiopathic hypoparathyroid cohort (Chawla et al., 2017; Mendonca et al., 2013). In contrast, two large registry studies reported no increased risk in overall fractures in post-surgical hypoparathyroid as well as non-surgical hypoparathyroid patients compared to controls (Underbjerg et al., 2014; Underbjerg et al., 2015).

Since hypoparathyroidism is rare, there have been limited studies assessing bone structure. Studies using histomorphometry and micro-CT have observed increased trabecular bone volume associated with preserved and/or better maintained trabecular micro-architecture and connectivity (Langdahl et al., 1996; Rubin et al., 2010; Rubin et al., 2008). Cortical morphology was assessed by histomorphometry in one study and demonstrated a trend towards higher cortical width and lower cortical porosity, neither of which reached significance (Rubin et al., 2008).

Abnormalities in the mineral and collagen components of the bone matrix have been observed in patients with hypoparathyroidism. Studies using quantitative backscattered electron imaging have reported increased bone mineralization density distribution parameters in trabecular bone (Misof et al., 2016). Studies using Raman and Fourier transform infrared imaging have demonstrated high pyridinoline/divalent collagen cross-link ratio, which indicates increased collagen maturity (Paschalis et al., 2012; Paschalis et al., 2009). These findings are consistent with low bone remodelling that has been well documented in states of chronic PTH deficiency.

The aim of the study was to assess bone microstructure and material composition in subjects with chronic PTH deficiency. Since bone remodelling is low in patients with hypoparathyroidism, I hypothesize that this condition is associated with higher cortical area
and density, lower cortical porosity and increased tissue mineralization density as well as preserved trabecular morphology relative to healthy controls.

5.4.3 Materials and methods

Subjects
Forty patients with hypoparathyroidism were recruited in total. Four primary and 14 secondary hypoparathyroid patients from Austin Hospital (Melbourne, Australia) and 10 primary and 12 secondary hypoparathyroid patients from Columbia University Medical Centre (New York, USA). The diagnosis of hypoparathyroidism was based on serum calcium and PTH being below the normal reference range on at least two occasions, the ongoing need for calcium and/or vitamin D supplementation to maintain serum calcium in the low normal range as well as medical documentations. Forty-nine healthy volunteers were recruited from the community in Melbourne as part of ongoing research in the department.

Image acquisition and analysis
Images were obtained using high-resolution peripheral quantitative computed tomography (HR-pQCT, XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland) and further analyzed by StrAx1.0 (University of Melbourne, Melbourne, Australia). Refer to chapter 4.

Biochemical analyses
PTH (1-84) and 25(OH) vitamin D were measured by electro-chemiluminescence immunoassay on Cobas 8000, Roche Diagnostics, Germany. Serum calcium and phosphate were analysed by a spectrophotometric immunoassay using NM-BAPTA and ammonium molybdate on Cobas 8000, Roche Diagnostics, Germany.

Statistical analysis
All statistical analyses were conducted using SPSS version 23.0 (SPSS Inc, Chicago, USA). Comparison of bone structural parameters between patients with hypoparathyroid patients and controls were performed using two sample student t tests. A sub-group analysis was performed for primary and secondary hypoparathyroid patient groups to controls using analysis of variance (ANOVA). The results were expressed as mean ± standard deviation for patient characteristics and mean ± standard error of the mean for bone variables. A frequency
distribution curve of mineralized bone matrix content of compact-appearing cortex was plotted for the all the hypoparathyroid patient cohort from the Austin Hospital and controls using the initial StrAxl software. The latest StrAxl version used to re-analyse the Austin Hospital patients and controls as well as patients from Columbia University Medical Centre did not provide the relevant data to plot frequency distribution curve of mineralized bone matrix content of compact-appearing cortex. A p value of <0.05 was considered to be statistically significant.

5.4.4 Results
As shown in Table 5.4.1, patients with hypoparathyroidism were matched by age and sex to controls. Height, weight and body mass index (BMI) were no different between patients and controls. The mean duration of disease was ~13 years. All patients were treated with conventional therapy of vitamin D and calcium for at least one year (range 1 to 40 years) to maintain serum calcium within the normal range or slightly below.
<table>
<thead>
<tr>
<th></th>
<th>Total HypoP (n=40)</th>
<th>Total Controls (n=49)</th>
<th>Male HypoP (n=13)</th>
<th>Male Controls (n=14)</th>
<th>Female HypoP (n=27)</th>
<th>Female Controls (n=35)</th>
<th>Reference ranges</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>50.1 ± 16.4</td>
<td>50.9 ± 14.7</td>
<td>50.3 ± 14.0</td>
<td>55.4 ± 12.0</td>
<td>50.0 ± 17.7</td>
<td>49.1 ± 15.4</td>
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<td>Height (m)</td>
<td>1.65 ± 0.08</td>
<td>1.65 ± 0.09</td>
<td>1.73 ± 0.07</td>
<td>1.74 ± 0.07</td>
<td>1.62 ± 0.06</td>
<td>1.61 ± 0.07</td>
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</tr>
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<td>Weight (kg)</td>
<td>72.8 ± 16.9</td>
<td>70.9 ± 13.8</td>
<td>85.5 ± 18.9</td>
<td>84.3 ± 13.5</td>
<td>69.6 ± 15.9</td>
<td>65.9 ± 9.9</td>
<td></td>
</tr>
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<td>BMI (kg/m²)</td>
<td>26.9 ± 6.1</td>
<td>26.0 ± 4.3</td>
<td>28.8 ± 5.6</td>
<td>27.9 ± 4.2</td>
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<td>15</td>
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<td>49:0</td>
<td>7:6</td>
<td>11:16</td>
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<td>Duration (years)</td>
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<td>11.9 ± 14.9</td>
<td>13.3 ± 13.2</td>
<td>13.3 ± 13.2</td>
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<td>Calcium (mg)</td>
<td>1700 ± 1131</td>
<td>2228 ± 1650</td>
<td>1363 ± 471</td>
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<td>Calcitriol (µg)</td>
<td>0.72 ± 0.24</td>
<td>0.61 ± 0.13</td>
<td>0.80 ± 0.27</td>
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<td>Cholecalciferol (IU)</td>
<td>2000 ± 907</td>
<td>2428 ± 975</td>
<td>1727 ± 786</td>
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<td>Aetiology (n)</td>
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<td>5:8</td>
<td>6:21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.18 ± 0.20</td>
<td>2.39 ± 0.11</td>
<td>2.15 ± 0.18</td>
<td>2.36 ± 0.16</td>
<td>2.20 ± 0.17</td>
<td>2.42 ± 0.15</td>
<td>2.10 – 2.60</td>
</tr>
<tr>
<td>Serum phosphate (mmol/L)</td>
<td>1.39 ± 0.22</td>
<td>1.21 ± 0.17</td>
<td>1.36 ± 0.19</td>
<td>1.18 ± 0.25</td>
<td>1.41 ± 0.16</td>
<td>1.25 ± 0.20</td>
<td>0.60 – 1.40</td>
</tr>
<tr>
<td>Serum PTH (pmol/L)</td>
<td>0.5 ± 0.4*</td>
<td>4.1 ± 0.9</td>
<td>0.4 ± 0.9*</td>
<td>4.9 ± 0.8</td>
<td>0.5 ± 0.8*</td>
<td>3.8 ± 0.9</td>
<td>1.6 – 6.9</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>59 ± 25</td>
<td>55 ± 23</td>
<td>55 ± 21</td>
<td>51 ± 19</td>
<td>62 ± 18</td>
<td>58 ± 27</td>
<td>&gt;75</td>
</tr>
</tbody>
</table>

