Emerging Issues in Osteoporosis

by
Emma Billington

A thesis submitted in fulfilment of the requirements for the degree of
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ABSTRACT

Background

Osteoporosis and fragility fractures affect a large proportion of the population and have significant health and economic consequences. This thesis addresses several emerging questions regarding bone metabolism and the entity of osteoporosis, specifically:

1. How is bone mineral homeostasis related to energy metabolism and cardiovascular health?
2. By what mechanisms do certain disease states and medications contribute to the development of secondary osteoporosis?
3. Why do different tools used to estimate fracture risk sometimes give discordant results for the same patient, and how should these be addressed in clinical practice?
4. How can we better understand the side effect profiles of osteoporosis treatments, and develop strategies to mitigate adverse effects?

Methods

Six studies are described herein:

1. An analysis of relationships between circulating parathyroid hormone (PTH), cardiometabolic risk factors, and bone mineral density (BMD) in healthy men
2. An assessment of associations between phosphate and adiposity in three cohorts, and between fibroblast growth factor 23 (FGF23) and weight in a fourth cohort
3. A meta-analysis of randomized controlled trials (RCTs) assessing the effects of thiazolidinedione (TZD) medications on BMD and bone turnover
4. An assessment of the effects of patient characteristics on absolute fracture risk estimates generated by the FRAX and Garvan fracture risk calculators

5. A crossover RCT evaluating the acute effects of calcium supplementation on blood pressure in postmenopausal women
6. A RCT assessing the effect of oral dexamethasone on prevention of the acute phase response (APR) following administration of zoledronic acid

Results & Conclusions

Results and their implications are summarized as follows:

1. PTH was positively associated with markers of cardiometabolic risk, including measures of adiposity and coronary artery calcification, but not with BMD. Adiposity may represent a cause of secondary hyperparathyroidism.
2. Serum phosphate was inversely associated with adiposity, independent of PTH. FGF23 and body weight were correlated, suggesting that FGF23 may mediate the relationship between phosphate and adiposity.
3. TZDs were associated with modest declines in BMD with no reversal after discontinuation. Avoidance of TZDs in those at high fracture risk may be prudent.
4. Estimates of fracture risk were higher, on average, with Garvan than FRAX. However, using a 3% ten-year hip fracture risk as a treatment threshold, the calculators were in agreement in 75% of cases. It is reassuring that the calculators often agree, but important to recognize that each incorporates different risk factors.
5. Supplementation with calcium as citrate resulted in attenuation of normal diurnal reductions in systolic blood pressure compared to placebo. Calcium supplements may work via this mechanism to increase cardiovascular risk.
6. Compared to placebo, a single dose of oral dexamethasone did not reduce the likelihood or severity of APR following zoledronic acid infusion. A higher dose or longer dosing interval may be required.

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I am appreciative of the assistance that I received from Associate Professor Andrew Grey, who critically reviewed the protocol and manuscript of the meta-analysis that is described in Chapter 5. Statistician Karl Pearson is quoted as saying that '*statistics are the grammar of science*,' and I am exceptionally grateful to Greg Gamble, who helped me to ensure that the 'scientific grammar' used throughout this thesis is sound. Thank you for teaching me the intricacies of SAS, and for your patience and ready advice. I have also been fortunate to receive advice and support from Dr. Mike Croxson, who helped to develop and facilitate the controlled trial described in Chapter 8.

I wish to extend my appreciation to my colleagues and friends from the Bone & Joint Research Group at the University of Auckland, who made me feel like a member of the group from day one. In particular, Dr. Anne Horne, who was always willing to share her experience from the front lines of clinical research, who provided invaluable support for the research outlined in Chapter 7, and who single-handedly ensured that the research study described in

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LIST OF ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APR	Acute phase response
ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
BMD	Bone mineral density
bsALP	bone-specific alkaline phosphatase
CAC	Coronary artery calcium
CaSR	Calcium-sensing receptor
CI	Confidence interval
CTX	β-C-terminal telopeptide of type I collagen
DXA	Dual-energy x-ray absorptiometry
eGFR	Estimated glomerular filtration rate
FGF23	Fibroblast growth factor 23
HORIZON	Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly
HR-pQCT	High resolution peripheral quantitative computed tomography
IGT	Impaired glucose tolerance
IL-1	Interleukin-1
IL-6	Interleukin-6
IQR	Interquartile range
M-CSF	Macrophage colony stimulating factor
NSAID	Non-steroidal anti-inflammatory
OR	Odds ratio
P1NP	Procollagen type-I N-terminal propeptide
PPAR-γ	Peroxisome-proliferator-activated receptor gamma isoform
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTH	Parathyroid hormone
RCT	Randomized controlled trial
RR	Relative risk
SD	Standard deviation

TGF-β	Transforming growth factor beta
TNF-α	Tumour necrosis factor alpha
TZD	Thiazolidinedione
WHI	Women's Health Initiative
WHO	World Health Organization
Wnt	Wingless

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Nature of contribution by PhD candidate	EOB designed study (with IRR), analyzed the data (with GDG), and wrote the chapter and manuscript.
Extent of contribution by PhD candidate (%)	80%

CO-AUTHORS

Name	Nature of Contribution
Greg D Gamble	Provided guidance and critical review of data analyses, critically revised manuscript.
Ian R Reid	Developed research question (with EOB), critically revised manuscript.

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

Name	Signature	Date
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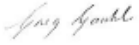
Greg Gamble		27/04/2017

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Extent of contribution by PhD candidate (%)	80%

CO-AUTHORS

Name	Nature of Contribution
Greg D Gamble	Provided guidance and critical review of data analyses, critically revised manuscript.
Sarah M Bristow	Provided data for analysis, critically revised manuscript.
Ian R Reid	Developed research question (with EOB), critically revised manuscript.

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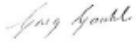
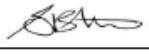
- that the candidate wrote all or the majority of the text.

Name	Signature	Date
Ian R Reid		April 26, 2017
Greg Gamble		27/04/2017
Sarah M Bristow		01/05/2017

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Extent of contribution by PhD candidate (%)	70%

CO-AUTHORS

Name	Nature of Contribution
Andrew Grey	Critically revised proposal and manuscript.
Mark J Bolland	Provided guidance regarding study design and drafting of research protocol, assisted with literature search, analyzed data, critically revised manuscript.

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

Name	Signature	Date
Mark Bolland		1/5/17
Andrew Grey		4/5/17

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Nature of contribution by PhD candidate	EOB designed study (with IRR), completed data analysis, and wrote the chapter and manuscript.
Extent of contribution by PhD candidate (%)	80%

CO-AUTHORS

Name	Nature of Contribution
Greg D Gamble	Provided guidance and critical review of the data analyses, critically revised manuscript.
Ian R Reid	Developed research questions, critically revised manuscript.

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- that the candidate wrote all or the majority of the text.

Name	Signature	Date
Ian R Reid		April 26, 2017
Greg Gamble		27/04/2017

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Nature of contribution by PhD candidate	EOB coordinated the study (with SMB), assisted with data analysis (with GDG), and wrote the chapter and manuscript
Extent of contribution by PhD candidate (%)	65%

CO-AUTHORS

Name	Nature of Contribution
Sarah M Bristow	Wrote study protocol, coordinated the study (with EOB), critically revised manuscript..
Greg D Gamble	Analyzed the data, critically revised the protocol and manuscript.
J de Kwant & A Stewart	Assisted with the running of the trial.
BV Mihov & AM Horne	Assisted with the running of the trial.
Ian R Reid	Principal Investigator, critically revised protocol and manuscript.

Certification by Co-Authors

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Name	Signature	Date
Ian R Reid	Ian Reid	April 26, 2017
Greg Gamble	Greg Gamble	27/04/2017
Sarah M Bristow		01/05/2017
Anne Horne		3|5|17
Jordan de Kwant		3/5/17
Angela Stewart		3.5.17

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Bobby Mihov		3/5/2017

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Nature of contribution by PhD candidate	EOB wrote the study protocol, coordinated the study (with AH), assisted with data analysis (with GDG), and wrote the chapter and manuscript
Extent of contribution by PhD candidate (%)	75%

CO-AUTHORS

Name	Nature of Contribution
Anne Horme	Coordinated the study (with EOB), critically revised manuscript.
Greg D Gamble	Analyzed the data, critically revised the protocol and manuscript.
K Maslowski	Assisted with the running of the trial.
M House	Assisted with the running of the trial and data management.
Ian R Reid	Principal Investigator, critically revised protocol and manuscript.

Certification by Co-Authors

The undersigned hereby certify that:

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- that the candidate wrote all or the majority of the text.

Name	Signature	Date
Ian R Reid	Ian Reid	April 26, 2017
Greg Gamble	Greg Gamble	27/04/2017
Meaghan House		2/5/17
Anne Horne	Anne Horne	2/5/17.
Katherine Maslowski		4/5/2017

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LIST OF PUBLISHERS' APPROVALS

The research described in Chapters 3 through 8 of this thesis has been published in peer-reviewed journals. Approval to include these publications in this dissertation has been obtained from the relevant publishers. Details are as follows:

1. **Chapter 3 (page 68):** Parathyroid hormone reflects adiposity and cardiometabolic indices but not bone density in normal men
    - **Published as:** Billington EO, Gamble GD, Reid IR. Parathyroid hormone is related to fat mass and cardiometabolic indices but not bone density in normal men. Bonekey Reports 2016; 5:852.
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2. **Chapter 4 (page 92):** Serum phosphate is related to adiposity in healthy adults
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3. **Chapter 5 (page 110):** The effect of thiazolidinediones on bone mineral density and bone turnover: A systematic review and meta-analysis
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4. **Chapter 6 (page 132):** Comparison of FRAX and Garvan fracture risk caculators for making treatment decisions in osteoporosis
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    - **Publisher:** Elsevier

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    - **Published as:** Billington EO, Bristow SM, Gamble GD, de Kwant J, Stewart A, Mihov BV, Horne AM, Reid IR. Acute effects of calcium supplements on blood pressure: randomized, cross-over trial in postmenopausal women. Osteoporosis International 2017; 28:119-125.
    - **Publisher:** Springer

6. **Chapter 8 (page 169):** The effect of single-dose dexamethasone on acute phase reponse following zoledronic acid: A randomized controlled trial
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CHAPTER 1:
INTRODUCTION

Osteoporosis is characterized by low bone mass, decreased bone strength, and an increased risk of low-trauma fracture [1]. Fragility fractures are the primary clinical consequence of osteoporosis, and up to 50% of postmenopausal women and 20% of men over the age of 50 will sustain one of these fractures in their remaining lifetime [2], resulting in significant morbidity and healthcare costs [3-6]. The terms ‘primary osteoporosis’ or ‘age-related osteoporosis’ can be used to describe the development of skeletal fragility in the context of aging, which is mediated by several factors, including a decline in circulating sex hormone concentrations, changes in calcium and vitamin D metabolism, and cellular senescence. A more specific term, ‘postmenopausal osteoporosis,’ refers to the presence of primary osteoporosis in women, resulting from the oestrogen deficiency that accompanies menopause. The term ‘secondary osteoporosis’ denotes osteoporosis that is not explained by age and/or age-related sex hormone deficiency alone and is often the result of illness or medication use.

Osteoporosis has affected humans for more than 4000 years, as evidenced by the findings of fractures and increased cortical porosity on examination of the skeletons of Egyptian mummies and other ancient populations [7, 8]. However, it was not until the late 1700s and early 1800s that the condition became widely recognized, when the surgeon Sir Astley Cooper noted that decreases in bone mineral density, known to be associated with aging, also resulted in an increased risk of fracture, and the pathologist Jean Lobstein remarked that the holes, or pores, in the bone of some patients were larger than in other patients. Lobstein subsequently coined the term ‘osteoporosis’ to describe this phenomenon [8]. In the late 1800s, research done in Germany indicated that fractures tended to affect females more than males, and it was postulated that osteoporosis and fragility fracture were the result of women tripping over their long skirts [9]. Further study by Fuller Albright in the 1900s demonstrated

that, in postmenopausal women, osteoporosis results primarily from oestrogen deficiency [10]. He pioneered the treatment of postmenopausal osteoporosis with oestrogen in 1941 [10].

Over the past 75 years, large strides have been made in the understanding and treatment of osteoporosis. In vitro and animal studies have provided us with a general understanding of bone biology, including the bone remodelling process and bone mineral metabolism. Postmenopausal osteoporosis has become a well-characterized entity, and we have learned that men, too, are at risk of osteoporosis and fragility fracture [11]. We have become aware that loss of bone mass can also occur as a consequence of other (secondary) factors, such as immobility, poor nutrition, comorbid diseases and concurrent medications [12]. Falls (albeit not necessarily over long skirts) have also been established as a risk factor for fracture [13, 14]. Technologies have been developed to assess bone turnover, bone density, and bone strength, and clinical prediction tools have been developed to evaluate an individual's risk of fracture. In addition, since Albright's reports of treating postmenopausal osteoporosis with oestrogen, a number of additional effective treatments for fracture prevention have been developed.

However, many unanswered questions remain. Our knowledge of bone biology is incomplete. Potential relationships between bone mineral homeostasis and cardiometabolic disease have been described, but the underlying physiology and clinical relevance of these relationships is uncertain. We are regularly discovering new secondary causes of osteoporosis, although in many cases the underlying pathophysiology remains elusive. While calcium and vitamin D supplementation have long been recommended for the prevention and treatment of osteoporosis, uncertainty has arisen regarding the efficacy and safety of these agents.

Pharmacologic osteoporosis therapies are also associated with side effects, some of which limit treatment uptake and adherence. The development of strategies to mitigate these side effects is desperately required. In the forthcoming literature review, **Chapter 2**, I summarize the current state of knowledge regarding the pathophysiology, diagnosis, and treatment of osteoporosis. Areas that pertain to the research contained in this thesis are emphasized.

The research presented in this thesis addresses several emerging uncertainties surrounding osteoporosis and bone metabolism. Specifically, **Chapter 3** presents the results from a study of the associations between circulating parathyroid hormone (PTH) levels, cardiometabolic risk factors, and bone mineral density (BMD) in a cohort of healthy men. In **Chapter 4**, relationships between inorganic phosphate and measures of adiposity in three independent cohorts of healthy men and women are reported, as are the associations between fibroblast growth factor 23 (FGF23) and weight in a fourth independent cohort of healthy women. In **Chapter 5**, I present the findings from a systematic review and meta-analysis of randomized controlled trials (RCTs) assessing the effects of thiazolidinedione medications, the use of which has been shown to increase fracture risk, on BMD and bone turnover markers. **Chapter 6** describes a cross-sectional cohort study evaluating the effects of patient characteristics on discrepancies in fracture risk estimates using two widely used fracture risk calculators (FRAX and Garvan). In **Chapter 7**, I present the results of a randomized crossover study designed to determine the acute effects of calcium supplementation on blood pressure in healthy postmenopausal women. **Chapter 8** describes a RCT assessing the effect of a single dose of oral dexamethasone on prevention of the acute inflammatory response that frequently occurs following the first infusion of zoledronic acid. The clinical significance of this research and areas for future exploration are discussed in **Chapter 9**. The research described in Chapters 3 through 8 has been published in peer-reviewed journals [15-20].

CHAPTER 2:
LITERATURE REVIEW

This literature review is divided into seven sections. **Section 2.1** provides a review of bone physiology, focusing on the constituents of bone, the bone remodelling cycle, and the hormonal regulation of calcium and phosphate homeostasis. **Section 2.2** summarizes existing data regarding relationships between bone mineral homeostasis and the development of cardiovascular and metabolic dysfunction, and **Section 2.3** addresses the causes and sequelae of disorders of PTH excess. **Section 2.4** provides an overview of the determinants of skeletal fragility and causes of secondary osteoporosis, and in **Section 2.5**, the epidemiology and consequences of osteoporosis are highlighted. **Section 2.6** outlines the tools available to assess bone density and predict fracture risk, and **Section 2.7** summarizes the currently available treatments for osteoporosis, with a focus on side effects. Throughout this review, the areas of uncertainty which are to be addressed in the remainder of this thesis are highlighted.

2.1 PHYSIOLOGY OF BONE & BONE MINERALS

Bone has a number of important roles in humans including facilitation of locomotion, protection of vital organs, support of bone marrow haematopoiesis, secretion of hormones, and storage of the minerals calcium and phosphorus. Within the skeleton, a multitude of tightly regulated physiologic processes serve to integrate the material constituents of bone and permit this important organ to fulfil its metabolic and structural responsibilities. Osteoporosis and other forms of metabolic bone disease develop when the normal bone physiology is interrupted. The following subsections describe the organic and inorganic

components of bone and the physiologic processes that are required to maintain skeletal homeostasis.

2.1.1 Bone composition

The human skeleton consists of 270 bones at birth. After fusion of several bones during growth, adults have a total of 206 bones [21]. Each bone is composed of both a cortical and a trabecular compartment. The cortical component forms the bone's hard outer layer. Cortical bone is laid down in a lamellar manner, has a smooth appearance, and is relatively resistant to bending and torsion [22]. Cortical bone is thus primarily responsible for the strength and stability of the skeleton. Inside the cortical shell sits a delicate network of trabecular bone, which has a spongy, cancellous appearance. While the number, spacing and thickness of the trabeculae are important determinants of skeletal strength [22], the trabecular bone also serves as the primary location for bone remodelling and mineral metabolism. The majority of bone turnover – around 70% - occurs at trabecular bone surfaces, although this type of bone only comprises 20-30% of the skeleton. Cortical bone, on the other hand, comprises 70-80% of the skeleton and accounts for only 30% of bone turnover [22]. Different bones have differing trabecular and cortical compositions. For instance, the vertebrae are composed of more than 75% trabecular bone, whereas the neck of the femur is approximately 65% trabecular, and the proximal radius is more than 95% cortical [23].

All bone, whether it be cortical or trabecular, is made up of both cellular and extracellular components. The extracellular component of bone can be further categorized into mineral and organic phases. The mineral phase makes up 60-70% of the dry weight of the human skeleton and lends hardness and rigidity to bone [24]. This phase is comprised of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], consisting largely of the minerals calcium and phosphorus [24]. The

organic phase consists primarily of type I collagen (90%) [25], and also contains a multitude of distinct noncollagenous proteins (~5%), lipids (~2%) and water. Type I collagen provides a structural blueprint for mineral deposition [25]. Although the specific roles of noncollagenous proteins, lipids, and water within the organic phase are incompletely understood, noncollagenous proteins appear to be important for maintaining the structural integrity of type I collagen, and are also involved in cell signalling and mineralization [26]. Together, the extracellular components of bone ensure that the skeleton is both strong and flexible.

Bone is constantly changing, or remodelling, in order to repair damage and preserve structure, and also to maintain adequate circulating concentrations of bone minerals [27, 28]. At any given time, approximately 20% of the trabecular bone surface is undergoing remodelling [29]. The cellular component of bone is essential for this process. Remodelling results from the coordinated actions of three types of bone cells: osteoclasts, osteoblasts, and osteocytes. The molecular mechanisms that underpin bone remodelling are numerous and complex, and each type of bone cell has a multitude of functions, not all of which pertain to remodelling. This scope of this literature review is limited to a discussion of the basic bone remodelling cycle, and the major contributions of each cell type to this process. This is reviewed in the next subsection.

2.1.2 Bone remodelling and the cellular components of bone

Bone remodelling is the primary mode of bone turnover in older individuals. Through remodelling, the majority of the skeleton is replaced over the period of a decade [30]. The remodelling process involves the removal of old bone from a region of the bone surface and replacement with new bone at the same site. This should be differentiated from the process of

bone *modelling*, which involves the formation of new bone at one site along with removal of old bone at a different skeletal location [31]. Modelling can result in large changes in bone size and shape, and is of great importance throughout childhood and adolescence [31]. Bone remodelling results from the concerted actions of the cells that resorb old bone (osteoclasts) and those that form new bone (osteoblasts). These two cell types are collectively known as a 'basic multicellular unit', and their remodelling activities are largely orchestrated by osteocyte cells, which reside within the bone matrix and send signals to the osteoclasts and osteoblasts in response to mechanical and humoral stimuli [32, 33]. The remodelling cycle is depicted in Figure 2.1 and consists of: 1) a period of activation, during which bone lining cells (quiescent osteoblasts) retract to expose a region of the bone surface for resorption, 2) resorption of the exposed bone matrix by osteoclasts, 3) a reversal phase during which the resorbed surface is prepared for the formation of new bone, and 4) a formation period where new osteoid is laid down by osteoblasts [34, 35]. This entire cycle generally lasts 6 to 9 months, with resorption taking 2 or 3 weeks and formation requiring several months [36]. The processes of bone resorption and formation are usually tightly coupled. Factors that increase bone resorption without a corresponding increase in bone formation, and those that decrease bone formation without a corresponding decrease in resorption lead to net losses in bone volume and increased skeletal fragility [28]. Imbalances in the rates of bone resorption and bone formation contribute to the development of primary osteoporosis and many forms of secondary osteoporosis.

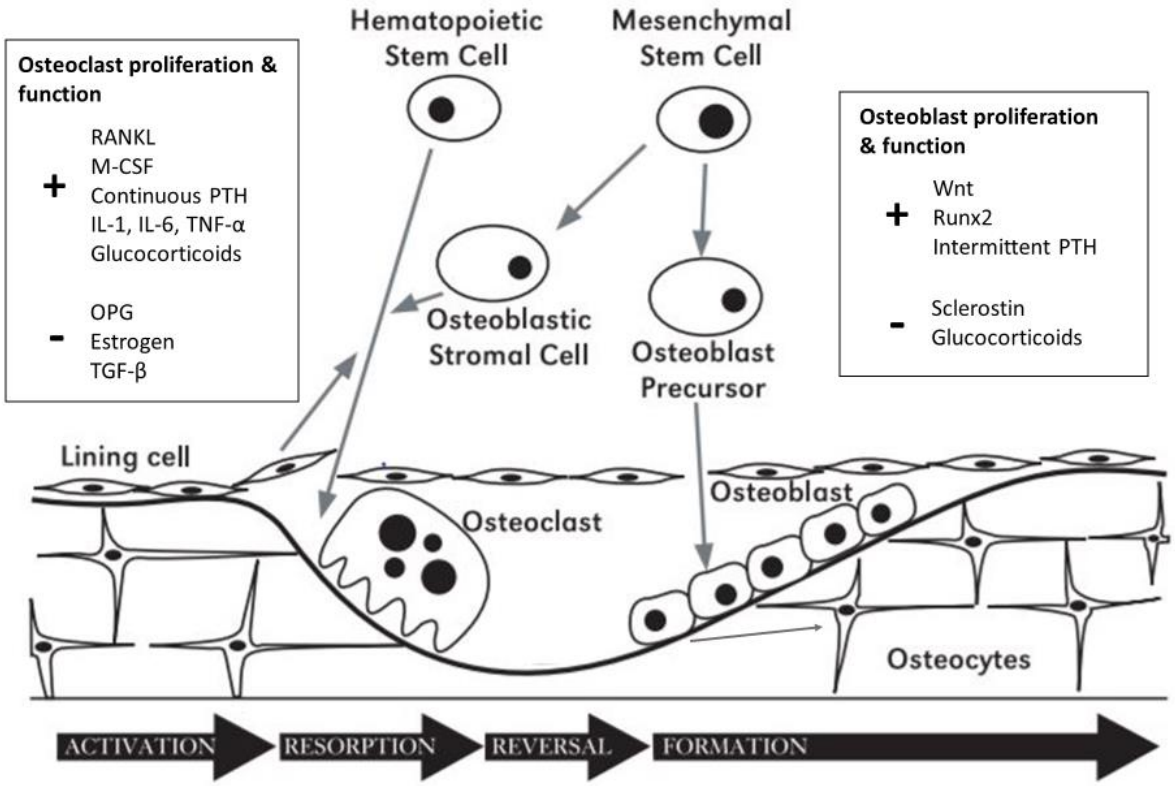


Figure 2.1 The bone remodelling cycle. The cycle consists of 1) a period of activation, during which bone lining cells retract to expose a region of the bone surface for resorption, 2) resorption of the exposed bone matrix by osteoclasts, 3) a reversal phase, and 4) a formation period where new osteoid is laid down by osteoblasts. The proliferation and function of osteoclasts and osteoblasts is regulated by a multitude of factors, some of which are listed in the insets. RANKL = receptor activator of nuclear factor-kappa B, M-CSF = macrophage colony stimulating factor, PTH = parathyroid hormone, IL-1 = interleukin-1, IL-6 = interleukin 6, TNF-α = tumour necrosis factor α, OPG = osteoprotegrin, TGF-β = transforming growth factor β. Adapted from "Bone Health and Osteoporosis. A Report of the Surgeon General," by the US Department of Health and Human Services, 2004 [37].

Bone resorption by osteoclasts

Bone resorption is carried out by osteoclasts. These cells are a member of the monocyte-macrophage phase, derived from the hematopoietic cell lineage [33, 38]. Osteoclasts and osteoclast precursor cells express the receptor activator of nuclear factor-kappa B (RANK). Simulation of RANK by its ligand (RANK ligand [RANKL]), which is secreted by both osteoblasts and osteocytes [39], results in osteoclast differentiation and recruitment to the region of the bone surface where resorption is to take place [40]. Osteoclast recruitment is also facilitated by macrophage colony stimulating factor (M-CSF) [41]. Following differentiation and recruitment, mature osteoclasts adhere to the bone, walling off a space between the cell membrane and the bone surface, called a sealing zone. The cells then secrete hydrochloric acid and acidic proteases such as cathepsin K into the sealing zone, resulting in degradation of the bone matrix and liberation of calcium, phosphate, and fragments of type I collagen [42].

Bone formation by osteoblasts

Following resorption of bone by osteoclasts, a short reversal phase occurs, during which time the resorption pit is prepared for formation of new bone [35]. The deposition of new bone is then carried out by osteoblasts, under the direction of osteocytes. Osteoblasts are derived from the mesenchymal stem cell line, and osteocytes are descendants of osteoblasts. A number of signal transduction cascades are involved in the differentiation of mesenchymal stem cells into osteoblasts (reviewed in [43]). Activation of the transcription factor Runx2 and induction of the canonical wingless (Wnt) pathway are two crucial steps in this differentiation process. Runx2 determines the fate of osteoblast precursor cells. Activation of this transcription factor promotes differentiation of mesenchymal stem cells into osteoblasts, rather than chondrocytes or adipocytes [43]. Some secondary causes of osteoporosis, such as

treatment with thiazolidinedione (TZD) medications, appear to increase fracture risk by altering Runx2 activity and promoting the differentiation of mesenchymal stem cells into adipose cells rather than osteoblasts [44-47]. Activation of the canonical Wnt pathway is also required for osteoblast differentiation. Wnt activation in osteoblast progenitor cells induces an intracellular signalling pathway, resulting in accumulation of the protein β-catenin within the cytoplasm and triggering osteoblast differentiation [43]. The glycoprotein sclerostin is an antagonist of the Wnt pathway, and its presence suppresses bone formation [48]. Accordingly, inhibition of sclerostin using a monoclonal antibody has been shown to increase bone formation, improve bone density, and reduce the risk of fracture [48].

Mature osteoblasts are responsible for the deposition of new bone and also play a role in the regulation of osteoclast activity. Osteoblasts secrete RANKL, as well as another important humoral factor, osteoprotegrin (OPG). While RANKL stimulates osteoclast differentiation and recruitment and promotes bone resorption [40], OPG does the opposite. OPG acts as a decoy receptor for RANKL, preventing binding of RANKL to RANK and thereby inhibiting osteoclast activity [38]. Factors that alter the ratio of circulating RANKL and OPG can therefore lead to the uncoupling of bone resorption and formation. Expression of RANKL is upregulated by interlukin-1, tumour necrosis factor α, glucocorticoids and vitamin D [36, 42], and downregulated by oestrogen [40]. OPG expression is upregulated by oestrogen and transforming growth factor β [36]. Another hormone, parathyroid hormone (PTH), which will be reviewed further in subsection 2.1.3, also has an important influence on the RANKL to OPG ratio. Sustained elevations in circulating PTH concentrations lead to increased production of RANKL and M-CSF, promoting bone resorption [42]. However, intermittent cyclical exposure to PTH has an opposite effect, resulting in the stimulation of bone formation at a greater rate than resorption and leading to net gains in bone density. This

physiology was exploited in the development of a recombinant PTH analogue (teriparatide), which is administered in an intermittent manner for treatment of osteoporosis [43, 49].

Osteoblasts initiate the formation of new bone by secreting type I collagen and noncollagenous proteins, including osteocalcin, osteopontin, and bone sialoprotein, onto the bone surface [32]. Type I collagen creates a scaffold onto which calcium and phosphate can deposit as part of the mineralization process. Mineralization is dependent on the presence of adequate amounts of calcium and phosphate, and the enzyme alkaline phosphatase [32, 50]. Osteoblast-secreted bone sialoprotein has also been shown to promote mineralization [51], [32, 50]. After osteoblasts complete their part in the bone formation process, each meets one of the following fates: 1) programmed cell death (apoptosis), 2) transformation into quiescent bone lining cells, or 3) 'burial' in the newly formed bone and further differentiation into osteocytes [52].

Regulation of bone remodelling by osteocytes

Previously believed to be inert cells residing in the bone matrix, recent evidence indicates that osteocytes are hormonally active, and that these cells are integral to the regulation of bone remodelling [52]. Osteocyte cell bodies sit within the bone matrix. From each cell body extends several long dendritic processes, forming an arachnoid network that connects these cells to the vasculature, the bone surface, and one another [52]. Osteocytes regulate bone remodelling in a number of ways. Like osteoblasts, these cells express surface receptors for PTH and Wnt, and have the ability to secrete RANKL and OPG. In fact, recent preclinical work suggests that the regulation of osteoclast differentiation and function via RANKL and OPG is influenced primarily by the secretion of these factors by osteocytes rather than osteoblasts [53]. Mechanical loading of osteocytes, such as with weight-bearing exercise,

results in the inhibition of sclerostin and activation of the Wnt pathway, as well as downregulation of RANKL. Ultimately, this leads to osteoblast differentiation and osteoclast inhibition [52, 54]. Not surprisingly, mechanical unloading results in increased expression of sclerostin and RANKL, having the opposite effects on osteoblast and osteoclast function [52, 53]. Osteocyte apoptosis also serves to regulate bone remodelling, promoting bone resorption via recruitment of osteoclasts [55]. Several stimuli, including local bone microdamage, oxygen deprivation due to immobilization, withdrawal of oestrogen, and glucocorticoid exposure can trigger osteocyte apoptosis [55]. In summary, osteocytes play an important role in assessing changes in the condition of the skeleton and signalling to osteoclasts and osteoblasts to ensure that bone remodelling adjusts accordingly.

2.1.3 Hormonal regulation of bone mineral metabolism

The skeleton serves as a reservoir for the elements calcium and phosphorus. These minerals are embedded within the bone as hydroxyapatite when new bone is formed and liberated into the blood when bone is resorbed. Phosphorus is highly reactive and only exists within the body as a component of phosphate complexes, not as phosphorus alone [56]. Accordingly, the term ‘phosphate’ will be used throughout this thesis when referring to phosphorus-containing compounds. This subsection will review calcium and phosphate homeostasis, focusing on the roles of the hormones PTH, fibroblast growth factor 23 (FGF23), and vitamin D. Figure 2.2 provides a schematic diagram indicating the origin of each of these hormones and their primary effects on calcium and phosphate homeostasis.

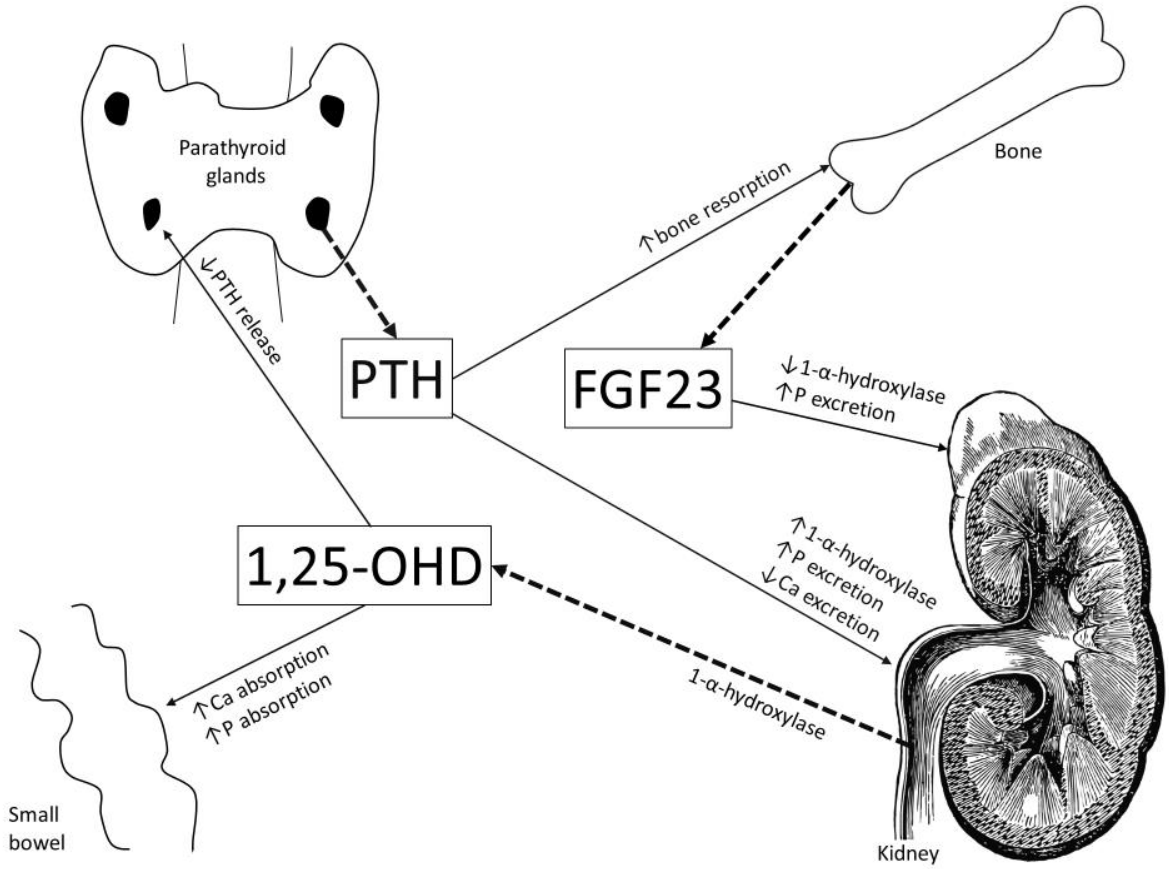


Figure 2.2. Principal hormones involved in bone mineral homeostasis. Dashed lines denote the primary origin of each hormone and solid lines indicate hormone actions as they relate to calcium and phosphate metabolism. PTH = parathyroid hormone, FGF23 = fibroblast growth factor 23, 1,25-OHD = 1,25-hydroxyvitamin D, Ca = calcium, P = phosphate.

Regulation of calcium homeostasis by PTH and vitamin D

On average, the adult human body contains approximately 1000g of calcium, 99% of which is stored within bone as hydroxyapatite, with the remaining 1% divided between the intravascular compartment, and the intra- and extracellular spaces of the soft tissues [57]. Calcium is necessary for a multitude of cellular functions, including muscle contraction, neuronal excitability, cell division, coagulation, and cell adhesion. Therefore, intravascular calcium concentration is very tightly regulated, with ionized (unbound) calcium concentration generally sitting within the range of 1.1 to 1.3 mmol/L [57]. Body calcium stores are maintained through the ingestion of calcium-containing foods. Most healthy individuals absorb around 200mg of elemental calcium from the gastrointestinal tract each day, with a comparable amount being excreted by the kidneys. On a daily basis, approximately 500mg of calcium is liberated from the bone via resorption and another 500mg is incorporated at sites of new bone formation [57].

Intravascular calcium concentrations are largely under the control of PTH, in conjunction with vitamin D [58], both of which increase circulating calcium levels. PTH is synthesized and released primarily by the chief cells of the parathyroid glands. Most individuals have four parathyroid glands, which sit adjacent to the thyroid gland in the anterior neck. The primary stimulus for PTH release is a reduction in circulating calcium levels. Low extracellular calcium concentrations stimulate the calcium-sensing receptor (CaSR), which is expressed by parathyroid chief cells. Stimulation of the CaSR results in release of pre-formed PTH and synthesis of new PTH molecules [58]. The release of PTH increases circulating calcium concentrations by several mechanisms. Sustained increases in PTH result in stimulation of bone resorption and liberation of calcium from the skeleton [42]. PTH enhances renal calcium reabsorption in the thick ascending loop of Henle and the distal collecting tubule

[57]. PTH also promotes expression of the enzyme 1-α-hydroxylase, which is synthesized primarily in the kidneys [57]. This enzyme converts the precursor molecule, 25-hydroxyvitamin D, into the active vitamin D hormone, 1,25-hydroxyvitamin D [59]. The 1,25-hydroxyvitamin D molecular is the specific ligand for the vitamin D receptor, and binding of this hormone to the receptor increases gastrointestinal calcium and phosphate absorption.

In humans, vitamin D synthesis requires several steps, beginning with the conversion of 7-dehydrocholesterol into cholecalciferol, a process that occurs in the epidermis and requires UVB radiation from sunlight. Cholecalciferol, also known as vitamin D3, can also be obtained in the diet and via supplement. Plant-derived ergocalciferol, or vitamin D2, is another vitamin D precursor molecule that can be obtained via supplement or ingestion of fortified food products. Cholecalciferol and ergocalciferol are hydroxylated in the liver to become 25-hydroxyvitamin D, which can then be converted by 1-α-hydroxylase into 1,25-hydroxyvitamin D. Alternately, 25-hydroxyvitamin D can be metabolized to the inactive compound, 24,25-hydroxyvitamin D, by the enzyme 24-α-hydroxylase. The 24-α-hydroxylase enzyme can also convert 1,25-hydroxyvitamin D into the inactive metabolite 1,24,25-hydroxyvitamin D [60]. As above, active 1,25-hydroxyvitamin D promotes gastrointestinal absorption of both calcium and phosphate [61] and has also been shown to promote bone resorption [62].

Regulation of phosphate homeostasis by PTH, FGF23 and vitamin D

Phosphates are essential to several biological processes, including bone mineralization and energy metabolism. Without adequate phosphate availability, bone mineralization cannot occur, leading to the development of osteomalacia [63]. Inorganic phosphates are required for

generation of the primary cellular energy currency, adenosine triphosphate (ATP). Organic phosphates are an important constituent of cell membranes [63]. The primary hormonal regulators of phosphate homeostasis are PTH, FGF23 and vitamin D. Vitamin D increases circulating phosphate levels, while PTH and FGF23 reduce phosphate concentrations by promoting renal phosphate excretion. Low levels of circulating phosphate stimulate the synthesis of vitamin D, which in turn promotes both intestinal phosphate absorption and renal phosphate reabsorption [64]. Although the primary driver of PTH release is low extracellular calcium concentrations, high serum phosphate also stimulates PTH release [65]. While PTH does increase 1,25-hydroxyvitamin D levels thereby increasing gastrointestinal phosphate absorption, it also promotes renal phosphate wasting, which has the net effect of lowering circulating phosphate concentrations [58].

FGF23 is another principal regulator of phosphate homeostasis. This hormone is secreted by cells of the osteoblast lineage, including both osteoblasts and osteocytes, in response to hyperphosphatemia, dietary phosphate loading, and high 1,25-hydroxyvitamin D levels [66]. In the kidney, FGF23 blocks phosphate reabsorption at the proximal tubule by inhibiting the type II sodium-phosphate cotransporters NPT2a and NPT2c [67]. This hormone also decreases expression of 1-α-hydroxylase and increases expression of 24-α-hydroxylase [68], leading to decreased 1,25-hydroxyvitamin D synthesis and increased catabolism. The effects of FGF23 on mineral homeostasis are exerted through the fibroblast growth factor receptor, and require binding of a co-receptor, αKlotho. Without the presence of αKlotho, FGF23 cannot exert its phosphaturic and vitamin D-lowering effects. For example, mice that lack αKlotho are phenotypically very similar to FGF23 knockout mice, with both manifesting a premature aging phenotype characterized by hyperphosphatemia, osteopenia, hypercalcemia and vascular calcification [69, 70].

2.1.4 Summary of bone physiology

Bone is a complex and metabolically active organ. Type I collagen serves as the bone's structural scaffolding, while noncollagenous matrix proteins help to maintain collagen fidelity and facilitate mineralization. The cellular component of bone consists of osteoclasts, osteoblasts, and osteocytes. Bone remodelling is the result of the concerted actions of these three cell types. Several humoral factors promote bone resorption, most notably RANKL, and, in cases of sustained exposure, PTH. Bone formation, on the other hand, is promoted by the Wnt/β-catenin pathway, and, in cases of intermittent exposure, PTH. Oestrogen may also promote bone formation via suppression of sclerostin [71]. Conditions that lead to uncoupling of bone resorption and formation can result in the development of osteoporosis.

Calcium and phosphate are incorporated within the bone mineral as hydroxyapatite during formation of new bone and liberated into the blood stream when bone is resorbed. Maintaining circulating concentrations of these minerals within a specific range is important, as each is required for multiple cellular functions. PTH, vitamin D, and FGF23 are the primary hormonal regulators of calcium and phosphate homeostasis. The net effect of PTH action is to increase circulating calcium concentrations and decrease circulating phosphate concentrations. The net effect of 1,25-hydroxyvitamin D is to increase circulating concentrations of both calcium and phosphate. FGF23 lowers serum phosphate concentrations and suppresses 1,25-hydroxyvitamin D production. The next section of this chapter outlines the growing body of evidence demonstrating relationships between these hormones and the minerals involved in bone mineral homeostasis, and both energy metabolism and the cardiovascular system.

2.2 RELATIONSHIPS BETWEEN BONE MINERAL METABOLISM, ENERGY METABOLISM AND THE CARDIOVASCULAR SYSTEM

In section 2.1, I provided an overview of bone physiology, as it pertains to osteoporosis. The present section explores the relationships between skeletal parameters, bone mineral homeostasis, and both energy metabolism and the cardiovascular system. This is an area of considerable ongoing research, and developing a better understanding of these relationships is likely to have important clinical implications for individuals with osteoporosis. For instance, fat mass (a marker of energy balance) is tightly correlated with BMD, and individuals with low body weight have an increased propensity for fragility fracture [72]. In addition, osteoporosis and atherosclerotic disease often coexist in the same individuals [73, 74]. Having low bone mass has been associated with an increased likelihood of coronary artery calcification and cardiovascular mortality in some populations [73-75] but not others [76]. It appears that these associations are mediated by hormonal cross-talk between the skeleton and several other organ systems, including the adipose tissue, the vasculature, and the pancreas [77]. However, in most cases the underlying mechanisms are not well understood. The next subsection summarizes existing data regarding the relationships between bone mineral homeostasis and energy metabolism.