Table 5.4.1 Baseline patient characteristics

Values expressed as mean ± SD. *p<0.05
Body mass index (BMI)
**Distal Radius**

Relative to controls, male patients with hypoparathyroidism had 10.0% larger total cortical area and 18.7% larger compact cortical area (both p<0.05). Outer transitional zone (OTZ) area, inner transitional zone (ITZ) area and medullary area were no different. Total cortical vBMD was 11.3% (p=0.12) higher due to 11.1% (p=0.11) lower total cortical porosity and 2.0% (p=0.11) greater mineral matrix density, all of which did not reach significance. Compact cortical porosity, OTZ porosity and ITZ porosity were all lower by 11.2% (p=0.18), 10.7% (p=0.15) and 7.6% (p=0.11) respectively, though not significantly so. Trabecular vBMD was 50.5% (p=0.10) higher without achieving significance due to 22.7% (p<0.05) better trabecular connectivity. Whereas, trabecular number, thickness and separation were similar in patients and controls (Table 5.4.2).

In female patients with hypoparathyroidism, total cortical, compact cortical, OTZ, ITZ and medullary area were no different to controls. Total cortical vBMD was 4.2% (p=0.19) higher due to 4.6% (p=0.19) lower total cortical porosity, with neither reaching significance, and 1.2% (p<0.05) greater matrix mineral density. Trabecular vBMD was 26.1% (p=0.13) higher, without reaching significance, due to 16.2% (p<0.05) more trabeculae. Trabecular thickness, separation and connectivity were similar in patients and controls (Table 5.4.2).

In patients with primary hypoparathyroidism, relative to controls, total cortical, compact cortical and OTZ area were larger by 15.3% (p<0.01), 24.5% (p<0.01) and 11.5% (p=0.06) respectively, although statistical significance was not achieved in some instances. ITZ and medullary area were no different to controls. Total cortical vBMD, total cortical porosity, matrix mineral density were similar in patients and controls. Trabecular vBMD and micro-architecture were no different in patients and controls, although there was a trend towards higher trabecular number (18.4%, p=0.06), which did not reach significance (Table 5.4.4).

In patients with secondary hypoparathyroidism, relative to controls, bone cross-sectional areas were comparable. Total cortical vBMD was 7.7% higher due to 7.6% lower total cortical porosity and 1.8% greater matrix mineral density (all p<0.05). Porosities in the compact cortex, OTZ and ITZ were lower by 6.6% (p=0.15), 6.8% (p=0.09) and 5.4% (p<0.05) respectively, although significance was not achieved in all instances. Trabecular vBMD was 45.6% (p<0.05) higher in patients than controls due to 84.2% (p=0.12) thicker
trabeculae and 18.9% (p=0.06) better trabecular connectivity, although significance was not achieved in some instances. Trabecular number and separation were no different to controls (Table 5.4.4).

In females, but not male patients, there was a positive correlation between disease duration and total cortical vBMD (r=0.35, p=0.07), medullary vBMD (r=0.64, p<0.01), matrix mineral density (r=0.54, p<0.01) and negative association with total cortical porosity (r=-0.34, p=0.09).

**Distal Tibia**

In male patients, all the bone cross-sectional areas (i.e. total cortical, compact cortical, OTZ, ITZ and medullary) were no different to controls. Total cortical vBMD was 11.7% (p=0.16) higher due to 9.5% (p=0.16) lower total cortical porosity and 1.5% (p=0.15) greater matrix mineral density, all of which were not significant. Compact cortical porosity and OTZ porosity were 11.9% (p=0.15) and 13.7% (p=0.07) lower than controls with neither reaching significance. While ITZ porosity was no different. Trabecular vBMD was 35.5% (p=0.19) higher than controls, though did not reach significance. Trabecular number, thickness, separation and connectivity were similar in patients and controls (Table 5.4.3).

Female patients had 6.0% larger total cortical area and 11.9% greater compact cortical area (both p<0.05) than controls. OTZ area, ITZ area and medullary area were no different. Total cortical vBMD was 6.6% (p=0.13) higher due to 5.6% (p=0.13) lower total cortical porosity, with neither reaching significance, and 1.5% (p<0.05) greater matrix mineral density. Compact cortical and OTZ porosity were similar, while ITZ porosity was 3.9% (p=0.09) higher than controls without achieving significance. Trabecular vBMD was 31.3% (p=0.11) higher due to 9.6% (p=0.19) more trabeculae with neither reaching statistical significance. Trabecular thickness, separation and connectivity were similar in patients and controls (Table 5.4.3).

The findings in the distal tibia for primary hypoparathyroid and secondary hypoparathyroid patients were similar to the distal radius (Table 5.4.4).
In female, but not male patients, longer disease duration was associated with greater total cortical vBMD ($r=0.52$, $p<0.01$), greater medullary vBMD ($r=0.59$, $p<0.01$), higher matrix mineral density ($r=0.41$, $p<0.05$) and lower total cortical porosity ($r=-0.52$, $p<0.01$).

There were no differences in bone structure at both distal radius and tibia in patients from Austin Health in comparison to Columbia University Medical Centre.