2.2.1. Relationships between bone mineral homeostasis and energy metabolism

Body weight, fat mass in particular, is an important determinant of BMD [72, 78]. Low fat mass is associated with low BMD and an increased risk of fracture [72]. From a mechanical standpoint, having low body weight results in reduced skeletal loading and less stimulus for new bone formation [77]. However, hormonal cross-talk between adipose tissue, the β-cells of the pancreas, and the bone also appears to mediate this relationship [77]. Specifically,

measures of adiposity and energy metabolism not only correlate with BMD, but also appear to be associated with circulating levels of bone minerals and their regulatory hormones.

In an evaluation of healthy individuals (n=1016) from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort, plasma calcium concentration was significantly positively associated with body mass index (BMI), waist circumference, and insulin resistance [79]. In a separate cohort of elderly men also from Uppsala, Sweden (n=961), higher serum calcium levels were associated with reduced insulin sensitivity [80]. In a study of middle-aged Korean men (n=213), serum calcium was positively associated with the presence of risk factors for metabolic syndrome, including waist circumference, lipid levels, glucose and blood pressure [81]. In 863 adults from the Insulin Resistance Atherosclerosis Study, calcium levels and calcium-phosphate cross-product were identified as risk factors for the development of type 2 diabetes [82]. Whereas serum calcium levels appear to be positively correlated with adiposity and glucose dysregulation, these parameters are inversely related to phosphate concentration. In a large observational study (n=46,798), Park *et al* determined that circulating phosphate concentrations were negatively correlated with the following metabolic parameters: BMI, waist circumference, fasting glucose, insulin, blood pressure, and triglyceride levels [83]. In a study of 255 individuals presenting for an annual medical check-up, Kalaitzidis and colleagues showed that those who met the diagnostic criteria for metabolic syndrome had lower phosphate concentrations than those who did not [84].

The phosphaturic hormone FGF23 also appears to be related to adiposity and glucose metabolism. In the population-based Swedish MrOS cohort of 964 men, body weight was positively correlated with FGF23 after adjustment for age ($r=0.18$, $p<0.0001$) and after

further adjustment for indices of mineral metabolism ($r=0.20$, $p<0.0001$) [85]. Similar relationships were seen with fat mass. In a community-based cohort of 70-year olds from Uppsala (n=946), correlations of FGF23 with body weight and fat mass were also found ($r=0.07$, $p<0.05$) [85]. In 2134 middle-aged men and women from the European Prospective Investigation of Cancer Germany cohort, waist circumference and BMI increased across the quartiles of FGF23 [86]. In the Health Aging and Body Composition study, a similar significant relationship between quartiles of FGF23 and BMI was observed, mean BMI being 26.6 in FGF23 quartile 1 and 28.0 in quartile 4 [87]. Finally, in patients with primary hyperparathyroidism, FGF23 levels are related to BMI ($p<0.001$) [88].

In 2006, Bolland *et al* demonstrated a positive relationship between fat mass and PTH [89], and subsequent research has confirmed positive correlations between PTH and both adiposity and insulin resistance [79, 90, 91]. Although evidence to support relationships between the bone minerals, their regulatory hormones, and energy metabolism is quickly accumulating, there are limited data addressing the direction of these associations and potential underlying mechanisms.

2.2.2 Relationships between bone mineral homeostasis and cardiovascular disease

Individuals with osteoporosis have an increased likelihood of having underlying coronary artery disease. For example, Choi and colleagues assessed bone density, coronary calcification, and coronary plaque burden in 467 individuals (339 females) presenting for routine medical check-ups, and found that low bone density was related to higher coronary calcification in women ($p<0.01$), and to higher coronary plaque burden in both women and men ($p<0.01$) [75]. In 863 postmenopausal women with no history of cardiovascular disease, Lee *et al* determined that individuals with low bone density (osteopenia or osteoporosis) were

significantly more likely to have a coronary artery calcium score ≥100 or obstructive coronary artery disease (≥50% stenosis of the major epicardial coronary arteries), relationships that remained significant after adjustment for age and cardiovascular risk factors [74]. In a recent systematic review and meta-analysis, Ye and colleagues identified 25 studies (n= 10,299) that interrogated the relationship between low bone density and atherosclerotic vascular abnormalities (a composite that included coronary artery calcification, cardiovascular disease, and coronary artery disease). Based on their meta-analysis of 14 studies (n=4933), they concluded that low BMD (osteoporosis or osteopenia) is an independent predictor of the development of atherosclerosis in elderly individuals (odds ratio [OR]: 1.81, p <0.0001) [92]. While the reasons for these associations are not well understood, it has been speculated that the development of both low bone density and cardiovascular disease could result from a common pathogenic process [73].

Assessment of the principal bone minerals, calcium and phosphate, has indicated that both minerals may promote the development of vascular dysfunction. Preclinical data suggest that circulating calcium encourages vascular calcification via stimulation of phosphate uptake by vascular smooth muscle cells, promotion of calcium-phosphate precipitation, and inhibition of expression of calcification inhibitors by vascular smooth muscle cells [93]. Circulating calcium may also influence blood pressure and blood coagulation. Acute increases in calcium concentrations tend to be associated with increases in blood pressure [94, 95]. This effect may be related to stimulation of the calcium-sensing receptor, which is expressed not only in parathyroid tissue but also throughout the vasculature [96], through activation of the renin-angiotensin-aldosterone system, or possibly indirectly, through modulation of PTH [97]. Increases in serum calcium could also promote influx of calcium into vascular smooth muscle cells, inducing contraction and thereby increasing vascular tone [98]. Calcium is required for

activation of the clotting cascade, and preclinical studies imply that increases in serum calcium levels result in increased coagulability [99, 100]. In a recent systematic review, Reid *et al* evaluated 8 human studies assessing the relationship between serum calcium concentrations and cardiovascular disease, finding a significant and relatively consistent 8% increased risk of cardiovascular disease for every one standard deviation increase in calcium [101].

Elevated serum phosphate concentrations have also emerged as a risk factor for vascular calcification and cardiovascular events [102]. In vitro studies have demonstrated that inorganic phosphate stimulates calcification of vascular smooth muscle cells [103]. A small study in humans (n=19) demonstrated that phosphate loading resulted in the development of endothelial dysfunction [104]. Observational data consistently demonstrate that high and even high-normal circulating phosphate levels are associated with an increased risk of cardiovascular events and mortality, both in those with known cardiovascular disease [105] and those without [106].

The role of FGF23 in the development of cardiovascular disease is less clear. As described in section 2.1, FGF23-null mice have a distinct phenotype which includes hyperphosphatemia and vascular calcifications [69], suggesting that the presence of this phosphatonin may be protective for the vasculature. Stubbs and colleagues have demonstrated that this calcified mouse phenotype can be rescued by the administration of a phosphate-deficient diet, which also serves to highlight the role of phosphate in the development of vascular calcification [107]. On the other hand, some literature suggests that FGF23 itself may have harmful cardiovascular effects. Preclinical data from Faul and colleagues demonstrates that FGF23 can induce left ventricular hypertrophy in mice, independently of its co-receptor, αKlotho

[108]. In humans, positive associations between FGF23 concentrations and left ventricular mass index, arterial stiffness, and cardiovascular events have been demonstrated in a cohort of Swedish adults [109-111], although data to confirm causation are lacking.

Recently, it has become clear that PTH levels, even within the normal range, are independently associated with markers of cardiovascular disease, including blood pressure, dyslipidaemia, left ventricular hypertrophy, and endothelial dysfunction [79, 112-129]. A number of studies have also reported associations between PTH and atherosclerotic burden [119, 130], as well as cardiovascular events and mortality [121, 128, 131, 132]. Whether these relationships represent an epiphenomenon or whether PTH plays a pathogenic role in the development of cardiovascular disease has yet to be established.

2.2.3 Summary of relationships between bone mineral metabolism, energy metabolism, and the cardiovascular system

Osteoporosis and low BMD are associated with low body weight and fat mass, and also with the development of cardiovascular disease. Associations between the minerals and hormones involved in bone homeostasis and both measures of energy metabolism and cardiovascular dysfunction and have also been observed. Specifically, increased adiposity and glucose dysregulation have been consistently associated with increased circulating calcium, PTH and FGF23, and with decreased circulating phosphate. In observational studies, cardiovascular disease and vascular risk factors are positively related to calcium, phosphate, PTH and FGF23 levels. While these relationships may have important clinical implications for individuals with osteoporosis and the general population, the underlying mechanisms are poorly understood, and in most cases, the direction of causality is not clear.

While the relationships described above are generally consistent across both small cohort studies and large population-based studies, it should be acknowledged that the use of both small and large sample sizes can lead to errors in the interpretation of results. For example, small sample sizes can result in failure to detect important clinically significant associations, while large population-based analyses may detect spurious associations between variables that meet criteria for statistical significance but have no biological relevance. Sample size can become particularly problematic when the outcomes of interest are assessed as part of post-hoc analyses on a cohort originally recruited to answer a different research question. In such cases, the sample size selected to assess the primary outcome may not be optimal for the parameters evaluated in the post-hoc study. This section describes the results of several meta-analyses, which can also contribute to errors by weighting larger studies with greater significance than smaller ones. Finally, the majority of the research described in this section pertains to healthy older Caucasian and Asian individuals, and it remains unclear whether the associations described in these studies can be generalized to other populations. Relationships between PTH, adiposity, and cardiometabolic risk factors are further interrogated in Chapter 3, while Chapter 4 examines the metabolic correlates of phosphate and FGF23. The research described in Chapter 7 attempts to elucidate the mechanism by which calcium supplementation might increase cardiovascular risk. The next section of this chapter expands upon my review of the skeletal and extraskeletal effects of PTH, in the form of a discussion of conditions associated with PTH excess.

2.3 DISORDERS OF PARATHYROID HORMONE EXCESS

As outlined in section 2.1, PTH is an important regulator of calcium and phosphate homeostasis, and exposure of osteoblasts to sustained high levels of this hormone promotes bone resorption. It is therefore not surprising that disorders resulting in PTH excess have

significant ramifications for bone mineral metabolism, and that prolonged PTH excess is an established secondary cause of osteoporosis.

In the general population, elevated circulating PTH concentrations are most often the result of either inappropriate autonomous PTH production by the parathyroid glands (primary hyperparathyroidism), or stimulation of excess PTH release by one or more secondary factors, such as vitamin D deficiency (secondary hyperparathyroidism). As described in section 2.2, PTH levels tend to be positively associated with adiposity and other cardiovascular and metabolic parameters. There is controversy regarding whether this relationship reflects a causative role of PTH in the development of cardiometabolic sequelae, or whether the presence of cardiometabolic dysfunction causes PTH excess. In this section of the literature review, disorders of PTH excess will be concisely reviewed.

2.3.1 Primary hyperparathyroidism

Individuals with primary hyperparathyroidism have autonomous hyperfunction of one or more of the parathyroid glands as the result of a single parathyroid adenoma, multiple adenomas, or multi-gland hyperplasia [133]. This develops spontaneously, with no obvious trigger. Primary hyperparathyroidism is a common endocrine disease, affecting women more frequently than men, with an overall prevalence on the order of 1 in 500 [133]. Biochemically, the disorder is characterized by elevated or inappropriately normal PTH concentrations in the setting of hypercalcemia [133-135]. Primary hyperparathyroidism is an established secondary cause of osteoporosis and fragility fracture, as well as nephrolithiasis, and renal impairment. The condition may also result in symptomatic hypercalcemia [136-139]. Consistent with the data presented in section 2.2, individuals with primary hyperparathyroidism appear to be at increased risk of cardiovascular events [136-139]. The

skeletal and renal sequelae of primary hyperparathyroidism are often progressive [140, 141], but can be reversed or at least ameliorated with parathyroidectomy [136, 140, 142-144]. Parathyroidectomy has also been shown to reverse blood pressure, lipid abnormalities, and markers of vascular dysfunction in some studies [91, 117, 118, 125, 145, 146], but not others [124, 147, 148].

2.3.2 Secondary and tertiary hyperparathyroidism

In secondary hyperparathyroidism, circulating PTH levels increase in response to a stimulus that is external to the parathyroid glands, most often vitamin D deficiency or renal dysfunction. In the case of renal dysfunction, pathogenesis is multifactorial, with contributions from hyperphosphatemia, low levels of circulating 1,25-hydroxyvitamin D (due to low availability of 1-α-hydroxylase, which is synthesized in the renal parenchyma) and resultant hypocalcaemia [149]. Medications such as lithium can also stimulate PTH release, as can renal tubular abnormalities that result in increased calcium excretion, and malabsorptive disorders such as celiac disease or inflammatory bowel disease in which gastrointestinal absorption of calcium is reduced [134]. While not traditionally considered causes of secondary hyperparathyroidism, characteristics such as age, fat mass, and blood pressure have previously been shown to be independently associated with circulating PTH concentrations [89, 112, 150, 151]. These variables may play a role in determining an individual's PTH level.

Generally, secondary hyperparathyroidism can be reversed by treating the underlying cause. However, in the setting of chronic kidney disease, a third entity – tertiary hyperparathyroidism – may develop. Tertiary hyperparathyroidism occurs most often in individuals who experience severe secondary hyperparathyroidism as the result of the

hyperphosphatemia and hypocalcaemia of kidney disease, during which time parathyroid hyperplasia and autonomy develop. Following correction of the secondary stimulus (for instance, after renal transplantation), these individuals continue to have high circulating PTH levels, despite normal renal calcium and phosphate handling [152, 153]. Although a detailed discussion of secondary and tertiary hyperparathyroidism in the setting of renal dysfunction is outside of the scope of this thesis, the concept that prolonged stimulation of the parathyroid glands can result in parathyroid autonomy is relevant to the research presented in Chapter 3.

2.3.3 Normocalcemic hyperparathyroidism

The entity of 'normocalcemic hyperparathyroidism' is characterized by persistent PTH elevation in the absence of hypercalcemia or recognized causes of secondary hyperparathyroidism. In recent years, there has been debate regarding the clinical relevance of this condition [114, 134, 154, 155]. It is unusual to define a disease in terms of levels of a single analyte, and given that reference ranges typically encompass the central 95% of a 'healthy' reference population [156], doing so implies that up to 2.5% of the population might have the disease [157].

Attempts to characterize the clinical ramifications of normocalcemic hyperparathyroidism have been met with conflicting results. In some case series, up to 50% of patients have osteoporosis, approximately 10% have nephrolithiasis, and up to 40% demonstrate disease progression [114, 154, 155, 158]. Cardiovascular outcomes in normocalcemic hyperparathyroidism have not been well studied, but some reports suggest that these individuals have an elevated risk of hypertension and cardiovascular disease, similar to what has been observed in primary hyperparathyroidism [159, 160]. Several patients from these cohorts have undergone parathyroidectomy and have been found to have either parathyroid

adenomas or hyperplasia [114, 154, 155, 158]. Therefore, it has been suggested that normocalcemic hyperparathyroidism represents an early subclinical form of primary hyperparathyroidism and that affected individuals are at risk of developing skeletal, cardiovascular, and renal consequences, and of becoming hypercalcaemic [114].

However, many of the individuals from these cohorts came to attention following referral for investigation of problems such as osteoporosis, raising the possibility that referral bias may account for the high prevalence of pathology reported [114, 154, 155]. In other cohorts, no association between normocalcemic hyperparathyroidism and adverse skeletal or renal outcomes has been identified, and there has been no evidence of progression to hypercalcaemic hyperparathyroidism [113, 161, 162]. Thus, it is unclear whether normocalcemic hyperparathyroidism is a discrete pathological entity with real associated morbidity, and there is currently no consensus regarding its management [137].

2.3.4 Summary of disorders of parathyroid hormone excess

In summary, PTH excess may be the result of primary parathyroid autonomy or a secondary stimulus to the parathyroid glands. Prolonged secondary hyperparathyroidism can lead to the development of parathyroid autonomy (i.e. tertiary hyperparathyroidism). Sustained elevations in PTH levels are known to increase bone resorption, leading to the development of secondary osteoporosis. PTH excess also appears to be associated with cardiometabolic dysfunction, although it is presently unclear whether PTH is a driver of this dysfunction or a by-product. Regarding normocalcemic hyperparathyroidism, uncertainty exists regarding the pathogenesis and clinical relevance of this entity. These unresolved issues will be further explored in Chapter 3.

2.4 CAUSES OF SKELETAL FRAGILITY

Previous sections of this chapter have provided a general overview of bone physiology, including composition of the skeleton, the bone remodelling process, and hormonal regulation of bone mineral homeostasis. Disruption of the normal physiology can lead to reductions in bone density and bone quality, predisposing affected individuals to fragility fracture. In this section, causes of skeletal fragility are reviewed. The first subsection addresses 'primary' or 'age-related osteoporosis', and encompasses the entity of 'postmenopausal osteoporosis' that occurs as a result of oestrogen deficiency. The second subsection discusses 'secondary osteoporosis', referring to osteoporosis that is not explained by age and/or age-related sex hormone deficiency alone and is often the result of underlying disease or medication use.

2.4.1 Primary (age-related and postmenopausal) osteoporosis

Most individuals achieve their maximal bone density, or peak bone mass, by the early-to-mid 20s [163]. Loss of bone mass generally begins around the early 30s, at which time the rate of resorption starts to exceed formation [164]. In women, bone loss accelerates around the time of menopause, often exceeding 2 to 5% per year in the perimenopausal period [165]. This translates into losses of 20 to 30% of bone density at trabecular sites and 5 to 10% at cortical sites in the decade following cessation of menses [23, 166]. Within 5 to 10 years of menopause, bone loss begins to slow again, continuing at a rate of 0.5 to 1.0% throughout the remainder of the lifespan [166, 167]. Men ultimately experience less bone loss than women, as they do not undergo an accelerated period of bone loss as women do following menopause. However, older men also tend to lose bone at a rate of 0.5 to 1.0% per year [164, 167].

Postmenopausal osteoporosis pertains to the uncoupling of bone resorption and bone formation that occurs in women following menopause, which is largely the result of oestrogen deficiency. As described in section 2.1, the presence of oestrogen suppresses bone resorption and promotes bone formation. Oestrogen suppresses the production of RANKL by osteoblasts [40] and upregulates OPG expression, in addition to suppressing the pro-osteoclast factors M-CSF, IL-1, IL-6, TNF-α, and prostaglandins [164]. In addition, oestrogen stimulates apoptosis of mature osteoclasts and osteoclast precursors [164]. The presence of oestrogen results in upregulation of growth factors such as insulin-like growth factor-1 and TGF-β in osteoblasts [166]. Oestrogen has more recently been shown to promote differentiation of mesenchymal stem cells into osteoblasts rather than adipocytes, and to reduce osteoblast and osteocyte apoptosis [168]. Recent evidence also indicates that oestrogen suppresses sclerostin, which, as described in section 2.1, is an antagonist of the anabolic Wnt signalling pathway [164, 169]. Thus, the net effect of the oestrogen deficiency that develops at the time of menopause is a rapid increase in bone resorption along with a reduction in bone formation.

Men experience gradual age-associated reductions in circulating sex hormone levels and corresponding bone loss, which puts older men at risk of developing osteoporosis. Over the course of the male lifespan, sex hormone binding globulin levels increase by more than 2-fold. This corresponds to a decrease in the amount of bioavailable testosterone, and a decrease in the amount of bioavailable oestrogen by approximately half [170]. The reduction in oestrogen concentrations is clinically important, as oestrogen status appears to be more reflective of BMD than testosterone in men, and correspondingly, oestrogen replacement has been shown to have a more marked effect on reducing bone turnover than testosterone replacement in men who are deficient in both hormones [171].

Additional age-related factors have been shown to promote bone loss, in particular, decreased growth hormone and insulin-like growth factor 1 production [164]. Non-hormonal factors such as cellular senescence are also likely contributors to the increased skeletal fragility seen in older individuals [164]. However, when bone loss and skeletal fragility exceed what is expected for an individual's age and sex, an assessment for secondary causes of osteoporosis is indicated. Several secondary causes are outlined in the following subsection.

2.4.2 Secondary osteoporosis

Secondary osteoporosis relates to the presence of osteoporosis and/or increased skeletal fragility that cannot be explained by age and expected age-related declines in circulating sex hormones alone. A multitude of causes of secondary osteoporosis have been identified. As described in previous sections of this literature review, these include conditions such as hyperparathyroidism, as well as the use of medications such as TZDs. Most secondary causes of osteoporosis fall into one or more of the following categories: genetic conditions, lifestyle factors, underlying disease states, and medication use. Although not meant to be an exhaustive list, some of the most common and relevant causes of skeletal fragility are laid out in Table 2.1. A complete discussion of the numerous secondary causes of osteoporosis and the underlying pathophysiology is outside of the scope of this thesis, although a detailed review of the topic has recently been published [12]. It is also important to note that a person's peak bone mass has a large impact on bone strength and propensity to fracture later in life. Determinants of peak bone mass are multifactorial, and include genetic factors, age of puberty, body mass, and weight-bearing physical activity [172, 173].

Table 2.1 Risk factors for osteoporosis and fragility fractures

Category	Risk Factor
Lifestyle factors	Immobilization Low body weight Smoking Excess alcohol consumption Falls
Underlying disease states	
Endocrine	Hyperparathyroidism Untreated hyperthyroidism Hypogonadism Type 1 and type 2 diabetes mellitus Cushing's syndrome Acromegaly
Malabsorptive	Inflammatory bowel disease Short gut syndrome Celiac disease
Hematologic	Multiple myeloma Mastocytosis Thalassemia Ankylosing spondylitis
Rheumatologic	Rheumatoid arthritis Systemic lupus erythematosus
Other	Hyponatremia Chronic kidney disease Chronic liver disease Spinal cord injury Organ transplantation Human immunodeficiency virus Idiopathic hypercalciuria Anorexia nervosa
Medications	Glucocorticoids Anticonvulsants Heparin Aromatase inhibitors Gonadotropin-releasing hormone agonists Selective serotonin reuptake inhibitors Proton pump inhibitors Thiazolidinediones Depot medroxyprogesterone acetate

The mechanism(s) by which the factors listed in Table 2.1 increase skeletal fragility are well-established in some cases and poorly understood in others [12]. A more complete understanding of how these conditions contribute to the development of skeletal fragility is necessary to guide the development of strategies for prevention and treatment. To provide an example relevant to the research that is presented in Chapter 5, TZD medications improve insulin sensitivity and lower blood glucose levels in people with type 2 diabetes, however post-hoc analyses of large randomized clinical trials have demonstrated that treatment with TZDs increases the risk of fracture by 1.5 to 2 times [174-176]. The mechanisms by which treatment with TZDs might increase fracture risk are unclear at present, although preclinical data indicates that these medications might promote the differentiation of mesenchymal stem cells into adipose cells rather than osteoblasts [44-47]. Given that the prevalence of type 2 diabetes exceeds 10% in many developed nations [177], and taking into account that most patients with diabetes who require glucose-lowering medications will require them indefinitely, it is necessary to gain a better understanding of the mechanism by which TZDs affect the skeleton and whether these effects are reversible. The effects of TZDs on BMD and markers of bone turnover are further addressed in Chapter 5.

2.4.3 Summary of causes of skeletal fragility

The most common cause of skeletal fragility is postmenopausal osteoporosis, which results primarily from the rapid decline in endogenous oestrogen production at the time of menopause. Although men do not experience an equivalent period of accelerated bone loss, they do experience gradual age-related declines in testosterone and oestrogen, contributing to steady bone loss in older age. Changes in growth hormone production and cellular senescence also contribute to the development of osteoporosis with advancing age. Beyond age-associated contributors, a number of other factors that interrupt normal skeletal physiology

can lead to the development of osteoporosis, although the pathogenic mechanisms are not clear in many cases. Specifically, the use of the insulin-sensitizing TZD medications are known to increase the risk of fracture, but the mechanism is unknown. The research described in Chapter 5 attempts to elucidate the mechanism by which TZDs increase fracture risk.

2.5 EPIDEMIOLOGY & CONSEQUENCES OF OSTEOPOROSIS

Osteoporosis is characterized by the presence of low bone mass and/or decreased bone quality which predisposes an individual to an increased risk of fragility fracture [1]. Fragility, or low trauma fractures are generally defined as fractures that occur spontaneously or as the result of a trauma that would not have been expected to result in a fracture in a healthy individual [178]. These fractures are the primary clinical consequence of osteoporosis and account for up to 80% of broken bones in postmenopausal women [178]. Although it is possible to fracture any bone, fractures of the spine, hip and wrist in particular are strongly associated with the presence of reduced bone mass, and their epidemiology has been well characterized [1]. Therefore, this section will focus primarily on the epidemiology and consequences of fragility fractures sustained at these sites.

2.5.1 Epidemiology of osteoporosis

It is estimated that up to 50% of postmenopausal women and 20% of men over the age of 50 will sustain at least one fragility fracture [2]. In New Zealand specifically, it has been estimated that there were approximately 84,000 osteoporotic fractures in 2007, including 3800 hip fractures and almost 28,000 vertebral fractures [179], with fracture rates being approximately 70% higher in women than men [179]. The incidence of fragility fractures varies not only with sex, but also with geographic location and ethnicity. For instance, rates

of hip fracture are lower in North America and Australasia than Scandinavia, and lower still in Asia [1]. Age is another important determinant of fracture rates. In women, fracture incidence begins to rise significantly in the perimenopausal period. At this time, the risk of forearm fracture rises steeply, reaching a plateau approximately 15 years after menopause [180]. Hip and vertebral fracture rates rise linearly from the time of menopause. While hip fracture incidence begins to increase exponentially from about age 65 onwards, vertebral fracture incidence continues to rise linearly throughout the lifespan [180]. In men, an increased incidence of hip and vertebral fracture becomes apparent approximately one decade later than women [180]. In both women and men, the incidence of vertebral fractures is difficult to quantify accurately, as up to two-thirds are asymptomatic, and less than 10% require hospital admission [181].

2.5.2 Consequences of fragility fracture

Fragility fractures are associated with significant morbidity, mortality, and cost [2, 182]. Mortality in the year following hip fracture approaches 30% in women [183, 184], and has been reported as high as 37% in men [184]. Excess mortality has also been observed following other types of osteoporotic fracture [185]. In those who survive, hip fracture can lead to an increase in morbidity and disability, and a drastic decline in independence [186]. Of community-dwelling individuals that sustain a hip fracture, more than 40% are living in long term care facilities a year later [187], and less than half regain their baseline level of function [188]. Vertebral fractures can result in both acute and chronic pain. These fractures can also lead to the development of thoracic kyphosis, which limits mobility, can have a detrimental effect on body image, and may lead to the development of restrictive lung disease [189]. The health care costs in the year following fracture are estimated on the order of USD 30,000 or hip fracture and USD 4000 to USD 11,000 for other types of fracture [182]. In New

Zealand, the total direct cost of managing osteoporosis was NZD 330 million in 2007, with projected costs of NZD 458 million in 2020 [179]. In the United States, osteoporotic fractures account for more hospitalizations and related costs than myocardial infarction, stroke, or breast cancer in women aged 55 or older [190].

The occurrence of a fragility fracture is an independent risk factor for the development of further fractures. Sustaining a fracture increases the risk of a subsequent fracture by approximately 2-fold [191]. In the case of vertebral fracture, the risk of subsequent fractures increases more than 4-fold, and the increased risk not only extends to further vertebral fractures but also to hip fracture [191, 192]. The risk of subsequent fracture is highest in the year following the incident fracture [193, 194].

2.5.3 Summary of the epidemiology and consequences of osteoporosis

In summary, older adults have a high probability of sustaining at least one fragility fracture, and these fractures have numerous health and economic consequences. Consequently, the identification and treatment of individuals at high risk of fragility fracture is of high priority. Tools for assessing bone mass and estimating fracture risk are reviewed in section 2.6 and strategies for fracture prevention discussed in section 2.7.

2.6 ASSESSMENT OF BONE DENSITY AND FRACTURE RISK

As outlined in section 2.5, fragility fractures are common, and they carry a significant burden. Fortunately, therapies are available to reduce fracture risk, and have been shown to be cost-effective when provided to those at the highest risk of fracture [182]. The current section will focus on the tools that are presently available to assess an individual's likelihood of fracture.

The diagnosis of osteoporosis and identification of individuals at high risk of fracture presents a challenge for clinicians. This disorder is characterized by decreased bone strength, low bone mass, and an increased risk of low-trauma fracture [1, 195]. However, in clinical practice, the identification of individuals meeting these criteria can be difficult. Techniques that measure bone strength and bone quality are currently limited to research use. While BMD can provide an estimate of bone mass, results can be difficult to interpret, as declining bone density is an expected consequence of aging and becomes clinically relevant only when it results in fracture. However, waiting until an individual has already sustained a fragility fracture to make the diagnosis of osteoporosis and institute treatment is not an optimal approach. Consequently, most clinicians use a combination of BMD and other risk factors to make the diagnosis of osteoporosis and estimate fracture risk. In the following subsections, I provide a concise review of the techniques that are available to assess bone mass, bone quality, and bone turnover, focusing on BMD. I also provide an overview of available clinical prediction tools that incorporate fracture risk factors to provide an estimate of fracture risk.

2.6.1 BMD and other techniques to assess bone mass and bone quality

Measurement of BMD with dual x-ray absorptiometry (DXA) is the primary modality for obtaining information about bone density in clinical practice. Widely utilized since the 1980s, this technique is rapid, non-invasive, and associated with a low radiation dose. DXA involves passing dual x-ray beams, each with a different photon energy, through an anatomical region of interest (i.e. the spine, hip or forearm). The two beams are differentially attenuated by the bone mineral and surrounding soft tissue, and analysis of these attenuation patterns can provide a measure of bone mineral content within the region, which can be then divided by the bone area to produce a two-dimensional assessment of BMD (in g/cm^2, also known as

areal BMD) [196]. Standard regions of interest for the assessment of areal BMD are the lumbar spine, the femoral neck, and the entire proximal femur (total hip) [197], although BMD is frequently measured at the forearm and total body as well.

In 1994, the World Health Organization (WHO) issued a report providing recommendations for diagnosing osteoporosis on the basis of BMD by DXA. The report indicated that osteoporosis may be diagnosed in individuals with a BMD T-score of -2.5 or less [198], where the T-score represents the number of standard deviations by which an individual's BMD varies from the young adult mean. A negative T-score indicates that BMD is below the young adult mean, whereas a positive score indicates BMD above the mean. The cutoff of -2.5 reflects the observation that the lifetime risk of hip fracture in Caucasian women aged 50-85 years is approximately 20%, and approximately 20% of women in this age group have a T-score of -2.5 or less [199, 200]. It should be noted that while T-scores are conventionally used for reporting BMD in postmenopausal women and men aged 50 or older, this is not the case for younger individuals. In premenopausal women and men aged <50 years, Z-scores are usually used. A Z-score reflects the number of standard deviations by which an individual's BMD varies from the age- and sex- matched mean. A Z-score of <-2.0 indicates that a person's BMD is below the expected range for age [197]. In women and men of all ages, a Z-score of <-2.0 should raise suspicion for secondary osteoporosis.

While assessment of areal BMD by DXA does provide valuable information about bone mass and fracture risk, this technique has limitations, and application of the WHO diagnostic criteria has the potential to create confusion for clinicians and patients alike. DXA does not provide information about bone quality or bone turnover and does not account for bone thickness, which are other important determinants of bone strength [201]. Perhaps most

importantly, BMD by DXA does not take into consideration other risk factors for fracture. It is therefore not surprising that BMD measurements account for less than 50% of an individual's fracture risk [202]. Correspondingly, less than half of fragility fractures occur in individuals with T-scores in the osteoporosis range, with the majority of fractures occurring in individuals with T-scores between -1.0 and -2.4 [203]. As a result, some individuals who meet the WHO criteria for the diagnosis of osteoporosis are actually at low risk of fragility fracture despite being given the label of 'osteoporosis', and by the same token, many individuals who do not meet the densitometric diagnostic criteria will be at high risk of fracture [202].

Additional imaging modalities have been examined for their ability to diagnose osteoporosis and predict fracture. Use of peripheral ultrasound of the heel is simple and non-invasive, but does not predict fracture risk as reliably as BMD by DXA [197]. Assessment of BMD using quantitative computed tomography at the spine and femur has similar predictive ability to DXA, although quantitative computed tomography is associated with significantly higher radiation exposure, and thus assessment of BMD by DXA is preferred [197]. High resolution peripheral quantitative computed tomography (HR-pQCT) has emerged as another promising tool for assessing both BMD and bone quality [204]. This technique involves scanning of the radius and/or tibia and can provide a three-dimensional (volumetric) assessment of BMD. Scans obtained with HR-pQCT are of high enough resolution to permit differentiation between cortical and trabecular bone compartments and provide information about bone microarchitecture. Data obtained with HR-pQCT can also be used to estimate bone strength using finite element analysis techniques [204]. Although HR-pQCT appears to be of value for prediction of fracture risk [205-208], its use is primarily limited to research at present, owing

in large part to the cost and scarcity of the required hardware, and to a paucity of normative data [209].

Non-radiologic techniques to assess bone quality and bone turnover include measurement of serum and urine markers of bone turnover, and bone biopsy. Measurement of bone turnover markers can provide an indication of bone formation and resorption. Serum levels of the byproduct of collagen synthesis, N-terminal propeptide of type 1 collagen (P1NP), and the osteoblastic enzyme, bone-specific alkaline phosphatase (bsALP), are indicative of bone formation. Serum levels of the collagen degradation products C-terminal telopeptide of type 1 collagen and (CTX) and urine levels of the N-terminal telopeptide of type 1 collagen (NTX) reflect bone resorption [210]. Chopin and colleagues recently reviewed the literature to determine whether bone turnover markers were predictive of bone loss and fracture in postmenopausal women. They concluded that persistently high bone turnover markers are associated with an increased rate of bone loss, as well as an increased risk of hip and non-vertebral fractures in women aged 75 and older [211]. However, the use of bone turnover markers to predict risk of fracture is hampered by several limitations. For example, measurement is subject to significant intra-individual and inter-individual variation, levels fluctuate both with meals and diurnally, and their use has not been validated in men [212]. At present, there is limited evidence that measurement of bone turnover markers improves the diagnosis of osteoporosis and identification of individuals at high fracture risk, beyond what can be achieved with BMD by DXA and assessment of clinical risk factors [210]. Developed in the 1950s, transiliac bone biopsy with tetracycline labelling remains the gold standard for assessment of bone turnover and bone quality [213]. In practice, bone biopsies are infrequently utilized, as they are invasive, costly, and require access to a pathologist with expertise in interpretation of bone histomorphometry [214].

2.6.2 Clinical prediction tools to estimate fracture risk

The presence of certain clinical risk factors is predictive of fracture risk [202]. The past decade has seen the development and validation of several tools which can be used by clinicians to estimate fracture risk [13, 14, 202, 215-217]. These tools take into account an individual's clinical risk factors for fracture, often in conjunction with BMD, and provide a fracture risk estimate. Most current osteoporosis guidelines encourage the use of these clinical decision aids for determining which individuals might benefit from pharmacologic therapy [218-220]. There is general agreement that individuals with a ten-year absolute risk of osteoporotic fragility fracture of ≥20% will benefit from pharmacologic therapy [219, 220]. Many practitioners also offer treatment if an individual's ten-year risk of hip fracture is ≥3%, as treatment of individuals above this threshold is expected to be cost effective [182, 220].

Widely utilized tools for fracture risk prediction include the FRAX calculator [202, 215], the Garvan nomogram [13, 14], QFracture [216], and the Canadian Association of Radiologists and Osteoporosis Canada (CAROC) tool [217]. While each of these algorithms have undergone extensive internal validation, application to external populations have indicated that these calculators often provide discrepant risk estimates for the same individuals [221-224], although the reasons for these discrepancies have not been fully explored. In New Zealand, clinical practice is often to estimate fracture risk using both FRAX (http://www.shef.ac.uk/FRAX/) and Garvan (http://garvan.org.au/promotions/bone-fracture-risk/calculator/). These two calculators incorporate different sets of clinical risk factors, and both have been validated in the New Zealand population [225]. The remainder of this subsection will provide an overview of FRAX and Garvan. The calculators are compared in Table 2.2.

Table 2.2 Features of FRAX and Garvan Fracture Risk Calculators

	FRAX	**Garvan**
Clinical Risk Factors	Age (40-90)	Age (50-96)
	Sex	Sex
	Weight	
	Height	
	Previous Fracture in Adulthood (yes or no)	Fractures since age of 50 (up to 3)
	Parent Fractured Hip	
	Current Smoking	
	Glucocorticoid use	
	Rheumatoid Arthritis	
	Secondary osteoporosis	
	Alcohol	
		Falls over last 12 months (up to 3)
Other Considerations	Incorporates competing risk of mortality	No competing risk of mortality
	Different algorithms can be utilized for different ethnicities and countries	No different algorithms for different ethnicities
	Likely involves interactions between variables	No interactions between individual variables
Type of Fractures Predicted		Hip fracture at 5y
		Any fragility fracture at 5y
	Hip fracture at 10y	Hip fracture at 10y
	Major osteoporotic fracture at 10y	Any fragility fracture at 10y

FRAX was developed by the WHO and was derived using data from nine population-based cohorts around the world, and then validated in an additional 11 cohorts [202, 215]. The FRAX algorithm has not been published. It incorporates 12 variables, and estimates can be generated with or without BMD data. It provides ten-year risk estimates for hip fracture and major osteoporotic fracture (clinical spine, hip, forearm or humerus fracture), taking into account the competing risk of mortality [226]. FRAX has been externally validated in cohorts from the US, Canada, UK, France, Poland, Japan and New Zealand, with varying results [217, 225, 227-231]. For the most part, it has been found to have moderate discriminative ability, but its calibration was suboptimal in several cohorts, and it tends to underestimate fracture risk, particularly in persons of older age [217, 223, 225, 227-231].

The Garvan nomogram was derived from analysis of the Dubbo Osteoporosis Epidemiology Study in Australia, and its algorithm has been published [13, 14]. The Garvan calculator incorporates four variables, and fracture risk can be determined with or without BMD data. It provides five- and ten-year risk estimates for hip fracture and fragility fracture [13, 14]. Unlike FRAX, the risk of competing mortality is not included in the Garvan algorithm. It has been externally validated in Canadian, New Zealand, Australian, and Norwegian populations, and found to have good overall calibration, but it tends to overestimate the risk of hip fracture, particularly in the highest risk individuals [222, 224, 225, 232]. Langsetmo *et al* also noted that the association between prior fracture and future fragility fracture was stronger in the Dubbo cohort than in their Canadian validation cohort, suggesting that the Garvan calculator may overemphasize the contribution of prior fracture to future risk when applied to some other populations [224].

FRAX and Garvan sometimes disagree when both are applied to the same individual. Sandhu *et al* found the correlation between fracture risk estimates from the two calculators to be only 0.60 in a retrospective study of Australian men and women. Most discrepancies occurred in individuals with a high probability of fracture. When validated for osteoporotic fracture, they found that the area under the curves (AUCs) for the Garvan calculator (0.76-0.84) were significantly higher than those for FRAX (0.54-0.78) [222]. Bolland *et al* evaluated FRAX and Garvan in a group of postmenopausal New Zealand women, and found that both tools had AUCs between 0.60 and 0.70 for hip fracture and osteoporotic fracture, suggesting that each had moderate discriminative ability. They found that the Garvan calculator was well calibrated for osteoporotic fracture, whereas FRAX consistently underestimated it. For hip fracture, they noted that both calculators were well calibrated at low risk estimates, but that in those aged >70 and those with T-scores ≤ -2.5, FRAX tended to underestimate fracture risk, whereas Garvan overestimated it [225].

When considering individual patients, differences between the two calculators are not always clinically relevant. For instance, in the highest risk individuals, both FRAX and Garvan estimates exceed the treatment thresholds (≥20% ten-year risk of major osteoporotic fracture and/or ≥3% ten-year risk of hip fracture) even if the absolute risk is much lower with FRAX than Garvan. Such patients are likely to receive treatment regardless of whether FRAX or Garvan is used to estimate fracture risk. The converse is true in patients at the lowest risk of fracture. Differences between the two calculators become clinically relevant when fracture risk estimates fall on opposite sides of a treatment threshold. When this happens, a clinician's decision to initiate pharmacologic osteoporosis treatment may vary depending on which calculator is used. Most prior comparisons of FRAX and Garvan have focused on average differences in risk estimates across patient groups [222, 225, 233], or on hypothetical patients

[234, 235]. However, it is important for clinicians who use these calculators to understand how the differences between them apply to individual patients in a 'real world' context. Therefore, in Chapter 6, I seek to determine which patient characteristics contribute to clinically relevant discrepancies between the two calculators in a clinical setting.

2.6.3 Summary of assessment of bone density and fracture risk

Multiple tools exist for assessing bone mass and bone quality, and for estimating fracture risk. While BMD provides important information about bone mass and can be used to predict fracture risk, it does not take into account bone quality or clinical risk factors. In order to improve identification of the individuals at the highest risk of fracture who are most likely to benefit from treatment, clinical prediction tools have been developed. These take into account clinical risk factors for fracture, with or without BMD, and provide an estimate of fracture risk. Two such tools, FRAX and Garvan, are frequently used in New Zealand but often produce discrepant fracture risk estimates when applied to the same individual. Chapter 6 explores the frequency with which these discrepancies occur and the influence of patient characteristics on discordance between the two calculators.

2.7 **TREATMENT OF OSTEOPOROSIS**

Prevention of fractures is the primary goal of osteoporosis therapy. This section summarizes available osteoporosis treatments, with a focus on safety and anti-fracture efficacy. The side effects of calcium supplementation and zoledronic acid are explored in the greatest detail, as the research described in Chapters 7 and 8 concerns the safety of these therapies. For the purpose of this review, osteoporosis treatments have been divided into the following three categories: lifestyle modification strategies, calcium and vitamin D, and pharmacologic agents.

2.7.1 Lifestyle modification strategies

As reviewed in section 2.4, observational studies have identified a number of risk factors for osteoporosis and fragility fracture. Some of these can potentially be modified with lifestyle changes, including immobility, falls, smoking, excess alcohol consumption, poor nutrition, and low body weight [12, 72, 202, 236]. In this subsection, the effects of strategies to modify these risk factors are reviewed.