The void bone matrix mineralization distribution curve for hypoparathyroid patients from the Austin Hospital was shifted to the right relative to controls at both the distal radius and tibia (Figure 5.4.1).
<table>
<thead>
<tr>
<th>Distal Radius</th>
<th>All HypoP (n=40)</th>
<th>All controls (n=49)</th>
<th>Male HypoP (n=13)</th>
<th>Male controls (n=14)</th>
<th>Female HypoP (n=27)</th>
<th>Female controls (n=35)</th>
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</thead>
<tbody>
<tr>
<td>Bone Cross-sectional Area</td>
<td></td>
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<tr>
<td>Total cortical (mm²)</td>
<td>97.9 ± 3.1</td>
<td>94.8 ± 2.5</td>
<td>121.7 ± 4.0*</td>
<td>110.6 ± 3.6</td>
<td>86.4 ± 1.6</td>
<td>87.6 ± 2.4</td>
</tr>
<tr>
<td>Compact cortical (mm²)</td>
<td>39.9 ± 1.7</td>
<td>36.3 ± 1.3</td>
<td>52.1 ± 2.4 *</td>
<td>43.9 ± 1.8</td>
<td>34.0 ± 1.2</td>
<td>32.8 ± 1.4</td>
</tr>
<tr>
<td>OTZ (mm)</td>
<td>26.9 ± 0.9</td>
<td>26.6 ± 0.7</td>
<td>32.9 ± 1.5</td>
<td>31.0 ± 1.3</td>
<td>24.1 ± 0.6</td>
<td>24.6 ± 0.6</td>
</tr>
<tr>
<td>ITZ (mm)</td>
<td>31.1 ± 1.3</td>
<td>31.9 ± 1.2</td>
<td>36.7 ± 2.4</td>
<td>35.7 ± 2.0</td>
<td>28.3 ± 1.3</td>
<td>30.3 ± 1.4</td>
</tr>
<tr>
<td>Medullary (mm²)</td>
<td>149.3 ± 7.5</td>
<td>149.5 ± 6.5</td>
<td>187.2 ± 14.0</td>
<td>177.9 ± 13.0</td>
<td>131.0 ± 6.6</td>
<td>136.7 ± 6.3</td>
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<td>Cortical Morphology</td>
<td></td>
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<tr>
<td>Total cortical vBMD (mgHA per cc)</td>
<td>788.8 ± 22.6*</td>
<td>739.9 ± 10.5</td>
<td>837.7 ± 54.7</td>
<td>752.4 ± 15.4</td>
<td>765.2 ± 20.1</td>
<td>734.2 ± 13.6</td>
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<tr>
<td>Total cortical porosity (%)</td>
<td>50.6 ± 1.6*</td>
<td>54.2 ± 0.8</td>
<td>47.4 ± 3.6</td>
<td>53.3 ± 1.2</td>
<td>52.1 ± 1.6</td>
<td>54.6 ± 1.1</td>
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<tr>
<td>Compact cortical porosity (%)</td>
<td>35.7 ± 1.4</td>
<td>37.8 ± 0.7</td>
<td>34.2 ± 3.1</td>
<td>38.5 ± 1.2</td>
<td>36.5 ± 1.5</td>
<td>37.4 ± 0.9</td>
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<tr>
<td>OTZ porosity (%)</td>
<td>38.6 ± 1.4</td>
<td>41.1 ± 0.6</td>
<td>36.7 ± 3.1</td>
<td>41.1 ± 0.9</td>
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<td>41.1 ± 0.8</td>
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<td>ITZ porosity (%)</td>
<td>80.6 ± 1.6*</td>
<td>84.2 ± 0.4</td>
<td>76.3 ± 4.0</td>
<td>82.6 ± 0.6</td>
<td>82.7 ± 1.4</td>
<td>84.8 ± 0.5</td>
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<tr>
<td>Matrix mineral density (%)</td>
<td>66.2 ± 0.3*</td>
<td>65.3 ± 0.1</td>
<td>66.6 ± 0.8</td>
<td>65.3 ± 0.2</td>
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<td>Trabecular Morphology</td>
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<tr>
<td>vBMD (mgHA per cc)</td>
<td>182.5 ± 22.4*</td>
<td>134.0 ± 6.6</td>
<td>245.4 ± 50.7</td>
<td>163.1 ± 9.8</td>
<td>152.2 ± 20.9</td>
<td>120.7 ± 7.6</td>
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<td>Number (/mm²)</td>
<td>2.76 ± 0.13</td>
<td>2.45 ± 0.09</td>
<td>2.99 ± 0.27</td>
<td>2.83 ± 0.12</td>
<td>2.65 ± 0.14*</td>
<td>2.28 ± 0.12</td>
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<td>Thickness (mm)</td>
<td>0.30 ± 0.10</td>
<td>0.19 ± 0.00</td>
<td>0.50 ± 0.30</td>
<td>0.20 ± 0.00</td>
<td>0.20 ± 0.01</td>
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<td>Separation (mm)</td>
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<td>Connectivity</td>
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<td>0.88 ± 0.05</td>
<td>0.78 ± 0.08</td>
<td>0.67 ± 0.04</td>
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Table 5.4.2 Bone morphology in hypoparathyroidism at the distal radius

Values expressed as mean ± SEM. *p<0.05
HypoP (hypoparathyroidism), OTZ (outer transitional zone), ITZ (inner transitional zone), vBMD (volumetric bone mineral density)
### Table 5.4.3 Bone morphology in hypoparathyroidism at the distal tibia

Values expressed as mean ± SEM. *p<0.05
HypoP (hypoparathyroidism), OTZ (outer transitional zone), ITZ (inner transitional zone), vBMD (volumetric bone mineral density)

<table>
<thead>
<tr>
<th>Distal Tibia</th>
<th>All HypoP (n=40)</th>
<th>All controls (n=49)</th>
<th>Male HypoP (n=13)</th>
<th>Male controls (n=14)</th>
<th>Female HypoP (n=27)</th>
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<td>Total cortical (mm²)</td>
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<td>210.0 ± 3.6*</td>
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<td>Compact cortical (mm²)</td>
<td>73.5 ± 2.5*</td>
<td>65.6 ± 2.5</td>
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<td>80.7 ± 3.8</td>
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<td>59.6 ± 2.5</td>
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<td>OTZ (mm²)</td>
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<td>70.1 ± 2.8</td>
<td>75.0 ± 2.8</td>
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<td>Medullary (mm²)</td>
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<tr>
<td>Total cortical vBMD (mgHA per cc)</td>
<td>719.7 ± 25.6*</td>
<td>663.7 ± 10.3</td>
<td>756.7 ± 54.1</td>
<td>677.3 ± 18.1</td>
<td>701.9 ± 27.7</td>
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<td>Total cortical porosity (%)</td>
<td>55.9 ± 1.9*</td>
<td>60.1 ± 0.8</td>
<td>53.4 ± 3.7</td>
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<td>42.7 ± 1.0</td>
<td>38.4 ± 3.3</td>
<td>43.6 ± 1.4</td>
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<td>OTZ porosity (%)</td>
<td>40.3 ± 1.8</td>
<td>44.0 ± 0.9</td>
<td>39.0 ± 3.1</td>
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<td>41.0 ± 2.2</td>
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<tr>
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<td>Matrix mineral density (%)</td>
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<td>66.5 ± 0.6</td>
<td>65.5 ± 0.2</td>
<td>66.3 ± 0.4*</td>
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<td>Trabecular Morphology</td>
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<td>vBMD (mgHA per cc)</td>
<td>199.6 ± 26.1*</td>
<td>148.7 ± 5.9</td>
<td>238.0 ± 53.1</td>
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<td>2.96 ± 0.14</td>
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<td>Thickness (mm)</td>
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</tr>
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<td>1.32 ± 0.29</td>
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<td>Connectivity</td>
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<td>0.88 ± 0.07</td>
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</table>
Table 5.4 Bone morphology in primary vs. secondary hypoparathyroidism

Values expressed as mean ± SEM. *p<0.05
OTZ (outer transitional zone), ITZ (inner transitional zone), vBMD (volumetric bone mineral density)
Voxels on the left contain only void volume (0%), more void than mineralized bone matrix volume, more mineralised bone matrix than void volumes, and only mineralized bone matrix volume on the right (100%). Relative to controls, patients with hypoparathyroidism had a right shift. These relative shifts were similar in both distal radius and tibia.