Exercise

The mechanical loading of bone that results from weight-bearing physical activity has a stimulatory effect on osteocytes and promotes bone formation [52, 54]. Exercise has also been shown to improve balance and reduce the risk of falls [237, 238], making it an enticing therapeutic option for osteoporosis. Correspondingly, observational data suggest that individuals who participate in regular exercise have a lower risk of hip fracture than their sedentary counterparts [239]. Remarkably, in the National Health and Nutrition Examination Survey (NHANES) I follow-up study, which evaluated 3600 women aged 40-77, those who reported moderate-to-vigorous physical activity had a 47% lower risk of hip fracture than those who did not participate in physical activity [240]. In the Study of Osteoporotic Fractures, which assessed 9700 postmenopausal white women, those who reported regular moderate-to-vigorous physical activity had a 42% lower incidence of hip fracture and 33% lower incidence of vertebral fracture compared to inactive women [241]. Unfortunately, there are no prospective RCTs of exercise interventions in which fracture is a primary outcome. Furthermore, the majority of data regarding the effects of exercise on skeletal outcomes comes from the general population of postmenopausal women, rather than people with known osteoporosis and/or prior fragility fracture [242]. In a 2011 Cochrane review which evaluated 43 randomized controlled trials, exercise interventions were not found to have a

significant effect on fracture risk in pooled analyses (OR: 0.61, 95% confidence interval [CI]: 0.23-1.64) [243]. In a 2013 meta-analysis of ten controlled exercise trials, exercise appeared to reduce overall fracture risk (relative risk [RR]: 0.49, 95% CI: 0.31-0.76) but there was a small number of fractures in the included trials, and evidence of publication bias was noted [244]. Despite a lack of high-quality prospective data to support an anti-fracture effect of exercise, regular physical activity is associated with multiple extraskeletal health benefits, and weight-bearing activity remains widely recommended for the treatment of osteoporosis and prevention of fracture [219, 220, 245]. An international task force has recently outlined priorities for further research in this area, and concluded that it is reasonable for most adults at risk of osteoporosis to engage in 150 minutes of moderate- to vigorous-intensity aerobic physical activity per week, as well as strength training activity two or three times a week and balance training two or three times a week [242].

Falls prevention

Falls are another established risk factor for fragility fracture [13, 14]. In a 2009 Cochrane review, a number of interventions were shown to reduce either rate of falls and/or risk of falling in community-dwelling older individuals [237]. These strategies included multiple-component exercise programs carried out in a group setting or in an individual's home, tai chi, falls assessment, and withdrawal of psychotropic medications. In individuals with pertinent risk factors, other interventions were also beneficial. For instance, eye surgery reduced rate of falls in individuals with cataracts, home safety interventions were beneficial in those with severe visual impairment and at the highest risk of falling, anti-slip shoe devices reduced the risk of falling in icy conditions, and pacemakers were associated with a reduced likelihood of falling in people with carotid sinus hypersensitivity [237]. Most of the studies described in the Cochrane meta-analysis were not designed to assess fracture incidence as a

primary outcome. The pooled results indicated that, of all of the interventions that reduced the risk and/or frequency of falling, only exercise interventions were associated with a reduced risk of fracture (RR: 0.36, 95% CI: 0.19-0.70) [237].

Additional lifestyle strategies

Smoking and excess alcohol consumption (≥3 units per day) are two well-established risk factors for fracture [202]. Low body weight is associated with an increased risk of fracture [72]. In addition, nutrition has been highlighted as a determinant of fracture risk in observational studies, with low protein intake [246] and low fruit and vegetable intake [247] being associated with increased risk [248]. Dietary calcium intake has also been associated with fracture risk, and this relationship is reviewed in further detail in the next subsection. Unfortunately, it has proven difficult to determine the effect of modifying these lifestyle factors on fracture risk. These factors are challenging to study in a randomized controlled capacity, and consequently there is a paucity of high quality data demonstrating that modification confers protection against fracture. However, given that smoking cessation, moderation of alcohol intake, consumption of a balanced diet, and maintenance of a healthy weight offer multiple health benefits beyond fracture prevention, these practices are widely recommended to individuals at risk of fracture [220, 245, 249].

In general, the lifestyle modification strategies addressed in this subsection are associated with minimal harm and can be safely recommended to all individuals at risk of fragility fracture. However, there is a paucity of controlled studies of these interventions with fracture as the primary outcome. Therefore, at present, lifestyle modification strategies cannot be recommended as a substitute for pharmacologic therapy in those at high risk of fracture.

2.7.2 Calcium and vitamin D

As outlined in sections 2.1 and 2.3, adequate availability of calcium is required for bone mineralization [32, 50], and 1,25-hydroxyvitamin D promotes intestinal calcium absorption. Deficiencies of either factor can also lead to secondary hyperparathyroidism [149]. Therefore, it is unsurprising that there has been much interest in the use of calcium and vitamin D for the prevention and treatment of osteoporosis.

Postmenopausal women have reduced intestinal calcium absorption and increased urinary calcium losses compared to their premenopausal counterparts [250, 251]. Studies carried out in middle-aged women and published in the 1970s indicated that a daily calcium intake on the order of 1200 to 1500mg might be required to maintain a neutral or positive calcium balance following menopause [252, 253]. These studies have influenced the recommendations provided by guidelines committees regarding optimal calcium intake [219, 220, 254], and have contributed to the broad adoption of calcium supplementation as a therapy for osteoporosis. For instance, the Institute of Medicine recommendations for dietary calcium and vitamin D intake, published in 2011, set the recommended dietary calcium intake at 1200mg/d for postmenopausal women and 1000mg/d for men over the age of 50 [254]. The majority of individuals do not achieve these targets through their diet [255-257], and so the use of calcium supplementation has been widespread—in the United States, it is estimated that almost half of the population takes a calcium supplement [255].

The Institute of Medicine 2011 report also proposed that serum 25-hydroxyvitamin D levels should be maintained at 50 nmol/L or greater, which avoids the development of secondary hyperparathyroidism in the vast majority of individuals, and recommended that this be achieved through a daily intake of 600 IU vitamin D for adults (800 IU for those over age 70)

[254]. Recommendations outlined by most osteoporosis guidelines committees are similar [219, 220, 245], although some advocate that 25-hydroxyvitamin D levels >75 nmol/L should be targeted [219, 258], and recommend supplementation of up to 2000 IU per day [259]. As with calcium, vitamin D supplementation is frequent in the general population [255].

It is well recognized that both calcium and vitamin D play integral roles in the maintenance of a healthy skeleton. However, recent appraisals of the literature on this topic indicate that, in individuals who are not at high risk for deficiencies of these nutrients, the evidence to support high dietary intake or supplementation of calcium and vitamin D for the treatment of osteoporosis is scarce [260, 261].

Effects of dietary calcium intake on BMD and fracture

In an observational study of almost 5000 community-dwelling adults, Bichoff-Ferrari *et al* determined that daily dietary calcium intake greater than 550mg was not associated with BMD, provided that 25-hydroxyvitamin D levels were adequate (>50 nmol/L) [262]. A recent meta-analysis carried out at the University of Auckland assessed 15 randomized controlled trials (n=1533) of dietary calcium intake and found increasing intake to be associated with statistically significant but clinically modest increases in bone density, on the order of 0.6 to 1.0% per year [263]. A meta-analysis published concurrently by the same group did not find increasing calcium intake from dietary sources to be associated with a reduction in fracture risk [260].

Effects of calcium and vitamin D supplementation on BMD and fracture

Recent observations regarding the efficacy of calcium and vitamin D supplementation have been similarly discouraging. In a 2014 meta-analysis of 23 randomized studies (n=4802),

Reid *et al* observed that vitamin D supplementation, when given without concurrent calcium, does not consistently increase BMD [261]. In a 2015 meta-analysis of 51 randomized trials (n=12,257), Bolland *et al* demonstrated that calcium supplementation, whether given alone or with vitamin D, is associated with a modest increase in BMD of 0.7 to 1.8% at one year, with no further increases in the studies in which follow-up was continued for two or more years [263]. A companion meta-analysis which evaluated 26 randomized trials assessing the impact of calcium supplementation with or without vitamin D on fracture incidence, demonstrated that supplementation had no effect on hip or forearm fracture, but did reduce the risk of vertebral fracture by 14% (RR: 0.86, 95% CI: 0.74-1.00) and all fractures by 11% (RR: 0.89, 95% CI: 0.81-096) [260]. However, there was significant heterogeneity between included studies, and the aggregate results were largely influenced by a positive study conducted by Chapuy and colleagues in a population of frail elderly individuals residing in residential care homes, with low baseline calcium intake and very low vitamin D levels [264, 265]. Sensitivity analyses demonstrated that the Chapuy study had a significant impact on the pooled results. Furthermore, when only the subgroup of studies deemed to be at low risk of bias was assessed, no effect of calcium supplement on fracture risk was observed [260].

Previously published meta-analyses reported similar findings, with Tang *et al* (2007) demonstrating a 12% reduction in the risk of all types of fractures (RR 0.88, 95% CI: 0.83-0.95) with calcium supplementation, given with or without vitamin D [266]. A network meta-analysis published by Murad and colleagues in 2012 found that combined calcium and vitamin D supplementation conferred a 19% reduction in the risk of hip fracture (OR 0.81, 95% CI: 0.68-0.96) in pooled analyses, but this benefit did not extend to fractures at other sites, or to supplementation with calcium or vitamin D alone [267]. In summary, the balance of the evidence suggests that, when given together, calcium and vitamin D supplementation

have modest effects on BMD. Calcium supplementation, given with or without vitamin D, might confer a small reduction in fracture risk, but this benefit is likely limited to populations at high risk of deficiency.

Safety of calcium and vitamin D supplementation

Recent studies have also raised concerns about the safety of supplementation with calcium and vitamin D. Vitamin D supplementation has been associated with hypercalciuria and hypercalcemia, although this is rarely observed at doses less than 4000 IU daily [268]. Intermittent administration of very high doses (for instance, 600,000 IU annually) has been associated with an increased risk of falls [269, 270]. Calcium supplementation increases the risk of nephrolithiasis [271] and is associated with hospitalizations for gastrointestinal symptoms [272]. A growing body of evidence suggests that individuals taking calcium supplements are at increased risk of myocardial infarction [273-275]. In the Auckland Calcium Study, 1400 postmenopausal women were randomized to receive calcium as carbonate or placebo and followed for 5 years. In this cohort, calcium supplementation was associated with an increased risk of myocardial infarction (RR: 2.12, 95% CI: 1.01 to 4.47) [274]. This finding was echoed in a subsequent meta-analysis, published in 2010 [273]. This meta-analysis, conducted by Bolland *et al*, excluded studies assessing co-administration of calcium and vitamin D, and therefore did not include results from a 2007 analysis of the Women's Health Initiative (WHI) cohort, which reported a neutral effect of calcium supplementation on cardiovascular risk [276]. Of relevance, more than half of the women in the WHI study were taking calcium supplements at baseline (average calcium intake was 1000mg at baseline) and continued to do so throughout the intervention period [271]. In 2011, Bolland and colleagues undertook additional analyses of the WHI study population [275] and found an interaction between cardiovascular outcomes and the use of calcium

supplements at the time of study enrolment. When only the subjects who were not taking non-protocol calcium supplements at baseline were considered, the intervention group who received calcium and vitamin D were found to have an increased risk of clinical myocardial infarction (hazard ratio: 1.22, 95% CI: 1.01-1.50). This finding was not evident in participants who were taking calcium supplements at baseline [275]. An updated meta-analysis by Bolland *et al* that included the WHI subjects not taking calcium supplements demonstrated a persistent increase in the risk of myocardial infarction (RR: 1.24, 95% CI: 1.07-1.45) [275]. Evidence from observational studies regarding the relationship between cardiovascular events and calcium supplements has been mixed, with some studies [277-279] but not others [280-282] reporting an association.

Although pooled analyses of RCTs suggest that calcium supplementation may precipitate myocardial infarction, the mechanism by which this might occur is not known. It has been postulated that the acute increase in serum calcium that accompanies ingestion of a supplement may play a role. Following ingestion of a 500 to 1000 mg calcium supplement, increases in serum calcium of approximately one standard deviation become evident within two hours and persist for at least 6 to 12 hours [283-285]. In observational studies, a one standard deviation increase in serum calcium (about 0.1 mmol/L) is associated with an increased risk of cardiovascular disease and death of 8 to 20% [101], indicating that changes of this magnitude are likely to be clinically relevant. As outlined in section 2.2, there are multiple potential mechanisms by which serum calcium may exert detrimental effects on the vasculature, including promoting the calcification of vascular smooth muscle cells, altering vascular integrity through activation of the calcium-sensing receptor [96], modulating the renin-angiotensin-aldosterone system [97], or increasing blood coagulability [99, 100]. In studies that have identified increased cardiovascular risk in calcium supplement users, this

risk increase becomes evident within one year of starting the supplement [273, 274], suggesting that effects of calcium on blood pressure or blood coagulation may more likely be responsible than effects on vascular calcification.

In observational studies, serum calcium levels have been shown to correlate positively with blood pressure [81, 286, 287]. In an analysis of genetic polymorphisms of the calcium-sensing receptor, which determines an individual's serum calcium 'set point', Jorde *et al* observed that individuals with polymorphisms that resulted in higher calcium levels had an increased risk of myocardial infarction. This risk was attenuated following adjustment for blood pressure, suggesting that blood pressure is a mediator of the relationship between serum calcium and cardiovascular dysfunction [288]. Correspondingly, in two studies, the acute increases in serum calcium concentration that followed an intravenous calcium infusion were accompanied by elevations in systolic blood pressure [94, 95]. Chapter 7 of this thesis describes a crossover RCT which was carried out to elucidate the mechanism by which calcium supplementation might increase cardiovascular risk. This study assessed the acute effects of calcium supplementation on blood pressure in 40 postmenopausal women.

2.7.3 Pharmacologic therapies

A number of pharmacologic agents have been shown to increase BMD and reduce fracture risk in postmenopausal women. The agents that are the most commonly used are listed in Table 2.2, which highlights the relative reductions in fracture risk observed in pivotal RCTs of each these medications [289-299], as well as their adverse effects [164, 300, 301]. It is important to note that the studies reflected in Table 2.2 were carried out in postmenopausal women, most between the ages of 50 and 80 with densitometric osteoporosis, for durations of 5 years or less. There is a paucity of RCTs with fracture as the primary outcome in

premenopausal or elderly (age >80) women [302], and in populations with osteopenic bone density. In men with low bone density, oral bisphosphonates, zoledronic acid and teriparatide have been shown to reduce fracture risk, while denosumab has been found to reduce vertebral fracture incidence in men receiving androgen-deprivation therapy for prostate cancer [303]. There is a paucity of head-to-head comparisons of pharmacologic agents for osteoporosis with fracture as a primary endpoint, meaning that there is no clear evidence for superiority of one medication over another. In a network meta-analysis, Murad *et al* found no statistically significant differences in efficacy between teriparatide, denosumab, and some of the more commonly used bisphosphonates [267].

These pharmacologic agents can be divided into two categories: medications that have a primarily antiresorptive mechanism, and agents whose principal effect is to increase bone formation. Hormone replacement therapy, selective oestrogen receptor modulators (raloxifene and bazedoxifene), the bisphosphonates (etidronate, alendronate, risedronate, ibandronate, zoledronic acid), denosumab can all be considered primarily antiresorptive. These medications inhibit the process of bone resorption (outlined in Section 2.1). The mechanism of strontium ranelate remains unclear, although this agent appears to exhibit both antiresorptive and anabolic effects [304]. Teriparatide, which is a recombinant PTH analogue, is the only anabolic agent that is currently approved for the treatment of osteoporosis. Subcutaneous administration of teriparatide on a daily basis results in preferential stimulation of bone formation over bone resorption, facilitating gains in BMD [49]. An overview of these medications is provided in the coming paragraphs, with a focus on the bisphosphonates and one of the most common side effects of zoledronic acid: the acute phase response (APR).

Table 2.3 Summary of the efficacy and safety of commonly used pharmacologic osteoporosis medications

Medication name	Fracture risk reduction[1]			Side effects
	Vertebral	Nonvertebral	Hip	
Antiresorptive agents				
Hormone replacement therapy [289, 291]	35-36%	NR	33-35%	Deep vein thrombosis and pulmonary embolism Possible increased risk of breast cancer
Raloxifene [290]	30-50%	NS	NS	Deep vein thrombosis and pulmonary embolism Worsening of hot flashes
Alendronate, Risedronate [292-294, 305]	30-47%	11-38%	18-51%	Gastrointestinal intolerance ONJ, AFF
Zoledronic acid [295]	70%	25%	44%	Acute phase response Transient hypocalcaemia Renal injury ONJ, AFF
Denosumab [296]	68%	20%	40%	Transient hypocalcaemia Rare risk of cellulitis ONJ, AFF
Mechanism unknown				
Strontium ranelate [297, 298]	41%	16%	NS	Rare risk of dermatologic reactions Probable increase in risk of myocardial infarction
Anabolic agent				
Teriparatide [299]	65%	53%	NS	Transient hypercalcemia, hyperuricemia Risk of osteosarcoma in rats, not reported in humans

[1]Relative risk reduction observed in pivotal randomized controlled trials carried out in postmenopausal women; medications further reviewed in [164, 300, 301]. NR = not reported, NS = non-significant, ONJ = osteonecrosis of the jaw, AFF = atypical femoral fracture

Hormone replacement therapy and selective oestrogen receptor modulators

The oestrogen deficiency of menopause can be ameliorated either with direct replacement of oestrogen (hormone replacement therapy), or via the administration of a selective oestrogen receptor modulator. Treatment with oestrogen was associated with increases in BMD and a reduction in fracture risk in the WHI RCTs, which assessed the effects of oestrogen and a progestin in women with an intact uterus as well as the effects of oestrogen alone in women with a hysterectomy [289, 291]. However, in the WHI population, combined therapy with oestrogen and a progestin was unexpectedly found to be associated with an increased risk of coronary artery disease (the primary endpoint), and the trial was stopped early due to an increased incidence of invasive breast cancer in the treatment group which had been prespecified as a primary adverse outcome [306]. The risk of venous thromboembolic events was also increased in the treatment group [306]. Therefore, the use of oestrogen for the treatment of osteoporosis is generally limited to those who also require oestrogen for the treatment of menopausal symptoms. As the risk of side effects appears to be lowest in the ten years following menopause, treatment is generally limited to this timeframe [220].

Raloxifene is a selective oestrogen receptor modulator. Like other medications in this class (tamoxifen, bazedoxifene), raloxifene has differential tissue-specific effects on oestrogen receptors, with an agonistic effect on the receptors in bone tissue and an antagonistic effect on breast tissue. This translates into a reduction in bone loss, as well as some protection against the development of breast cancer [307]. The use of raloxifene has been shown to reduce the risk of vertebral fracture, although the pivotal registration trial did not demonstrate significant reductions in nonvertebral or hip fractures [290]. The use of raloxifene has been associated with an increased risk of venous thromboembolism and worsening of hot flashes [290].

Bisphosphonates

The bisphosphonates are the most frequently used medications for the treatment of osteoporosis [308]. Bisphosphonates are analogues of inorganic pyrophosphate. Pyrophosphate consists of two phosphate molecules that are linked by an oxygen atom, while bisphosphonates consist of two phosphate molecules linked by a carbon atom [309]. The carbon atom has two additional side chain moieties, one of which is a hydroxyl (-OH) group, and the other which varies between bisphosphonate compounds, accounting for differences in mechanism of action and potency. Bisphosphonates are chemically stable and bind tightly to hydroxyapatite, meaning that they have a high affinity for bone mineral [309]. Following oral or intravenous administration of a bisphosphonate, the compound is preferentially incorporated into the bone mineral at sites of high bone turnover. What medication is not incorporated into the bone is renally excreted [309]. When the osteoclastic bone resorption process begins in a bisphosphonate-treated individual, locally bound bisphosphonate compounds are transported from the bone surface into the osteoclasts via endocytosis. First generation bisphosphonates (also called non nitrogen-containing bisphosphonates, as they do not contain a nitrogen group) include clodronate, etidronate and tiludronate. These medications become incorporated into nonhydrolyzable ATP analogues and inhibit ATP-dependent cellular processes within osteoclast cells, which results in osteoclast dysfunction and apoptosis [309]. The second- and third-generation bisphosphonates (alendronate, risedronate, ibandronate, pamidronate and zoledronic acid) have nitrogen-containing side chains. These bisphosphonates bind more tightly to hydroxyapatite than the first generation agents and also work through a different cellular mechanism. Nitrogen-containing bisphosphonates inhibit the enzyme farnesyl pyrophosphate, which acts within the mevalonate pathway. Inhibition of this enzyme results in the failure to prenylate several proteins that are required for osteoclast function, ultimately leading to apoptosis [310].

Bisphosphonates can be administered either orally or intravenously. Currently, the most commonly used oral bisphosphonates are alendronate and risedronate. These medications are hydrophilic and poorly absorbed, with <1% bioavailability [309]. Conversely, intravenous bisphosphonates, such as zoledronic acid, have 100% bioavailability [311]. The biologic half-lives of nitrogen-containing bisphosphonates are long, with oral nitrogen-containing bisphosphonates suppressing bone turnover for 6 to 24 months following cessation of therapy [312, 313]. Following intravenous administration, zoledronic acid has been shown to suppress markers of bone turnover and maintain BMD for up to five years, relative to placebo [314, 315].

Alendronate, risedronate and zoledronic acid have each been shown to reduce the risk of vertebral, nonvertebral and hip fracture in postmenopausal women with established osteoporosis [292-295, 305, 316]. In the Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly Pivotal Fracture Trial (HORIZON-PFT), over 7000 postmenopausal women (mean age 73) with either densitometric osteoporosis or both low bone density (T-score of -1.5 or less) and prevalent vertebral fracture were randomized to receive three annual infusions of zoledronic acid 5mg or placebo. Treatment with zoledronic acid was associated with a 70% reduction in vertebral fracture risk, 41% reduction in hip fracture risk, and 25% reduction in nonvertebral fracture risk compared to placebo [295]. Among more than 2000 patients (mean age 75) who received treatment with zoledronic acid or placebo beginning within 90 days of a hip fracture in the HORIZON Recurrent Fracture Trial (HORIZON-RFT), clinical fracture rates were reduced by 35% in the zoledronic acid group over almost 2 years of follow-up [317]. Zoledronic acid was also associated with a 28% reduction in mortality in HORIZON-RFT population [317]. A large proportion of HORIZON-RFT study participants received only a single dose of the study drug, as the study

was stopped after median follow-up of 1.9 years when prespecified efficacy boundaries were surpassed. In 1367 subjects from the HORIZON-RFT and HORIZON-PFT studies who received only a single infusion of zoledronic acid, there was a 32% reduction in clinical fracture incidence over a mean follow-up period of approximately 1.5 years compared to those who received placebo [318]. When the group who received a single zoledronic acid infusion was compared with those who had three or more annual infusions, the magnitude of clinical fracture risk reduction versus placebo was similar at three years (32% with a single infusion versus 34% for three or more infusions) [318]. These results suggest that even a single infusion of zoledronic acid confers a reduction in fracture risk, and demonstrate that the anti-fracture effects of this agent extend beyond a single year. These attributes make zoledronic acid an enticing first line treatment option.

Bisphosphonate side effects

The bisphosphonate medications reviewed above (alendronate, risedronate and zoledronic acid) have been associated with adverse events, some of which have limited their acceptability to patients and their potential for long-term use. With respect to relatively common short-term side effects, therapy with oral bisphosphonates has been associated with gastrointestinal intolerance in up to 20% of individuals [319], likely contributing to the high rates of discontinuation that have been observed within the first year of treatment [320]. Treatment with nitrogen-containing bisphosphonates, zoledronic acid in particular, can provoke an inflammatory reaction in some patients, known as the acute phase response (APR). Inhibition of the enzyme farnesyl pyrophosphate synthase by the aminobisphosphonates leads to the accumulation of upstream metabolites which activate gamma-delta T-cells and result in release of proinflammatory cytokines such as interferon-gamma, TNF-α and IL-6 [321-324]. While the clinical manifestations of the APR can be

variable, symptoms such as fever, arthralgia, nausea, headache and fatigue are most common [325]. APRs typically occur within 72 hours of infusion, often beginning in the first 24 hours [322]. They are almost always self-limited, resolving within several days [322, 325].

Estimates of APR incidence range from 42-76%, with most studies defining APR as an increase in temperature and/or the development of typical APR symptoms [322, 325-329] [330]. Development of APR is much more common following the administration of intravenous bisphosphonates than oral bisphosphonates, and occurs more frequently in bisphosphonate-naïve individuals than in persons with previous exposure to either intravenous or oral bisphosphonates [325]. For example, an individual who sustains an APR after their first infusion of zoledronic acid is unlikely to have a recurrence of these symptoms following subsequent infusions. However, it is not uncommon for patients to refuse further bisphosphonate therapy after sustaining a symptomatic APR. Although APRs are common, preventative strategies are not well established. Chapter 8 of this thesis reports the results of a RCT assessing whether administration of the glucocorticoid dexamethasone at the time of first zoledronic acid infusion can reduce the incidence or severity of the APR.

Short-term exposure to bisphosphonates has occasionally been associated with other adverse effects. Some patients (approximately 1%) develop uveitis following treatment with zoledronic acid. This requires urgent ophthalmologic assessment, but typically resolves rapidly following treatment with ocular corticosteroids. Uveitis is felt to represent a rare manifestation of the APR [325], although ocular inflammation may occur any time from days to years following treatment [331]. Bisphosphonates are renally cleared, and the development of significant renal impairment has been reported, albeit rarely, following treatment with zoledronic acid [332]. The use of alendronate and zoledronic is contraindicated if creatinine

clearance is less than 35 mL/min, and use of risedronate is not recommended when creatinine clearance is less than 30 mL/min. However, treatment with oral bisphosphonates does not appear to worsen renal function, even when administered to patients with creatinine clearance below 30-35 mL/min [333], and the delivery of a lower dose of zoledronic acid (such as 2.5mg) over an extended timeframe (45 minutes versus the usual 15 minutes) may reduce the likelihood of nephrotoxicity [334]. Oral and intravenous bisphosphonates transiently lower circulating calcium concentrations, and there have been case reports of symptomatic hypocalcaemia following treatment with these agents, particularly in individuals who are vitamin D deficient [335].

In the HORIZON-PFT, an increased incidence of atrial fibrillation was reported in the group treated with zoledronic acid [295], although following a review of post-marketing surveillance data, the US Food and Drug Administration concluded in 2008 that a causal link could not be established [336], and subsequent investigations into the relationship between bisphosphonate use and arrhythmia have produced generally reassuring findings [337]. In 2009, the Food & Drug Administration released a report documenting several cases of oesophageal cancer in patients exposed to oral bisphosphonates [338]. However, subsequent research has not provided further evidence for an association or causal link between bisphosphonate use and oesophageal cancer [339]. Although many of the short-term side effects described here do not tend to have a large impact on patient care, the potential for gastrointestinal intolerance with the oral bisphosphonates and APR with zoledronic acid do present barriers to treatment uptake and adherence. The use of intravenous zoledronic acid avoids the issue of gastrointestinal intolerance, although effective strategies to reduce the incidence of APR following treatment with this medication are required.

Although the research contained in this dissertation does not address this topic directly, an important emerging issue in osteoporosis relates to two potential long-term side effects of bisphosphonates: osteonecrosis of the jaw (ONJ) and atypical femoral fractures (AFFs). Concern about these side effects has resulted in restriction of the use of antiresorptive agents over the long-term [308], and has contributed to low rates of treatment uptake and adherence [340, 341]. ONJ is defined as an area of exposed bone in the maxillofacial region which does not heal within 8 weeks following identification by a health care provider [342]. The incidence of ONJ with bisphosphonate use in the osteoporosis population is estimated at less than 90 cases per 100,000 patient years of therapy [342]. The risk is felt likely to increase with duration of exposure, but this has not been definitively demonstrated [342]. The association between bisphosphonates and ONJ first came to light in the oncology population, where patients are often exposed to cumulative bisphosphonate doses more than 10 times that of the osteoporosis population. Accordingly, in the oncology population, the incidence of ONJ in bisphosphonate-treated individuals is closer to 1-2%, compared to an estimated 0.001-0.15% in osteoporosis, suggesting a dose-response relationship [342]. However, existing evidence has been unable to confirm a causal link between bisphosphonate exposure at the lower doses used to treat osteoporosis and ONJ, with several studies suggesting that bisphosphonate use is not associated with an increased risk of ONJ in individuals with osteoporosis [343-345]. The mechanism by which bisphosphonates might cause the development of ONJ is not clear [342]. To date, no studies have confirmed that the risk of ONJ decreases following bisphosphonate discontinuation. ONJ can often be treated conservatively with oral antibiotic rinses or systemic antibiotics, but sometimes requires surgical debridement [342].

AFFs are subtrochanteric fractures of the femur, usually transverse and originating at the lateral cortex (complete diagnostic criteria are reviewed in [346]). Data from a large public health database in the United States suggest that the risk is on the order of 2 per 100,000 patient years with less than two years of bisphosphonate therapy, increasing to around 100 cases per 100,000 patient years with eight to ten years of treatment [347]. A nationwide study from Sweden suggests that, in women, exposure to bisphosphonates for more than four years is associated with a risk of 11 AFFs per 10,000 patient years [348]. The risk of AFF appears to decline following antiresorptive discontinuation, potentially by as much as 70% in the first year, although data are limited [348].

As the risk of AFF (and potentially ONJ) appears to increase with duration of bisphosphonate exposure and to decline rapidly after treatment cessation, and because bisphosphonates suppress bone turnover for months to years after they are stopped, the concept of a 'drug holiday' has arisen. Although there is little evidence to guide this practice, many osteoporosis experts will reassess the need for ongoing bisphosphonate therapy after three to five years of treatment, and at that point consider whether a treatment-free interval of two or more years (or one year, in the case of risedronate) might be appropriate [334].

Denosumab

Denosumab is a fully-humanized monoclonal antibody against RANKL. As described in section 2.1, RANKL stimulates osteoclast proliferation and function. Denosumab prevents association of RANKL with its cognate receptor, RANK, on the membrane of pre-osteoclast cells. This results in suppression of osteoclastogenesis [349]. Denosumab has been shown to reduce the risk of vertebral, nonvertebral and hip fractures in postmenopausal women [296], and vertebral fractures in men receiving androgen deprivation therapy for prostate cancer

[350]. Denosumab therapy has been associated with transient hypocalcaemia in some patients [351], and an increased risk of cellulitis was observed in the pivotal registration trial [296], although this has not been reported in longer term studies. Episodes of both ONJ and AFF have been reported with the use of denosumab [352, 353], although as with bisphosphonates, current data suggest that these complications are very rare. Unlike the bisphosphonates, denosumab does not have a prolonged biological half-life in bone. Within six months of cessation of denosumab, bone turnover markers are seen to rebound, transiently exceeding baseline levels. These changes in bone turnover markers correspond with declines in BMD [354]. In 2016, Lamy *et al* described nine women who sustained spontaneous vertebral fractures within a year of denosumab cessation [355]. Their group recently published an updated case series which describes the occurrence of rebound-associated vertebral fractures in a total of 24 women who discontinued denosumab [356]. It has been suggested that perhaps this agent should be continued indefinitely once started [357].

Strontium ranelate

Treatment with strontium ranelate has been shown to modestly reduce the incidence of both vertebral and nonvertebral fractures [297, 298]. Use of this agent is occasionally associated with skin irritation, and in rare cases, Drug Rash with Eosinophilia and Systemic Symptoms (DRESS syndrome) [358]. Of further concern, the use of strontium ranelate has been associated with an increased risk of venous thromboembolism and myocardial infarction in RCTs [359]. The modest efficacy of this agent, combined with these potential adverse effects makes it less attractive as a first line therapy for fracture prevention. Strontium ranelate is currently used in Europe, but not New Zealand or North America.

Teriparatide

At present, the recombinant PTH analogue, teriparatide, is the only anabolic agent available for the treatment of osteoporosis. Teriparatide consists of the first 34 N-terminal amino acids of the endogenous 84 amino acid PTH molecule [299]. Treatment with teriparatide results in increased bone formation and resorption, although formation outweighs resorption, particularly in the first several months of the treatment course. This results in a net accrual of bone mass [49]. This agent has been shown to reduce the risk of vertebral and nonvertebral fractures [299]. A significant reduction in hip fracture incidence was not observed in the teriparatide registration trial, which assessed 1637 postmenopausal women, although the total number of hip fractures was very low (n=9) [299]. Treatment with teriparatide is limited to 18 to 24 months, following the observation of a dose- and duration-dependent incidence of osteosarcoma in rats treated with this agent [360]. To date, concerns that the use of this medication might confer an increased risk of osteosarcoma have not been borne out in human RCTs or postmarketing surveillance [361]. In humans, the use of teriparatide has been associated with transient hypercalcemia [299], as well as nausea, abdominal pain, and headache [362]. BMD begins to decline within months of treatment cessation [363], and antiresorptive therapy is generally initiated at the time of teriparatide discontinuation, with the view of prolonging its anti-fracture effects.

2.7.4 Summary of treatment of osteoporosis

A number of treatments are available to prevent bone loss and reduce the risk of fracture. Lifestyle strategies such as smoking cessation, moderation of alcohol intake, maintenance of a balanced diet and a healthy weight and regular weight-bearing physical activity have multiple health benefits and may reduce the risk of fracture. In individuals at risk of falls, interventions to prevent falling may also reduce fracture risk. In individuals who are not at

high risk of deficiency, there is a lack of compelling evidence to support the consumption of high amounts of dietary calcium, or the widespread use of calcium and vitamin D supplementation to reduce the risk of fragility fracture. Recent evidence has linked the use of calcium supplements with an increased risk of myocardial infarction, a relationship that may be mediated by the effect of calcium on blood pressure. A study exploring this hypothesis is presented in Chapter 7. A number of pharmacologic therapies are available to reduce the risk of fragility fracture. Zoledronic acid is an enticing first-line treatment option, although the first infusion of this agent often results in the development of an APR. Chapter 8 describes the effect of the glucocorticoid, dexamethasone, on the incidence and severity of APR following treatment with zoledronic acid.

CHAPTER 3:
PARATHYROID HORMONE REFLECTS ADIPOSITY AND CARDIOMETABOLIC INDICES BUT NOT BONE DENSITY IN NORMAL MEN

3.1 INTRODUCTION

Hyperparathyroidism, resulting from either autonomous parathyroid hyperfunction or a secondary stimulus of PTH production, is a common endocrine problem. As described in Chapter 2, potential consequences of PTH excess include accelerated bone loss and fragility fracture, nephrolithiasis, renal impairment, increased cardiovascular risk, and in the setting of primary hyperparathyroidism, symptomatic hypercalcemia [137]. Vitamin D deficiency, renal dysfunction, hypercalciuria, malabsorption and the use of medications (e.g. lithium) are recognized causes of secondary hyperparathyroidism [134]. While not typically considered causes of secondary hyperparathyroidism, characteristics such as age, fat mass, and blood pressure have previously been shown to be independently associated with circulating PTH concentrations [89, 112, 150]. These variables may play a role in determining an individual's PTH level.

The determinants of PTH become particularly important when considering the entity of normocalcemic hyperparathyroidism [114, 154, 155]. This condition is characterized by persistent PTH elevation in the absence of hypercalcemia or recognized causes of secondary hyperparathyroidism. As I reviewed in Chapter 2, it is unusual to define a disease in terms of levels of a single analyte. Given that reference ranges typically encompass the central 95% of a 'healthy' reference population [156], doing so implies that up to 2.5% of the population might have this condition [157]. Furthermore, there is disagreement about where the upper end of the PTH reference range should lie, particularly in individuals without conditions

known to cause secondary hyperparathyroidism that are common in the general population, such as vitamin D deficiency or renal dysfunction [157, 364].

It has been suggested that normocalcemic hyperparathyroidism represents an early, subclinical form of primary hyperparathyroidism, and that affected individuals are at risk of developing sequelae [114]. This notion is supported by some case series, in which up to 50% of patients have osteoporosis, approximately 10% have nephrolithiasis, and up to 40% demonstrate disease progression [114, 154, 155, 158]. Some reports also suggest that people with normocalcemic hyperparathyroidism have an elevated risk of hypertension and cardiovascular disease, similar to what has been observed in primary hyperparathyroidism [159, 160]. However, many of these individuals came to attention following referral for investigation of problems such as osteoporosis and nephrolithiasis, raising the possibility that referral bias may account for the high prevalence of pathology reported [114, 154, 155]. In some other populations, there is no evidence of an association between normocalcemic hyperparathyroidism and adverse skeletal or renal outcomes, or progression to hypercalcaemic hyperparathyroidism [113, 161, 162]. Thus, as outlined in Chapter 2, it is presently unclear whether normocalcemic hyperparathyroidism is a discrete pathological entity with real associated morbidity, and there is currently no consensus regarding its management [137].

It is also uncertain whether the deleterious associations of PTH that have been documented in some cohorts with normocalcemic hyperparathyroidism manifest because PTH exceeds a certain ‘cut point’, or whether the disease associations are linear. In the present investigation, I have identified a cohort of healthy men, free of referral bias, and assessed relationships

between serum PTH and specific pathologies (skeletal and cardiometabolic), in order to determine whether these pathologies are associated with high-normal PTH levels.

3.2 METHODS

This analysis uses data from 151 community-dwelling men who participated in a clinical trial at the University of Auckland. In the original study [365], men aged 40 years or older were recruited via newspaper advertisements. Exclusion criteria included major active disease (including coronary heart disease, hypertension, diabetes mellitus, untreated thyroid disease, liver disease, malignancy, or known metabolic bone disease), current lipid-lowering therapy, serum creatinine >200 μmol/L, severe vitamin D deficiency (25-hydroxyvitamin D <25 nmol/L), and BMD Z-score <-2.0 at the spine or total hip. A total of 909 men answered the newspaper advertisement and 570 returned a study questionnaire. The most common reasons for exclusion of interested potential participants were medications (n=62), medical conditions (n=62) and abnormal blood tests (n=20). Eligible participants were randomized to receive calcium supplements (600 or 1200mg per day) or placebo for a two-year period. PTH was measured at baseline in 50 subjects per group, providing the cohort for the present study. The study was approved by the local ethics committee, and informed consent was obtained.

3.2.1 Data Acquisition

Height was measured with a Harpenden stadiometer, and weight was determined using electronic scales. Blood pressure was measured using a Dinamap automatic monitor. The protocol involved a five minute rest period followed by three blood pressure measurements, taken three minutes apart. Results were averaged. Dietary calcium intake was assessed with a validated food frequency questionnaire [366].

BMD and body composition were measured at baseline and again every six months for two years, using a Prodigy dual-energy x-ray absorptiometer (DEXA, GE-Lunar, WI, USA). BMD was assessed at the spine, hip and the total body. To obtain BMD estimates at cortical sites, BMD data at the arms and legs were extracted from the total body scans.

Fasting blood samples were collected at baseline. 25-hydroxyvitamin D was measured using either a radioimmunoassay (DiaSorin, Stillwater, MN, USA) or a chemiluminescent assay (Nichols, San Juan Capistrano, CA, USA), both assays meeting the performance targets for the Vitamin D External Quality Assessment Scheme. Inter-assay coefficients of variation have been shown to be 8.8% for the Diasorin assay and 7.5% for the Nichols assay at our centre [400]. Results using the Nichols assay were converted to Diasorin results using the following equation: Diasorin = Nichols x 0.75+5.6. The derivation of this equation has been previously described [400]. Serum procollagen type 1 N-terminal propeptide (P1NP) and PTH were measured using Roche autoanalyzers (Roche Diagnostics, IN, USA). Total cholesterol, HDL cholesterol and triglycerides were measured using a Roche-Hitachi 747 autoanalyzer (Roche Diagnostics, Mannheim, Germany). LDL cholesterol was calculated using the Friedewald formula.

Eighty-six men from this cohort (42 men from the placebo group and 44 who received calcium 1200mg per day throughout the two year study period) underwent coronary computed tomography using a 64-slice scanner (LightSpeed VCT, General Electric, Milwaukee, WI, USA), an average of 3.5 years after study entry. Scans were analysed using the Agatston system with manufacturer-supplied software, and coronary artery calcium (CAC) scores were determined [76].

3.2.2 Statistical Analysis

Associations between PTH and baseline parameters (in the entire cohort), and between PTH and change in BMD (in the placebo group only) were assessed using Pearson's correlation since these variables satisfied the criteria for normality. Interactions with age and estimated glomerular filtration rate (eGFR) were evaluated. Parameters that were correlated with PTH in bivariate analysis (p-value <0.15) were included, along with established predictors of PTH, in a stepwise multivariate regression analysis to obtain a parsimonious model that reflects the strongest determinants of PTH. This approach relies more on external clinical/scientific judgement for model selection than the typical automated stepwise procedure which has a tendency to overfit models which subsequently lack generalisability, may include spurious predictors and exclude known important predictors. Variance inflation factor and tolerance (1/variance inflation factor) were calculated for each model to assess multicollinearity. Variance inflation factor >10 was considered indicative of multicollinearity.

Participants were stratified into tertiles of PTH concentration. Differences between tertiles were assessed using the chi-squared test for categorical variables and analysis of variance (ANOVA) for continuous variables. Significant main or interaction effects were further explored using Tukey's post hoc test, to preserve the overall pairwise error rate. Tests for linear or quadratic trend were conducted using orthogonal contrasts. Continuous variables that differed significantly between PTH tertiles were also assessed using analysis of covariance (ANCOVA), with age, 25-hydroxyvitamin D and eGFR included as covariates.

All data analysis was done with SAS v9.4 (SAS Institute, NC, USA), and figures were created using Prism v6.0 (GraphPad Software Inc, CA, USA). The threshold for statistical significance was $p < 0.05$, with no adjustment for multiple testing in these hypothesis-generating analyses, beyond that of *post hoc* analyses in the context of a significant main effect.