**5.4.5 Discussion**

I report that patients with PTH deficiency due to secondary rather than primary hypoparathyroidism have better maintained cortical and trabecular bone relative to controls as well as higher matrix mineral density. Patients with hypoparathyroidism had a tendency towards higher total cortical vBMD due to lower total cortical porosity and greater matrix mineral density, although statistical significance was not reached in many instances when analysed by sex, but significance was achieved when analysed as an entire cohort or by disease type with secondary rather than primary hypoparathyroidism. Trabecular vBMD also tended to be higher and associated with preserved trabecular microstructure and connectivity, though significance was not consistently achieved when analysed by sex but when analysed as a whole cohort or disease type with secondary rather than primary hypoparathyroidism. Moreover, disease duration was associated with better maintained bone structural parameters in female, but not male patients. There was a rightward shift of the void bone matrix...
mineralization distribution curve for patients with hypoparathyroidism relative to controls at both the distal radius and tibia.

**Bone microstructure**

My data suggests that secondary, rather than primary hypoparathyroidism was associated with better maintained cortical and trabecular bone structure as well as higher matrix mineral density has not been confirmed in other studies. Most studies have grouped primary and secondary hypoparathyroidism together and analysed as a whole as there were lower numbers of patients with primary hypoparathyroidism. Two fracture outcome studies have reported no increased risk in overall fractures in post-surgical hypoparathyroid as well as non-surgical hypoparathyroid patient cohorts, the risk for upper limb fractures was reported to be reduced in post-surgical hypoparathyroid patients but increased in non-surgical hypoparathyroid patients. The reduced risk of upper limb fractures in patients with post-surgical hypoparathyroidism is consistent with the finding of better maintained bone structure in this patient cohort. While the increased risk of upper limb fractures in patients with primary hypoparathyroidism may in part be explained by the effects of the disease on skeletal growth.

My results confirm the findings of other studies with higher trabecular bone volume associated with preserved bone micro-architecture demonstrated in patients with PTH deficiency. A small histomorphometry study with only 12 patients (8 females and 4 males) with hypoparathyroidism (9 post-surgical and 3 idiopathic) with mean age ~59 (range 36 – 70) years and disease duration range 2 – 53 years, reported a trend towards higher trabecular bone volume associated with preserved trabecular micro-architecture compared to age- and sex-matched controls (Langdahl et al., 1996). Though with only 12 patients, this study was likely underpowered to detect any true differences in bone structure.

A larger histomorphometry study involving 33 patients (24 females and 9 males) with hypoparathyroidism (18 post-surgical, 13 autoimmune and 2 DiGeorge) with mean age ~48 years and mean disease duration ~17 years reported significantly greater trabecular bone volume and trabecular width associated with preserved trabecular
micro-architecture compared to age- and sex-matched controls (Rubin et al., 2008). The same authors also analysed the bone biopsy specimens from this cohort of patients using micro-CT. The histomorphometric findings were corroborated with micro-CT demonstrating higher trabecular bone volume accompanied with superior trabecular micro-architecture and connectivity in patients with hypoparathyroidism relative to controls (Rubin et al., 2010).

My study and others have observed greater cortical bone volume and lower cortical porosity in patients with PTH deficiency. Unlike Langdahl et al, Rubin et al also analysed cortical morphology using histomorphometry. The study reported a trend towards increased cortical width and reduced cortical porosity in patients with hypoparathyroidism. There was also a large study involving 60 patients (48 females and 12 males) with hypoparathyroidism (35 post-surgical, 24 idiopathic and 1 DiGeorge) with mean age ~46 years and mean disease duration ~11 years that demonstrated increased cortical vBMD and reduced cortical porosity using HR-pQCT. No differences in bone strength were observed between patients and controls using finite element analysis. However the control group used for comparison were not appropriate as they were younger in age (20 –29 years), which may reduce the likelihood of detecting true differences in bone structure and bone strength (Cusano et al., 2016).

Material properties
 Although increased areal BMD and preserved or better maintained bone structure has been reported in patients with PTH deficiency, the effects on bone strength remain uncertain. Furthermore, increased fracture risk has been reported in some but not all studies. This paradox led me and other studies to investigate abnormalities in bone material composition in patients with hypoparathyroidism.

Increased matrix mineralization density in patients with PTH deficiency was evident in my study and others. This finding in part contributed toward the higher cortical vBMD. Furthermore, the void bone matrix mineralization distribution curve for my cohort of patients was shifted to the right relative to controls, suggesting a more highly mineralized skeleton compared to controls. A recent study recruited 30
hypoparathyroid patients treated with one (n=14, mean age ~55 years) or two years (n=16, mean age ~47 years) with PTH(1-84). Quantitative backscattered electron imaging was used to measure bone mineralization density distribution variables. At baseline, both one year and two year PTH(1-84) treatment groups demonstrated increased bone mineralization density distribution parameters in trabecular bone but not cortical bone (Misof et al., 2016). In contrast, other preliminary studies have reported the mean mineralization density in patients with hypoparathyroidism to be no different to controls using quantitative backscatter electron imaging (Paschalis et al., 2012; Paschalis et al., 2009). This result is not consistent with the low bone turnover generally associated with hypoparathyroidism. Though with only 19 patients, the study may have had insufficient power to detect true differences.

**Bone turnover**

The mechanism underlying increased cortical and trabecular bone mass as well as higher matrix mineralization density in patients with PTH deficiency is low bone turnover. Although bone remodelling markers were not performed, my results are consistent with a low bone remodelling state in patients with hypoparathyroidism. The low bone turnover in patients with PTH deficiency has been well documented. Biochemical markers of bone remodelling are generally suppressed or in the lower half of the normal range compared to age- and sex-matched individuals (Rubin et al., 2011; Sikjaer et al., 2011; Winer et al., 1998). Furthermore, histomorphometric indices in patients with hypoparathyroidism indicate reduced bone turnover on all three bone surfaces: endocortical, intra-cortical and trabecular (Rubin et al., 2008).

**Limitations**

One limitation is the small sample size in each group when the cohort of patients were analysed by sex. HR-pQCT image acquisition from two different centres may introduce some variability. However, to minimize this, the same patient imaging protocol was used and daily calibration was performed at both sites. Image analysis was performed at the one centre (i.e. Austin Health). The other limitation is the cross-sectional study design, which means causality cannot be proven. Hence prospective studies are needed to confirm the findings. Although a non-threshold based analysis...
method was used to quantify bone morphology, which improves bone segmentation, accurately defining the cortico-trabecular margin is always a major challenge.

In conclusion, patients with PTH deficiency due to secondary hypoparathyroidism rather than primary hypoparathyroidism have better maintained cortical and trabecular bone morphology as well as higher matrix mineralization density relative to controls. Similar results were found when the data was analysed by sex. Furthermore, longer disease duration was associated with better maintained bone structure. More prospective studies are needed to see how these skeletal abnormalities affect bone strength and fracture risk.
Discussion and Conclusions

Chapter Six
Chapter 6: Discussion and Conclusions

Fractures are a significant healthcare issue globally, affecting both sexes throughout the lifespan with two peak incidences occurring during childhood and later in life. Although historically there is strong focus on vertebral fractures, non-hip, non-vertebral fractures utilizes 36 – 66% of all fracture expenditure due to its greater prevalence (Ioannidis et al., 2013).