3.3 RESULTS

3.3.1 Baseline characteristics and PTH correlations

Table 3.1 sets out the baseline characteristics of the study population and their correlations with PTH. PTH was positively related to BMI, fat mass, diastolic blood pressure, triglycerides, total and LDL cholesterol, and was inversely associated with dietary calcium intake, 25-hydroxyvitamin D and urinary calcium excretion. The significant correlations between PTH and baseline cardiometabolic indices are illustrated in Figure 3.1. Relationships between cardiometabolic indices and PTH were then assessed after adjustment for age, 25-hydroxyvitamin D and eGFR. This was done using linear regression models, with PTH as the dependent variable, and age, 25-hydroxyvitamin D, eGFR and the cardiometabolic parameter of interest as independent variables. In these adjusted models, PTH remained significantly associated with BMI ($p=0.005$), fat mass ($p=0.004$), diastolic blood pressure **(**$p=0.006$), triglycerides ($p=0.03$), LDL cholesterol ($p=0.004$), total cholesterol ($p=0.004$), and dietary calcium intake **(**$p=0.02$). Because adiposity is known to influence blood pressure and lipid levels, relationships between PTH and these variables were further adjusted for fat mass. Following this adjustment, PTH remained significantly associated with LDL ($p=0.01$) and total cholesterol ($p=0.004$), the relationship with diastolic blood pressure was attenuated ($p=0.04$), and the association with triglycerides was no longer significant ($p=0.23$).

Table 3.1 Baseline characteristics of men (n=151) and Pearson correlation with parathyroid hormone (PTH) levels

Characteristic	Mean (SD) / n (%)	Range	Correlation with PTH (r)	P-Value for Correlation
Age (y)	58.0 (10.2)	41.6-88.0	0.13	0.12
Height (cm)	176.6 (6.6)	159.8-191.6	-0.15	0.07
Weight (kg)	82.3 (10.7)	62.6-123.3	0.12	0.14
BMI (kg/m^2)	26.4 (2.9)	19.8-38.2	**0.24**	**0.004**
Total body fat mass (kg)	19.3 (6.9)	4.9-42.4	**0.23**	**0.005**
Total body lean mass (kg)	59.0 (6.6)	45.5-76.1	-0.05	0.57
Systolic blood pressure (mmHg)	132 (14)	98-169	0.16	0.05
Diastolic blood pressure (mmHg)	79 (8)	58-106	**0.22**	**0.006**
Dietary calcium intake (mg/d)	854 (413)	179-2338	**-0.19**	**0.02**
Physical activity (MJ/d)	32.2 (5.5)	25.9-56.6	-0.14	0.11
History of smoking (n, %)	69 (46%)			
Current smoker (n, %)	6 (4%)			
Previous fractures ≥40y (n, %)	12 (8%)			
Anti-hypertensive medication (n, %)	14 (9%)			
PTH (pmol/L)	4.1 (1.3)	1.8-8.5		
Albumin-adjusted calcium (mmol/L)	2.33 (0.09)	2.10-2.59	0.11	0.18
25-hydroxyvitamin D (nmol/L)	91.3 (33.6)	33.0-256.0	**-0.23**	**0.004**
Phosphate (mmol/L)	1.01 (0.13)	0.70-1.40	-0.08	0.31
eGFR (mL/min/1.73m^2)	73.3 (11.3)	51.7-111.9	-0.09	0.25
P1NP (ug/L)	39.1 (15.2)	14.0-140.0	-0.10	0.23
Urinary calcium excretion (mmol/L)	2.05 (1.80)	0.10-9.80	**-0.18**	**0.03**
Fasting blood glucose (mmol/L)	5.0 (0.5)	4.1-6.9	0.13	0.11
Triglycerides (mmol/L)	1.31 (0.66)	0.50-3.60	**0.19**	**0.02**
Total cholesterol (mmol/L)	5.61 (0.82)	3.40-7.50	**0.25**	**0.002**
HDL cholesterol (mmol/L)	1.46 (0.35)	0.80-2.70	-0.05	0.54
LDL cholesterol (mmol/L)	3.55 (0.72)	1.60-5.30	**0.23**	**0.005**
Total body BMD (g/cm^2)	1.26 (0.08)	1.08-1.51	0.05	0.58
Lumbar spine BMD (g/cm^2)	1.26 (0.15)	0.92-1.69	0.10	0.24
Total hip BMD (g/cm^2)	1.08 (0.13)	0.83-1.36	0.07	0.37
Femoral neck BMD (g/cm^2)	1.01 (0.13)	0.72-1.35	0.00	0.97
Bilateral arm BMD (g/cm^2)	0.99 (0.07)	0.83-1.22	0.06	0.44
Bilateral leg BMD (g/cm^2)	1.45 (0.11)	1.14-1.80	-0.05	0.54

Statistically significant correlations are bolded.

BMI = body mass index, PTH = parathyroid hormone, eGFR = estimated glomerular filtration rate, P1NP = procollagen type 1 N-terminal propeptide, HDL = high density lipoprotein, LDL = low density lipoprotein, BMD= bone mineral density

Laboratory reference ranges for biochemical parameters: PTH 1.7-7.3 pmol/L, total calcium 2.10-2.55 mmol/L, 25-hydroxyvitamin D 50-150 nmol/L, phosphate 0.7-1.5 mmol/L, P1NP 20-85 µg/L, urinary calcium excretion 2.5-7.5 mmol/day, fasting blood glucose 3.5-5.4 mmol/L, triglycerides <2.0 mmol/L, total cholesterol <5.0 mmol/L, HDL cholesterol >1.0 mmol/L, LDL cholesterol <3.4 mmol/L.

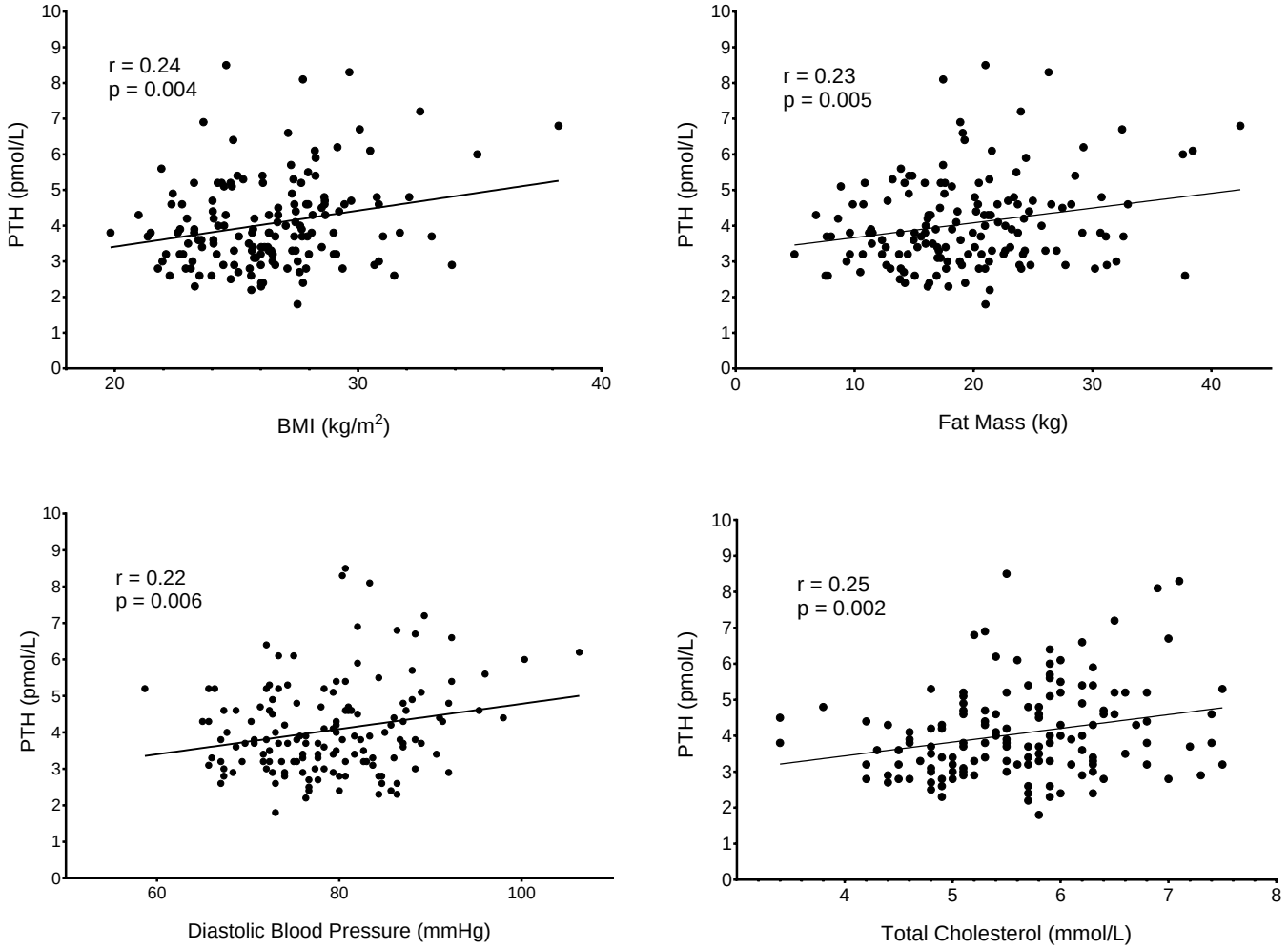


Figure 3.1 Correlations between serum parathyroid hormone (PTH) and cardiometabolic risk factors. r = Pearson correlation coefficient. BMI = body mass index.

3.3.2 Relationship between PTH and coronary artery calcium

The 86 patients who had CAC scores assessed were divided into clinically relevant categories [367] based on whether their CAC score was 0 (n=22), 1-100 (n=38), or >100 (n=26). Mean PTH, by CAC score category, is shown in Figure 3.2. There were significant differences in mean PTH between CAC score groups (p=0.002, ANCOVA adjusted for treatment group). Men with CAC score >100 had significantly higher baseline PTH levels than men with a CAC score of 0 (4.4 pmol/L vs 3.2 pmol/L; p=0.001, post hoc Tukey). PTH and CAC score category were linearly related (p=0.0005, linear contrasts). These results remained significant after adjustment for fat mass, age, 25-hydroxyvitamin D and eGFR (p=0.02 for difference between CAC score categories and p=0.008 for linear contrasts), as well as following adjustments for lipid concentrations (triglycerides, total cholesterol, and LDL).

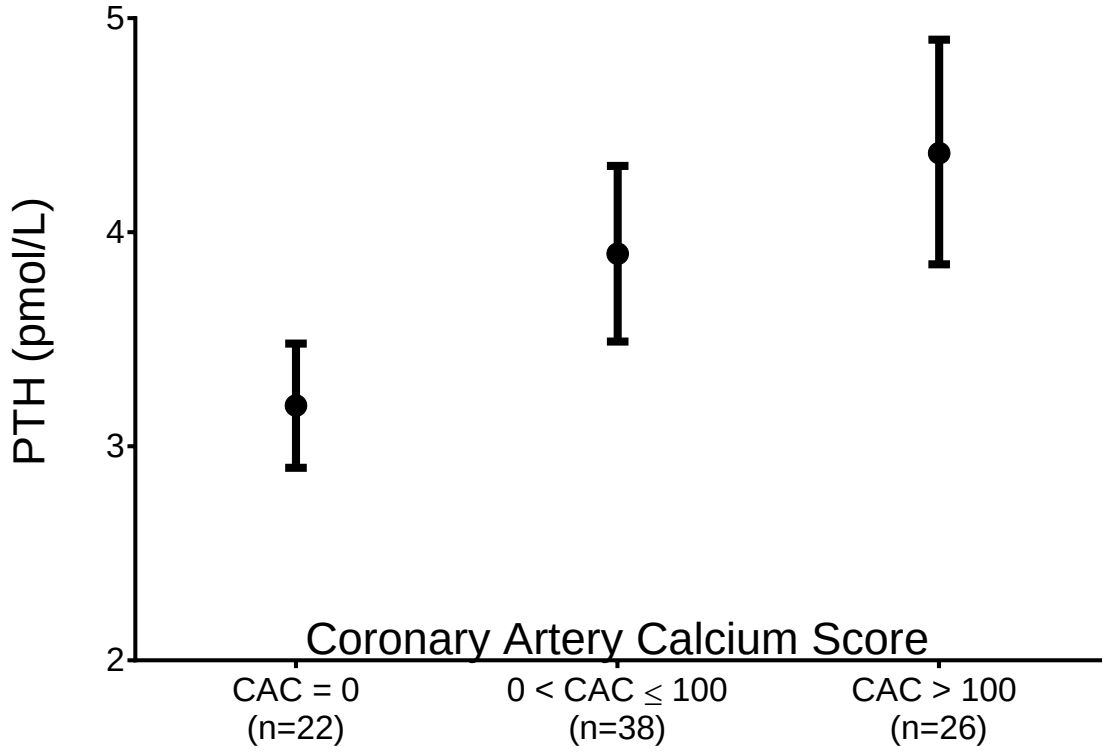


Figure 3.2 Relationship between serum parathyroid hormone (PTH) level and coronary artery calcium (CAC) score. CAC score categories are shown on the x-axis, where a score of 0 indicates low cardiovascular risk. PTH values are means and error bars represent 95% confidence intervals. p=0.0005 for linear trend (ANOVA, linear contrasts).

3.3.3 Comparison of cohort characteristics among PTH tertiles

Cardiometabolic indices and bone mineral density are compared across PTH tertiles in Table 3.2. BMI, fat mass, blood pressure, fasting blood glucose and total cholesterol levels were significantly increased in the highest PTH tertile. After adjusting for age, 25-hydroxyvitamin D, and eGFR, systolic blood pressure and total cholesterol no longer differed significantly across tertiles. The prevalence of cardiometabolic risk factors was assessed using established cutoffs [368, 369]. A higher proportion of men in the top tertile had LDL cholesterol levels ≥3.5 mmol/L, diastolic blood pressure ≥85 mmol/L and coronary artery calcium scores >0 (Figure 3.3). Of the 86 men who had CAC scores assessed, CAC score was >0 in 60% of men in the lowest PTH tertile, 76% in the middle PTH tertile, and 95% in the highest PTH tertile. Of the 14 men taking anti-hypertensive medication, one had a PTH level in the lowest tertile, five had PTH levels in the middle tertile, and eight had PTH in the highest tertile (p=0.06 for difference).

BMD was comparable across tertiles of PTH at all sites. The proportion of men who had a prior fracture since age 40 was not different between tertiles (10% in the lowest tertile, 6% in the middle tertile, 8% in the highest tertile, p=0.79).

Table 3.2 Comparison of cardiometabolic indices and bone mineral density according to parathyroid hormone (PTH) tertile

Variable	Lowest Tertile (n=51)	Middle Tertile (n=49)	Highest Tertile (n=51)	Unadjusted p-value[1]	Adjusted p-value[2]
PTH range (pmol/l)	1.8-3.3	3.4-4.3	4.4-8.5	-	-
Age (y)	56.2 (10.3)	57.3 (9.8)	60.4 (10.3)	0.11	-
25-OHD (nmol/L)	98.3 (33.0)	95.0 (38.5)	80.7 (26.5)	**0.02**	-
BMI (kg/m^2)	26.0 (2.6)	25.7 (2.7)	27.4 (3.2)	**0.01**[3]	**0.002**[3,4]
Fat mass (kg)	18.7 (6.7)	17.9 (6.1)	21.4 (7.4)	**0.02**[3]	**0.03**[3]
SBP (mmHg)	131 (12)	129 (14)	136 (14)	**0.04**[3]	0.16
DBP (mmHg)	77 (7)	78 (7)	81 (10)	**0.02**[4]	**0.02**[4]
eGFR (mL/min/1.73m^2)	74.9 (12.0)	74.4 (12.0)	70.5 (9.5)	0.10	-
Blood glucose (mmol/L)	4.98 (0.43)	4.94 (0.42)	5.19 (0.56)	**0.02**[3]	**0.04**[3]
Triglycerides (mmol/L)	1.22 (0.67)	1.35 (0.71)	1.37 (0.59)	0.47	0.57
Total cholesterol (mmol/L)	5.36 (0.79)	5.51 (0.80)	5.85 (0.82)	**0.03**[4]	0.05
LDL cholesterol (mmol/L)	3.43 (0.72)	3.48 (0.67)	3.74 (0.74)	0.07	0.09
HDL cholesterol (mmol/L)	1.46 (0.36)	1.42 (0.30)	1.49 (0.39)	0.66	0.67
CAC score >0 (n/N, %)	21/35 (60%)	22/29 (76%)	21/22 (95%)	**0.01**[4]	-
Total body BMD (g/cm^2)	1.25 (0.08)	1.25 (0.09)	1.26 (0.08)	0.96	0.40
Lumbar spine BMD (g/cm^2)	1.25 (0.15)	1.25 (0.15)	1.27 (0.14)	0.69	0.41
Total hip BMD (g/cm^2)	1.08 (0.13)	1.07 (0.13)	1.09 (0.13)	0.78	0.31
Femoral neck BMD (g/cm^2)	1.01 (0.13)	1.01 (0.14)	1.00 (0.13)	0.92	0.69
Bilateral arm BMD (g/cm^2)	0.99 (0.06)	0.99 (0.07)	0.99 (0.07)	0.98	0.36
Bilateral leg BMD (g/cm^2)	1.47 (0.11)	1.44 (0.12)	1.45 (0.11)	0.46	0.51

Variables are reported as mean (standard deviation), with the exception of CAC score, which is reported as the number (percentage) of men with CAC score >0. Statistically significant p-values are bolded.
[1]In unadjusted analyses, continuous variables were compared using analysis of variance (ANOVA), and CAC scores were compared using Chi squared analysis.
[2]In adjusted analyses, continuous variables were compared using analysis of covariance (ANCOVA), with age, 25-hydroxyvitamin D and estimated glomerular filtration rate included as covariates.
[3]Significant difference between top tertile and middle tertile identified using the method of Tukey.
[4]Significant difference between top tertile and bottom tertile identified using the method of Tukey.
25-OHD = 25-hydroxyvitamin D, SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = body mass index, eGFR = estimated glomerular filtration rate, LDL = low density lipoprotein, HDL = high density lipoprotein, CAC = coronary artery calcium, BMD = bone mineral density.

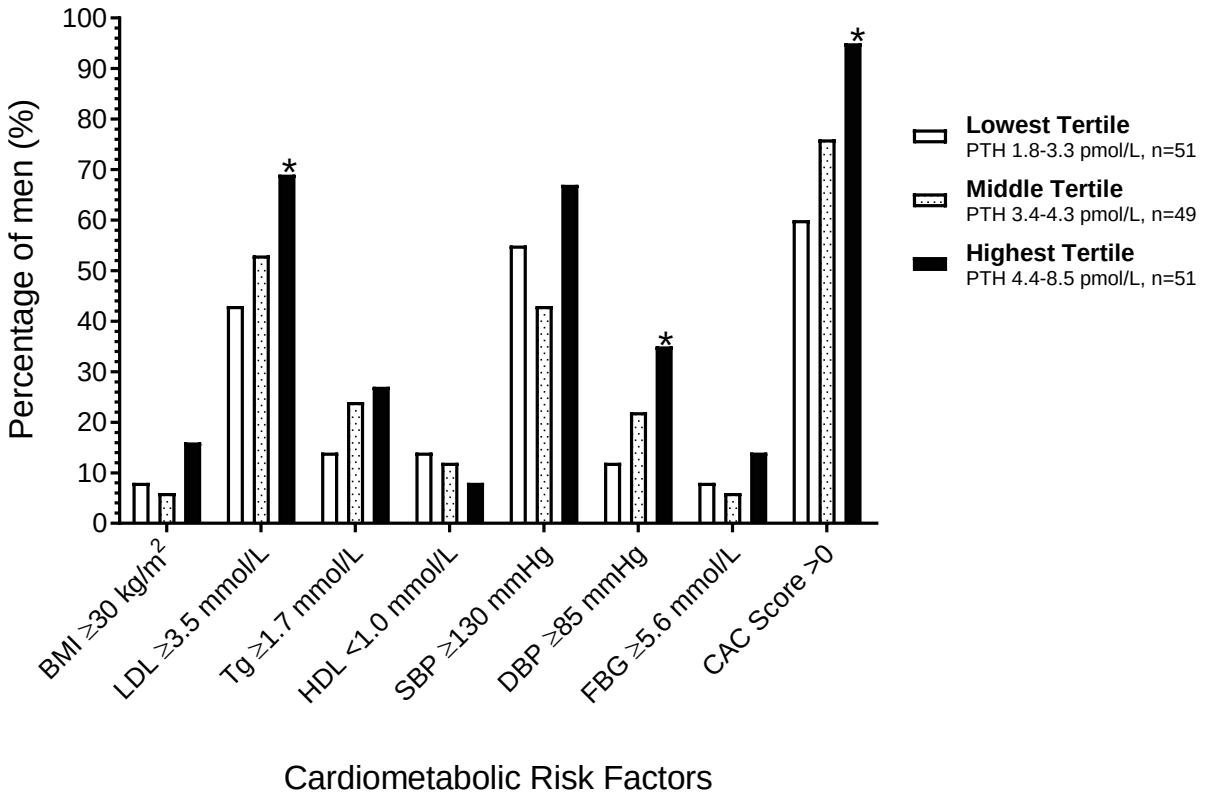


Figure 3.3 Proportion of men (n=151) with cardiometabolic risk factors, stratified by parathyroid hormone (PTH) level.
*Denotes a significant difference between tertiles, $p < 0.05$ (Chi squared)
BMI = body mass index, LDL = low density lipoprotein cholesterol, HDL = high density lipoprotein cholesterol, SBP = systolic blood pressure, DBP = diastolic blood pressure, FBG = fasting blood glucose, CAC = coronary artery calcium

3.3.4 Relationship between PTH and change in BMD

Correlation between baseline PTH level and change in BMD over 2 years was assessed in 50 men who had been randomized to the placebo arm of the trial. There was no association between PTH and change in BMD at the total body (r=-0.04, p=0.79), the total hip (r=0.00, p=0.98), the femoral neck (r=0.10, p=0.49), the spine (r=0.19, p=0.20), the arms (r=-0.18, p=0.21) or the legs (r=0.00, p=0.99).