Early on bone fragility was crudely detected on radiographs as osteopenia or fractures, which are features present when bone loss is advanced. Later on, densitometry was developed to try and detect individuals at risk before fracture and before severe irreversible bone destruction occurred. However, densitometry has a number of limitations and is restricted to measuring areal BMD, which does not identify microstructural deterioration causing bone fragility. Many patients suffer fracture despite have ‘normal’ BMD or osteopenia which suggests that DXA is not a sensitive method at detecting bone fragility (Siris et al., 2004).

Deterioration in bone microstructure is associated with disproportionate loss in bone strength. Bone loss causing trabecular perforation has a far more profound effect on bone strength than bone loss through trabecular thinning. Studies using finite element analysis have demonstrated 2 to 5 times greater loss in bone strength from loss of trabeculae compared to trabecular thinning (Silva et al., 1997). Small increases in cortical porosity also has disproportionately large reduction in bone strength (Burr, 2003).

Although bone microstructural deterioration is not captured by DXA, recent advances have led to the development of high resolution peripheral quantitative computed tomography (HR-pQCT) which is able to measure three-dimensional bone microstructure in-vivo with high resolution, good precision and low radiation (Boutroy et al., 2005; Laib et al., 1998). However, there are some limitations to this technology. Firstly, HR-pQCT is not widely available. There are only 3 machines in Australia with its use being restricted to research. Secondly, measurement sites are difficult to duplicate. The variability in the positioning of the region of interest due to differences in forearm length needs to be considered when interpreting data.
investigating age related differences, sexual dimorphism or racial differences as there is heterogeneity in bone geometry and bone morphology within a small region of bone (Ghasem-Zadeh et al., 2017).

Advances have also occurred in software analyzing images acquired using HR-pQCT. One of the major hurdles in analyzing bone microstructure is accurate segmentation (separation) of cortical from trabecular bone. This is particularly problematic in more advanced age when cortical bone loss by intracortical remodeling causes trabecularization of the cortex as pores coalesce fragmenting the cortices. The fragments resemble trabeculae. It is the accurate assignment of these cortical remnants to cortical bone rather than trabecular bone that poses a major challenge. The implications of erroneously including these cortical remnants to trabecular bone is that, firstly, trabecular vBMD is overestimated and secondly, cortical porosity is under-estimated. As such, the loss of trabecular bone as well as the increase in cortical porosity may not be fully appreciated in disease states with high bone turnover or during ageing.

One method devised to improve the accuracy of bone segmentation is through the addition of transitional zone, which creates a cortico-trabecular transitional compartment. StrAx, is a software that uses non-thresholding algorithm to analyze images acquired using HR-pQCT, which can more accurately assign cortical remnants to cortical rather than trabecular bone (Zebaze et al., 2013). This analysis technique has provided a different perspective on bone microstructural changes seen in certain endocrine disorders.

Fractures complicate many endocrine disorders. Increase fracture risk has been observed in patients with type 1 and type 2 diabetes mellitus. Meta-analyses have reported ~6 fold and ~2 fold increase relative risk of hip fractures in type 1 diabetes and type 2 diabetes mellitus respectively (Janghorbani et al., 2007; Vestergaard, 2007). PTH excess due to untreated primary hyperparathyroidism (PHPT) appears to be associated with increased risk in morphometric vertebral fractures as well as non-hip, non-vertebral fractures (Khosla et al., 1999; Vestergaard et al., 2000). In contrast, there is uncertainty regarding fracture risk in patients with PTH deficiency as fracture
outcome studies are inconsistent with some studies reporting increase morphometric vertebral fractures while other studies report no increase in overall fractures in patients with hypoparathyroidism (Chawla et al., 2017; Mendonca et al., 2013; Underbjerg et al., 2014; Underbjerg et al., 2015).

The overall aim was to investigate the changes in bone structure and material composition underlying bone fragility in a number of endocrine diseases such as and type 1 and type 2 diabetes mellitus, untreated primary hyperparathyroidism and hypoparathyroidism. This was achieved by using HR-pQCT to acquire images of the distal radius and distal tibia in cases and controls. Bone structure was assessed by the standard analysis protocol from the manufacturers, which uses a threshold based algorithm the in type 1 and type 2 diabetes study. StrAx was used to measure bone structure and matrix mineral density in the PTH excess and PTH deficiency study.

The main findings for my type 1 diabetes study were that patients with diabetes had no significant deficits in cortical and trabecular bone relative to controls. The results are consistent with areal BMD in the lumbar spine and femur, which was no different in cases and controls. Patients with diabetes had a tendency towards lower bone formation and bone resorption markers, which did not reach significance in all instances. Further work will be done re-assessing the effects of diabetes using the StrAx algorithm.

The main findings for my type 2 diabetes study were that patients with diabetes had similar cortical morphology whereas trabecular bone was better maintained than controls. These results are consistent with higher aBMD in the spine and hip as well as lower bone remodelling markers observed in diabetic patients. Future studies will be done re-examining the effects of diabetes using the StrAx algorithm.

The main findings for my PTH excess study were that PTH excess is deleterious to both cortical and trabecular bone. In patients with untreated PHPT, the normal or high trabecular density reported in several studies is likely to be the result of inclusion of cortical remnants in the medullary compartment. In patients with untreated PHPT, there was a left shift of the void-matrix mineralization density distribution curve relative to controls due to increased cortical porosity which reduced cortical vBMD.
Cortical and trabecular deficits may not be completely reversible with surgical treatment.

The main findings for my PTH deficiency study were that patients with PTH deficiency due to secondary rather than primary hypoparathyroidism have better maintained cortical and trabecular bone relative to controls as well as higher matrix mineral density. There was a rightward shift of the void bone matrix mineralization distribution curve for patients with hypoparathyroidism relative to controls.

In future, given the ageing population and the growing health care issue of fragility fracture, use of more advanced technology that has the ability to measure bone microstructure may better identify individuals at risk of bone fragility and target treatment accordingly. However, large prospective studies need to be undertaken to validate the use of HR-pQCT in the clinical setting. More machines also need to be made available for its clinical uptake. In addition, using percentage of limb length instead of a fixed length in determining measurement site for HR-pQCT may need to be considered given the variability in population height and limb length.

In conclusion, measuring areal bone mineral density was a good start to identifying subjects at risk of bone fragility, but it fails to detect deterioration in bone microstructure, which is a highly sensitive indicator of bone fragility. There have been many advances in image acquisition and analysis, which has come a long way towards improving bone segmentation. Applications of these technological advances to better identify individuals at risk and to target treatment is still to be determined.
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Appendix One

New insights into the effects of primary hyperparathyroidism on the cortical and trabecular compartments of bone


1 Department of Medicine and Endocrinology, Austin Health, University of Melbourne, Heidelberg, Australia
2 Department of Medicine and Division of Endocrinology, Metabolic Bone Disease Unit, College of Physicians and Surgeons, Columbia University, New York, USA

Original Full Length Article

ABSTRACT

In primary hyperparathyroidism (PHPT), protracted elevation of serum parathyroid hormone (PTH) is held to be associated with cortical, but not trabecular, bone loss. However, an alternative explanation for the apparent preservation of trabecular bone is fragmentation of the cortex by intracortical remodeling. The cortical fragments resemble trabeculae and so may be erroneously included in the quantification of trabecular bone density.

To test this hypothesis, we compared bone microarchitecture in 43 patients with untreated PHPT (mean 62.9 years, range 31–84) with 47 healthy age-matched controls and 25 patients with surgically treated PHPT (0.16 years, SD = 0.21). Images of the distal radius and tibia were acquired using high-resolution peripheral quantitative CT and analyzed using StrAx10, a new software program that quantifies bone morphology in vivo. Results were expressed as the mean number of standard deviations (SD) from the age-specific mean (2 scores, mean ± SEM).

In subjects with PHPT, total tibial cortical area was reduced (-0.26 ± 0.08 SD; p = 0.002). Cortical volumetric bone mineral density (vBMD) was reduced (-0.29 ± 0.06 SD; p < 0.001) due to higher cortical porosity (0.32 ± 0.06 SD; p < 0.001) and lower tissue mineralization density (-0.21 ± 0.06 SD; p = 0.002). Medullary area was increased (0.26 ± 0.08 SD; p = 0.002) and trabecular vBMD was reduced (-0.14 ± 0.04 SD; p = 0.001).

In subjects who underwent successful parathyroidectomy, cortical area (-0.18 ± 0.10 SD; NS) and medullary area (0.18 ± 0.10 SD; NS) did not differ from controls. Cortical vBMD was reduced (-0.15 ± 0.05 SD; p = 0.003) due to high porosity (0.15 ± 0.05 SD; p = 0.006), values numerically lower than in untreated PHPT. Tissue mineralization density (-0.26 ± 0.04 SD; p = 0.001) and trabecular vBMD were reduced (-0.16 ± 0.04 SD; p = 0.001). The results were similar in the distal radius.

In PHPT, chronically elevated endogenous PTH does not spare trabecular bone; it causes bone loss and microarchitectural deterioration in both cortical and trabecular compartments of bone.

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Introduction

Bone remodeling is surface dependent; it is initiated upon the three (intracortical, endocortical and trabecular) components of a bone’s inner (endosteal) envelope. During young adulthood remodeling is balanced, the volume of old or damaged bone matrix excreted beneath the surface is replaced with an equal volume of new bone matrix [1]. During advancing age, remodeling becomes unbalanced; less bone is deposited than was removed so that each time a remodeling event occurs, local structural deterioration ensues [2].

After menopause and in diseases such as primary hyperparathyroidism (PHPT), structural deterioration accelerates because the surface extent of remodeling increases and the negative bone multicellular unit (BMU) balance worsens [3]. Remodeling upon trabecular surfaces thins and removes them. Remodeling upon endocortical surfaces thins the cortex and enlarges the medullary cavity. Intracortical remodeling of the cortex adjacent to the medullary canal cavitates it; Haversian canals coalesce warning the porosity, thinning the cortex from ‘within’ and producing cortical remnants that look like trabeculae (‘trabecularization’) [4]. Intracortical remodeling and porosity account for 70% of total bone lost with age [4].
Parathyroid hormone (PTH) excess in PHPT produces cortical bone loss but is supported to preserve bone at trabecular sites [3–10]. This notion is based on finding normal or increased so-called ‘trabecular’ bone volumetric bone mineral density (vBMD) when assessed by noninvasive imaging and normal volumetric/total volume (BV/TV) using histomorphometry [3–10].

These disparate findings in the cortical and trabecular compartments are unusual because the three (endocortical, trabecular and intracortical) components of the endosteal (inner) envelope are contiguous; they are connected forming one inner surface of the mineralized bone matrix volume; remodeling with loss of bone due to the negative bone balance is generally similar on each of these three components [1].

Thus, an alternative interpretation is that the normal or increased trabecular vBMD in PHPT is an artifact produced by inclusion of cortical remnant because they resemble true trabecular, namely those of growth plate origin [4]. Cortical remnants are unlikely to confer normal bone strength because ‘trabecularized’ bone is chaotic and lacks the cancellous architectural design of trabecular bone [4]. This view would also reconcile the increased vertebral fracture risk in PHPT, an anomaly if endogenous PTH preserved or increased trabecular bone volume in PHPT [6].

We hypothesized that (i) endogenous PTH in PHPT produces both cortical and trabecular bone loss; (ii) cortical remnants, which resemble trabecular, are erroneously included in the analysis of trabecular density, and (iii) there are residual deficits in both cortical and trabecular bone following successful parathyroidectomy. To test these hypotheses, we analyzed images of the distal radius and tibia acquired by high-resolution peripheral quantitative CT (HR-pQCT), using StrAxt1.0, a new software program that quantifies bone morphology in-vivo.

Materials and methods

Patient cohort

Patients with untreated and surgically treated PHPT were recruited from Austin Hospital (Melbourne, Australia) and Columbia University Medical Center (New York, USA). We studied 43 untreated PHPT, 25 treated PHPT, and 47 age-matched healthy controls (Table 1). The healthy participants, previously published were recruited from the community [4]. The mean duration post-parathyroidectomy was 53 ± 53 years. The study was approved by the Human Research Ethics Committee of Austin Health and the Institutional Review Board of Columbia University Medical Center. All subjects gave written, informed consent.

Table 1

<table>
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<td>Serum phosphate (mmol/L)</td>
<td>1.57 ± 0.23</td>
<td>1.50 ± 0.16</td>
<td>1.50 ± 0.20</td>
<td>0.60–1.40</td>
</tr>
<tr>
<td>Serum PTH (pmol/L)</td>
<td>13.0 ± 1.7</td>
<td>6.4 ± 4.7</td>
<td>4.1 ± 6.9</td>
<td>1.6–6.9</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>50.9 ± 13.8</td>
<td>55.4 ± 18.1</td>
<td>54.3 ± 23.3</td>
<td>&gt;75</td>
</tr>
<tr>
<td>% supplemented with cholecalciferol</td>
<td>46</td>
<td>47</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>% supplemented with calcium</td>
<td>0</td>
<td>28</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD.

* Untreated PHPT vs. Treated PHPT p < 0.05.

† Untreated PHPT vs. Control p = 0.05.

§ Represents the desirable level of 25(OH) vitamin D at our institution.

Image acquisition & analysis

Images of the non-dominant distal radius and tibia were acquired using high-resolution peripheral quantitative CT (HR-pQCT, XtremeCT, Scanco Medical AG, Britttisellen, Switzerland) which has an isotropic voxel size of 82 μm [12]. Daily quality control was carried out by scanning a phantom containing rods of hydroxyapatite (QRM Moehrendorf, Germany). Radiation exposure was ~3 μSv per measurement. Images were retrieved and analysed using a new software (StrAxt1.0, StrAnCorp, Melbourne, Australia) [13]. Analysis was restricted to the 40 most proximal of the 110 slices in the region of interest as they have thicker cortices allowing accurate assessment of porosity. Results for bone compartments, cortical morphology and trabecular vBMD were derived from StrAxt1.0 analysis. Trabecular morphology (ie, trabecular number, thickness, and separation) was derived from Scanco Medical AG software analysis.

Using curve profile analysis, bone is segmented from the soft tissue background and then into its compact-appearing cortex, outer and inner transitional zone, and medullary (trabecular) compartment. Once deposited, matrix rapidly undergoes primary mineralization reaching ~80% of its peak mineralization density within days to weeks. StrAxt1.0 excludes voxels with attenuation between 80–100% of the maximum attenuation produced by 1,200 mg hydroxyapatite (HA)/cc, fully mineralized bone, when quantifying porosity, because these voxels contain younger bone at various stages of secondary mineralization responsible for the heterogeneity in mineralization that could be mistakenly interpreted as porosity. These voxels are unlikely to contain Haversian canals (poles in cross section) because few are less than 25 μm diameter. Porosity is quantified by estimating the void fraction of each of the remaining voxels. To do so, the mineralized bone matrix volume of each voxel is quantified using an interpolation function derived from two references: (i) the attenuation of voxels containing fully mineralized bone matrix (equivalent to that produced by 1,200 mg HA/cc) are assigned a value of 100% and (ii) voxels that are empty with an attenuation equivalent to background are assigned a value of 0%. The volume fraction of a voxel that is void (otherwise known as porosity) is 100% minus the mineralized bone matrix fraction. The porosity of the compartment is the average void volume fraction of all composite voxels which vary in their

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proportions of void and mineralized bone matrix, not only the completely empty voxels [13].

To quantify tissue mineralization density, only voxels with attenuation values between 80 and 100% of the attenuation produced by 1200 mgHA/cc are used. The difference between the attenuation produced by voxels containing only mineralized bone and the attenuation produced by background represents the attenuation produced by the mineral within the bone matrix. Tissue mineral density is the ratio of the attenuation produced by the mineral in the bone matrix to the attenuation produced by 1200 mgHA/cc. Frequency distribution curves depicting the composition of the cortex according to voxel content (empty, mineralized bone matrix only, varying proportions of each) were also computed [13].

Segmentation and quantification of porosity is accurate with $\tau^2$ ranging from 0.88 to 0.90 at the distal radius and tibia which assessed bone morphology in vivo compared to gold standard micro-CT (19 µm voxel size). Reproducibility, the root mean square error of the coefficient of variation is 0.5 to 3.0% (Fig. 1).

Total cortical area, the compact-appearing cortical area, ITZ and ITZ and medullary area were expressed as a percentage of the total CSA to adjust for bone size. Tissue mineralization density was determined by the attenuation produced by voxels whose volume was occupied by bone matrix and no void volume.

**Biochemical analyses**

Serum intact PTH and 25(OH) vitamin D were measured using an electro-chemiluminescence immunoassay (Roche Modular E170, Switzerland). Serum calcium and phosphate were analyzed by automated techniques (unciel Dxc 800, Beckman Coulter Inc, USA).

**Statistical analyses**

Measurements were expressed as mean Z scores (±SEM), the number of standardised deviations (SD) from age-, sex-, height- and weight-adjusted mean of zero based on the linear regression in 47 healthy controls. T-tests assessed whether the Z scores differed from zero. Group comparisons were made using ANOVA. The contribution of cortical porosity and tissue mineralization density to the variance in cortical vBMD was determined using stepwise regression analysis. A frequency distribution curve of mineralized bone matrix content of compact-appearing cortex was plotted for the three groups. A p value of <0.05 was considered to be statistically significant. Analyses were conducted using SPSS version 20 software (SPSS Inc, Chicago, USA).

**Results**

Untreated PHPT vs. controls

As shown in Fig. 2 and Table 2, for the distal tibia, relative to controls, untreated patients with PHPT had reduced total cortical area (compact-appearing plus transitional zone) ($-0.26 ± 0.08$ SD, $p = 0.002$) and increased medullary area ($0.26 ± 0.08$ SD, $p = 0.002$). The compact-appearing cortical area and outer transitional zone area were each reduced ($-0.31 ± 0.08$ SD, $p < 0.001$ and $-0.43 ± 0.15$ SD, $p < 0.001$).

![Fig. 1. Segmentation and quantification of HR-pQCT images by Sobel feature analysis.](image-url)
SD, p = 0.007 respectively). The inner transitional zone area was not reduced.

Total cortical vBMD was reduced (−0.29 ± 0.06 SD, p < 0.001) due to increased cortical porosity in the compact-appearing (0.13 ± 0.04 SD, p = 0.003) and in the transitional zones (OTZ, 0.16 ± 0.05 SD, p = 0.002 and ITZ, 0.32 ± 0.04 SD, p < 0.001) and (b) reduced tissue mineralization density (−0.21 ± 0.06 SD, p = 0.002) of the surrounding bone matrix. Of the reduction in cortical vBMD, 97.5% was attributable to the increased cortical porosity with only 2.5% attributable to lower tissue mineralization density. Cortical vBMD and cortical porosity correlated inversely with $r^2 = 0.975$ before and 0.89 after adjustment for tissue mineralization (both p < 0.001). The void bone matrix mineralization distribution curve was shifted left relative to controls (Fig. 4). Trabecular vBMD was reduced (−0.14 ± 0.04 SD, p < 0.001) relative to controls. Trabecular separation was increased (0.07 ± 0.03 SD, p = 0.03) but trabecular number (−0.06 ± 0.05 SD; p = 0.259) and the trabecular thickness (0.10 ± 0.06 SD; p = 0.110) did not differ from controls (Fig. 3).

**Treated PHPT vs. controls**

In successfully surgically treated patients, relative to controls, total cortical area was not reduced and medullary area was not increased. The compact-appearing cortical area was reduced (−0.22 ± 0.09 SD; p = 0.03). The outer and inner transition zone areas were not different to controls. Total cortical vBMD was reduced (−0.15 ± 0.05 SD; p = 0.003). Overall, total cortical porosity was increased due to increased ITZ porosity, porosity in compact-appearing cortex and OTZ were no different to controls. Tissue mineralization density distribution was shifted to the left relative to controls. Trabecular vBMD was reduced relative to controls (−0.16 ± 0.04 SD; p < 0.001) due to reduced trabecular number (−0.19 ± 0.05 SD; p < 0.001) not thickness (0.09 ± 0.08 SD; NS). Trabecular separation was increased (0.24 ± 0.09 SD; p < 0.010) (Fig. 3).

**Trained PHPT vs. untrained PHPT**

In successfully surgically treated patients, total cortical area, compact-appearing cortical area and outer transitional zone area were 3, 10, and 15% larger than in untreated patients respectively; none reaching significance. The inner transitional zone area was no different. Cortical vBMD was 85% greater than in the untreated patients (p = 0.075). Total cortical porosity, compact-appearing cortex, outer and inner transitional zone porosity were higher relative to controls, but 9, 22, 16, and 2% less than in the untreated patients with none reaching significance. Tissue mineralization density was low relative to controls, but no different when compared to the untrained group (p = NS) (Fig. 2 and Table 2). Trabecular vBMD, number, thickness and separation were no different to the untreated patients. Results for the distal radius were attenuated but showed a similar trend to that of the distal tibia.

**Discussion**

We report that endogenous PTH excess as seen in PHPT is deleterious to both cortical and trabecular bone in the distal tibia. Specifically, (i) mean cortical bone area was reduced due to remodeling upon the
intracortical surface eroding the cortices from ‘within’ producing porosity and upon the endocortical surface eroding it ‘outwards’ enlarging the medullary cavity. (ii) mean tissue mineralization density of the surrounding compact-appearing cortical bone matrix was reduced. (iii) there was a left shift of the void-matrix mineralization density distribution curve relative to controls due to a increased cortical porosity which reduced cortical vBMD. (iv) Mean trabecular vBMD was reduced. (v) Patients with surgically corrected PHPT had residual but somewhat less severe deficits in several tests. The results were similar at the distal radius but attenuated.

Cortical deficits

These results, and those using peripheral quantitative computed tomography [14,15] and high resolution pQCT [16], suggest that endogenous PTH excess results in structural deterioration of both cortical and trabecular bone and that these deficits may be partly reversible. Patients with PHPT had reduced cortical bone area for two reasons. Firstly, intracortical remodeling removed more bone than it replaced leaving void (porosity). Secondly, endocortical resorption eroded the cortex enlarging the medullary area. While endocortical resorption is commonly regarded as a main mechanism responsible for cortical thinning in PHPT and in aging, the main mechanism we propose that is responsible is intracortical remodeling which thins the cortex from the ‘inside’ by cavitating the inner compact cortex [4]. The transitional zone area increases at the price of compact appearing cortex as a result of intense remodeling in this region.

During remodeling in advancing age, >95% of the bone removed by each BMU is replaced with new bone in these patients. This newly deposited (younger) bone matrix has a lower tissue mineralization density than the bone matrix removed so that there is a left shift in the tissue mineralization density distribution as more void is produced and an increasing proportion of the newly formed osteons have a lower tissue mineral density. Nevertheless, as primary mineralization occurs rapidly and achieves >80% of full mineralization of collagen, little of the deficit in cortical vBMD was due to reduced tissue mineralization density of the newly deposited bone; most of the deficit in cortical vBMD was the result of increased intracortical porosity produced by the negative BMU balance.

Trabecular defects

Intracortical remodeling in the inner cortex produces cortical remnants which resemble true trabeculae (i.e., of growth plate origin). These remnants are erroneously called ‘trabecular’ are usually included in quantification of trabecular vBMD. Most imaging methods are not able to distinguish cortical remnants from true trabeculae and therefore show increased or normal trabecular bone volume in PHPT. When these cortical remnants were excluded from the analysis, trabecular vBMD was reduced due primarily to an increase in trabecular separation as the negative BMU balance and high remodeling intensity completely perforate and remove complete plates. In untreated subjects, trabecular density was reduced with trabecular number and thickness were not reduced. Trabecular density was measured using SkXtLab which recognizes the transitional zone and confines measurement of trabecular density to the medullary compartment [13]. Analysis of the trabecular number and thickness was done using the Skanno method [12], which does not recognize the transitional zone and so does not account for cortical trabecularization which erroneously increases trabecular number and likely increases trabecular thickness (as remnants of the cortex may be thick and contain osteons). Finding that ‘trabecular’ number was lower in the successfully surgically treated patients is consistent with the notion that reversal of porosity in the inner cortex, which re-corticalizes the fragments (regarded as trabeculae), reduces what is mistakenly called ‘trabecular’ bone. Evidence of this will require prospective studies. The lack of differences between untreated and successfully surgically treated subjects may reflect the lack of power and that some deficits are irreversible following successful surgery.

Increased bone remodeling intensity causes bone deficits

Increased bone formation rate determined using dynamic histomorphometry is reported in PHPT [3-10,13,18-20]. This measurement is the product of the surface extent of bone formation
(reflecting activation frequency or the intensity of bone remodeling) and the mineral apposition rate (MAR). Remodeling intensity and the degree of negativity of the BMDs vary from surface to surface, but there is no evidence that remodeling events produce a net positive balance upon trabecular surfaces (resulting in trabecular bone gain) and a net negative balance upon the endocortical and intracortical surfaces (resulting in cortical bone loss). The increased bone formation surfaces upon trabecular in PHPT is the result of high surface extent of remodeling — there are more BMDs per unit surface area. This will not preserve trabecular bone volume unless the negative BMDs balance is corrected. It will not increase trabecular bone volume unless the volume of bone deposited is greater than the volume removed by each of the increased numbers of BMDs. Evidence of increased mean wall thickness or MAR is not compelling. Thus, there is little convincing evidence to support the notion that PTH preserves or increases trabecular bone volume.

Residual bone deficits post-parathyroidectomy

In many of the parameters measured in the post-parathyroidectomy group, deficits were still present but less so than in untreated patients. The residual deficits suggest that the effects of PTH excess in PHPT are not entirely reversible because the negative BMDs balance irreversibly removes complete trabeculae that cannot be reconstructed and produces large coalescent pores that cannot be refilled during the bone formation phase of bone remodeling because of the negative bone balance.

A cautionary note is needed because this was not a prospective study and preoperative morphology was not known. The extent to which these changes can be shown to be reversible will require prospective studies with long follow-up after parathyroidectomy. Another limitation is that all the controls were recruited in Melbourne, however there were no detectable differences between the Melbourne and New York cases.

Conclusion

In conclusion, contrary to prevailing notions, PTH excess in PHPT is deleterious both to cortical and trabecular compartments of bone. The normal or high trabecular density reported in several studies is likely to be the result of inclusion of cortical remnants in the medullary compartment. It is likely that these cortical remnants do not function as competent trabecular elements. Data on increased fracture risk in PHPT, albeit limited, may be explained by these observations.

A. TIBIA

B. RADIUS

Fig. 4. Frequency or void bone matrix distribution curve of voxels within the compact-appearing cortex of the distal tibia (A) and distal radius (B). Voxels on the left contain only void volume (0%), more void than mineralized bone matrix volume, more mineralized bone matrix than void volume, and only mineralized bone matrix volume on the right (100%). Relative to controls, untreated PHPT had a left shift relative to untreated PHPT, treated PHPT had a right shift. These relative shifts were similar in both distal tibia and radius.
Disclosures

R. Zelase, A. Ghassem-Zadeh and E. Seeman are inventors of the Strex1.0 software. There is no other information to disclose.

Acknowledgment

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References