3.3.5 Multivariable regression

Using stepwise multivariable linear regression, a model was constructed to examine independent determinants of PTH concentration. The decision was made *a priori* to include the following established predictors of PTH in one model: age, 25-hydroxyvitamin D, eGFR, urinary calcium excretion, dietary calcium intake. The model created using this forced selection method is shown in Table 3.3 and also includes BMI, total cholesterol and diastolic blood pressure as meeting the $p<0.15$ inclusion criterion. Although eGFR was included in the model, it did not reach the $p<0.15$ inclusion criterion. This model accounts for 26% of variation in PTH concentrations.

Stepwise regression was then repeated without mandating the inclusion of specific variables. The final model was chosen on the basis of goodness of fit and parsimony and comprised six variables: 25-hydroxyvitamin D, urinary calcium excretion, dietary calcium intake, BMI, total cholesterol and diastolic blood pressure (Table 3.3). This model also accounts for 26% of variation in PTH concentrations. There was no indication of multicollinearity on formal testing in either model. Calcium score was not included in regression analyses, as only 86 participants underwent CAC assessment.

Table 3.3 Multivariate regression models showing the strongest predictors of parathyroid hormone (PTH) concentration

Independent Variable	**Forced variable selection**[1]		**Stepwise selection**[2]	
	Standardized β Coefficient	p-value	Standardized β Coefficient	p-value
25-hydroxyvitamin D	-0.19	0.01	-0.24	0.002
Dietary calcium intake	0.14	0.03	-0.19	0.02
Urinary calcium excretion	-0.17	0.03	-0.17	0.03
Total cholesterol	0.23	0.003	0.19	0.02
BMI	0.21	0.009	0.14	0.08
Diastolic blood pressure	0.14	0.08	0.15	0.07
Age	0.17	0.04	-	-
eGFR	0.12	0.16	-	-

[1] Established predictors of PTH were forced *a priori* into the model. r^2 for model =0.26, p-value for model <0.0001

[2]This model was determined using stepwise selection, with no variables forced into the model. r^2 for model =0.26, p-value for model <0.0001

eGFR = estimated glomerular filtration rate, BMI = body mass index

3.4 DISCUSSION

Results of this study, carried out in a non-referral cohort of community-dwelling men, indicate that PTH levels are not associated with BMD, and that within a heathy population, the individuals with the highest PTH levels do not experience excess bone loss over two years. However, this evaluation highlights important associations between PTH and BMI, fat mass, blood pressure, lipid levels, and coronary artery calcification. These associations are not limited to men with the highest PTH levels. Rather, my findings demonstrate a continuous relationship of PTH with adiposity and cardiometabolic risk factors, indicating that adiposity should be considered as a potential cause of secondary hyperparathyroidism and implying that high or high-normal PTH levels might represent a marker of underlying cardiometabolic disease in some individuals.

As reviewed in Chapter 2, a number of studies have reported associations between PTH and adiposity [89, 90], systolic and diastolic blood pressure [79, 112, 287], and lipid levels [79, 287]. PTH has also been recognized as a predictor of cardiovascular events and mortality in the general population [119, 121]. Individuals with normocalcemic hyperparathyroidism have been shown to have increased BMI, triglycerides, LDL cholesterol, and blood glucose [91]. The findings of this study confirm the relationships between PTH and BMI, fat mass, blood pressure and lipid levels, and also highlight an association between PTH and coronary artery calcification. To my knowledge, the relationship between PTH and coronary calcification has not been previously reported, although evaluations of the PIVUS cohort and the Uppsala longitudinal study of adult men have demonstrated positive relationships between PTH and both

atherosclerotic burden [119] and arterial stiffness [112]. In the present study, associations between PTH and cardiometabolic risk factors were present throughout the entire cohort, indicating that this relationship is not unique to those with the highest PTH levels. Total cholesterol, BMI, and diastolic blood pressure proved to be important predictors of PTH concentration in multivariate models. Although obese individuals often have vitamin D deficiency, which drives PTH production, this has been unable to fully explain PTH variance in previous studies [370, 371], and the associations of PTH with fat mass/BMI were independent of 25-hydroxyvitamin D in the present study.

The mechanism(s) linking these cardiometabolic indices and PTH remains uncertain. It is possible that adiposity or related metabolic abnormalities may stimulate PTH production either directly or indirectly. Similar to other studies that have assessed the determinants of PTH [89, 150, 151], the models that I have presented were only able to explain part of the variance, pointing to other possible influences. PTH has been shown to be related to serum leptin [370]. PTH levels are increased in obesity [370], and leptin levels are higher in patients with primary hyperparathyroidism [372], but are not reduced by parathyroidectomy [372], suggesting that PTH is not driving leptin secretion, but that possibly the reverse may be the case. PTH increases secretion of IL-6 and TNF-α [373], which are pro-inflammatory and likely to increase vascular disease. The administration of leptin to leptin-deficient mice results in 3- to 5-fold increases in circulating parathyroid hormone levels [374, 375], and while similar changes have not been seen in limited human studies [376, 377], evaluation of human parathyroid explants has demonstrated that leptin and its cognate receptor are expressed by parathyroid chief cells in both normal and hyperplastic parathyroid tissue, and that local leptin exposure stimulates PTH release [378].

Thus, a possible pathogenic pathway would be that higher body weight, itself independently associated with hypertension and hyperlipidaemia, results in increases in PTH levels.

Another potential mediator of the relationship between adiposity and PTH is FGF23. Levels of this hormone correlate with measures of adiposity [85], and, as outlined in Chapter 2, there is evidence indicating that FGF23 has a complex relationship with PTH (reviewed in [379]). In preclinical studies, PTH has been shown to stimulate FGF23 expression via activation of nuclear receptor-associated protein-1 (Nurr1) and also potentially through Wnt signalling. There is also evidence to suggest that FGF23 exerts an effect on PTH. While some preclinical data suggest that FGF23 may suppress PTH production, treatment of mouse parathyroid tissue with FGF23 has been shown to increase cell proliferation and PTH secretion [380]. In addition, clinical conditions of FGF23 excess, such as renal failure and tumour-induced osteomalacia, are associated with elevated circulating PTH concentration [379]. Furthermore, in a patient with a translocation involving a region of chromosome 13 with a breakpoint adjacent to the *αKlotho* gene, which resulted in increased αKlotho and FGF23, hyperparathyroidism was observed, suggesting a possible stimulatory influence of FGF23 on PTH in certain clinical scenarios [379, 381].

With respect to the relationships that we observed between PTH and blood pressure, lipid levels and coronary artery calcification in the present study, it is possible that these associations are simply products of the relationship between PTH and adiposity. However, recent data indicate an independent and potentially bidirectional relationship between PTH and the renin-angiotensin-

aldosterone system, which may explain associations between PTH and blood pressure [382]. There are at least three case reports of patients with primary hyperparathyroidism who have subsequently developed primary hyperaldosteronism [97]. Furthermore, in a study of 16 patients with primary hyperparathyroidism, circulating aldosterone concentrations decreased following parathyroidectomy [383]. On the other hand, PTH levels are elevated in persons with primary hyperaldosteronism, and have been shown to normalize following surgical or medical treatment of the hyperaldosteronism [384]. Taken together, these data indicate the possibility of a positive feedback loop between PTH and aldosterone [97].

The direction of effect in the relationship between PTH and cardiometabolic risk factors cannot be established from these cross-sectional analyses. The existing literature is also unable to provide a definitive answer to this question, and raises the possibility of bidirectional effects. If high PTH influences the development of cardiometabolic risk, then risk factors may be expected to improve following normalization of PTH. Some prospective studies of individuals undergoing parathyroidectomy for primary hyperparathyroidism have reported improvements in blood pressure, lipids, endothelial function, and left ventricular mass [118, 145], while others have failed to demonstrate a change [385, 386]. To my knowledge, no studies have documented reductions in weight, cardiovascular events, or mortality following parathyroidectomy for primary hyperparathyroidism [91]. On the other hand, if cardiometabolic risk influences PTH, then PTH concentrations would be expected to mimic fluctuations in cardiovascular and metabolic parameters. Previous work by Grey and Bolland suggests that weight gain predates PTH elevation in women with primary hyperparathyroidism [387], and a study in obese adolescents found that PTH decreased significantly following weight loss [388]. I recently

undertook an analysis of 21 obese adults (median BMI 40.9 kg/m^2) who underwent sleeve gastrectomy for weight loss. In this cohort, PTH concentration decreased by a median (95% CI) of 1.1 (-1.7 to 0.2) pmol/L, or 23%, between baseline and one year post-op (p=0.009) (unpublished data, to be presented at the 2017 American Society of Bone & Mineral Research Annual Meeting). PTH levels have also been found to decrease when individuals with diabetes are treated with a TZD or metformin, although it is not clear whether this is the direct result of improved glycaemic control, or mediated by the effect of the medication on bone turnover [389]. In a large longitudinal cohort study of female nurses who did not have primary hyperparathyroidism at baseline, those with hypertension were approximately 1.5 times more likely to develop primary hyperparathyroidism than normotensive women [390]. The findings of the present study highlight the important need for further research examining the relationship between PTH and cardiometabolic risk, and characterizing the phenotype associated with normocalcemic hyperparathyroidism.

I did not observe an association between PTH and BMD or fracture history in the present cohort. Furthermore, changes in BMD over two years were unrelated to PTH. These findings are in contrast with some previous reports of people with PTH levels in the upper normal range, and persons with normocalcemic hyperparathyroidism. The prevalence of osteoporosis is high amongst individuals with normocalcemic hyperparathyroidism who were identified following referral to a metabolic bone disease clinic, exceeding 50% in some reports [114, 154, 155]. In a non-referral population, Berger and colleagues found that community-dwelling individuals in the upper PTH reference range (PTH ≥5.6 pmol/L) had reduced BMD at the femoral neck and total hip compared to those with lower PTH levels [391]. In a longitudinal assessment of the MrOS-

Sweden cohort, men with baseline PTH ≥4.2 pmol/L experienced greater annual bone loss at the hip than men with lower PTH levels [392]. However, my findings corroborate the results of several other large studies, carried out in unselected populations. In another analysis of the MrOS-Sweden cohort, BMD was comparable among men with normocalcemic hyperparathyroidism and normal controls [162]. In a subgroup of the WHO MONICA study, men and women with normocalcemic hyperparathyroidism were no more likely to have had a fracture than normal individuals, and calcaneal ultrasound showed similar attenuation in both groups [113]. In a cohort of women presenting for osteoporosis screening, normocalcemic hyperparathyroidism was not associated with lower BMD or increased risk of fracture [393]. The reason for these inconsistencies is unclear. One possible explanation is that the entity of normocalcemic hyperparathyroidism actually encompasses a heterogeneous group of individuals who have the same biochemical profile but different causes and consequences [162]. This group may include: 1) normal individuals whose PTH levels are within the top 2.5% of the population and therefore fall outside of the reference range, 2) individuals with parathyroid autonomy (i.e. a *forme fruste* of primary hyperparathyroidism) and, 3) individuals with a secondary elevation in PTH due to an unknown (but possibly cardiometabolic) stimulus. As there is no way to reliably differentiate between these groups at present, existing research including the present study has reported aggregate results, which may account for conflicting findings between studies. A possible paradigm for the pathogenesis of secondary hyperparathyroidism is presented in Figure 3.4.

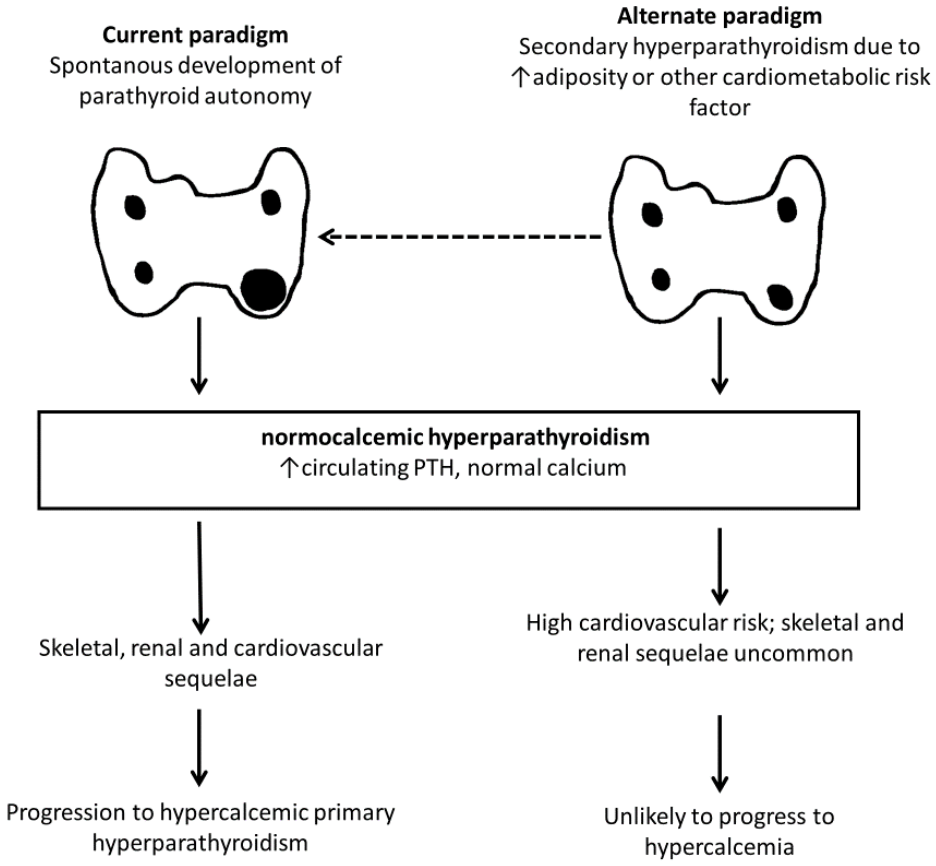


Figure 3.4 Paradigms for the pathogenesis of normocalcemic hyperparathyroidism. PTH = parathyroid hormone.

Similar to previous reports [150, 151], the present study identified significant inverse associations between PTH and 25-hydroxyvitamin D, dietary calcium intake, and urinary calcium excretion. Age is another established determinant of PTH [151], and although I did not identify a significant correlation between age and PTH in bivariate analyses, the two parameters were significantly associated in the multivariate regression model, suggesting that the relationship between PTH and age is influenced by other determinants of PTH. Serum calcium concentration and renal function directly influence PTH secretion. PTH concentrations are often correlated with serum calcium and with GFR [150, 151], although the relationship with GFR tends to be attenuated in cohorts with normal renal function [150]. I did not identify significant associations between PTH and either of these parameters, which may be because our cohort lacked men with significant calcium aberrations or renal dysfunction, or due to the relatively small sample size.

This study has limitations. The men assessed were a homogeneous and relatively healthy group. This has the advantage of limiting referral bias, which might have resulted in overestimation of skeletal consequences of high and high-normal PTH in other studies [114, 154, 155]. However, these results are not necessarily generalizable to men with renal dysfunction, vitamin D deficiency, or known vascular disease. The findings of relationships between PTH and cardiometabolic risk factors are also limited by the cross-sectional nature of these correlations, which precludes determination of causation, and introduces the potential for confounding. In addition, ionized calcium levels were not measured so it is possible, albeit unlikely, that some of the individuals studied could have had primary hyperparathyroidism.

In summary, this research demonstrates that in healthy, community-dwelling men, PTH levels are related to adiposity and cardiometabolic risk factors, but not associated with low BMD or rates of bone loss. These findings support the notion of PTH as a marker of cardiovascular risk, and suggest that cardiovascular risk may be elevated in normocalcemic hyperparathyroidism. While correlation does not imply causation, cardiovascular risk evaluation may be prudent in individuals with high or high-normal PTH. Further study is required to elucidate the directions of the relationships between PTH, adiposity and cardiometabolic indices, and to assess long-term outcomes in individuals who are found to have an isolated high or high-normal PTH level.

CHAPTER 4:
SERUM PHOSPHATE IS RELATED TO ADIPOSITY IN HEALTHY ADULTS

4.1 INTRODUCTION

As reviewed in Chapter 2, inorganic phosphate is an essential component of many biological processes. Adequate phosphate concentrations are required for bone mineralization, and phosphate has a crucial role in energy metabolism, being required for generation of the primary cellular energy substrate, ATP [50, 63]. Consequently, circulating phosphate levels are widely measured in both clinical practice and research. While undertaking the analyses described in Chapter 3, I incidentally observed an inverse correlation between serum phosphate levels and fat mass in a cohort of healthy men. In large databases, such associations can easily occur by chance, so I went on to look for similar associations between serum phosphate and measures of adiposity in two further independent data sets, and used what other variables were available in these databases (e.g. PTH) to gain insight into the possible mediators of this relationship. The present study reports on the relationships between serum phosphate and measures of adiposity in a population of healthy men, and in two independent cohorts of healthy women.

Although FGF23 levels were not available in any of the three populations studied, this hormone is an important regulator of serum phosphate concentrations [394] and has been associated with fat mass in other cohorts [85, 86], suggesting that it may be a mediator of the relationships reported in this study. For this reason, I also looked for associations between FGF23 and BMI in a fourth cohort in whom FGF23 levels were available.

4.2 METHODS

4.2.1 Participants

This analysis uses baseline data from four independent cohorts of adults who participated in clinical trials at the University of Auckland [365, 395-397]. A brief description of each cohort is given below. The trial interventions took place after the collection of the data used in these analyses. All studies were approved by the local ethics committee, and informed consent was obtained from each participant at the time of study enrolment.

Male Cohort

This cohort (n=323) of community-dwelling men, aged 40 and older, were recruited by newspaper advertisement and randomized to receive calcium supplements (600 or 1200mg per day) or placebo for a two-year period [365]. Exclusion criteria included major active disease (including coronary heart disease, hypertension, diabetes mellitus, untreated thyroid disease, renal dysfunction, liver disease, malignancy, or known metabolic bone disease), current lipid-lowering therapy, 25-hydroxyvitamin D <25 nmol/L, and BMD Z-score <-2.0 at the spine or total hip.

Female Cohort 1

This cohort (n=185) of healthy postmenopausal women, age < 75 years, were recruited via newspaper advertisement and randomized to receive hydrochlorothiazide 50mg or placebo daily for a two-year period [395]. Exclusion criteria included disorders of calcium metabolism, previously treated osteoporosis, systemic illness (including renal, thyroid or hepatic

dysfunction), glucocorticoid use, use of calcium supplements at a dose of >500mg per day, and use of hormone replacement therapy within 12 months.

Female Cohort 2

This cohort (n=1471) of healthy postmenopausal women, aged 55 or older, were recruited by advertisement and randomized to receive either 1000mg elemental calcium (as citrate) or placebo daily for five years [396]. Women receiving therapy for osteoporosis or taking calcium supplements were ineligible. Additional exclusion criteria included major ongoing disease, malignancy, metabolic bone disease, 25-hydroxyvitamin D <25 nmol/L, and BMD Z-score of <-2.0 at the spine.

FGF23 Cohort

This cohort (n=20) of healthy women, at least 5 years postmenopausal, were recruited from a group of women who had volunteered for other bone health studies and been ineligible because of normal bone density. Participants were randomized to receive either 1000mg elemental calcium (as carbonate) or to a placebo containing no calcium for three months [397]. Exclusion criteria included known cardiovascular disease, recent treatment with medications known to affect calcium concentrations or bone metabolism, and active systemic illness.

4.2.2 Measurements

In all cohorts, height was measured with a Harpenden stadiometer, and weight was determined using electronic scales. Dietary calcium intake was assessed with a validated food frequency questionnaire [366]. Baseline blood pressure was measured using either a Dinamap automatic

monitor (Johnson & Johnson, Tampa, FL) (Male Cohort, Female Cohort 2) or a manual sphygmomanometer (Female Cohort 1), after a five to ten minute rest period. In the Male Cohort and in Female Cohort 2, three blood pressure readings were taken three minutes apart, and results were averaged.

BMD and body composition were measured using dual-energy x-ray absorptiometry with a Lunar Prodigy (Male Cohort) [365], Lunar DPX-L (Female Cohort 1) [395], or Lunar Expert (Female Cohort 2) [396] instruments (GE-Lunar, Madison, Wisconsin). BMD was assessed at the spine, hip and the total body.

Each participant provided a fasting serum sample at baseline, and concentrations of phosphate, total calcium, creatinine, glucose, and lipids were assessed [365, 395, 396]. Glomerular filtration rate was estimated using the MDRD equation [398].

In the Male Cohort, 25-hydroxyvitamin D was measured using either a radioimmunoassay (DiaSorin, Stillwater, Minnesota) or a chemiluminescent assay (Nichols, San Juan Capistrano, California), both assays meeting the performance targets for the Vitamin D External Quality Assessment Scheme (DEQAS) [399]. Results using the Nichols assay were converted to DiaSorin results [400]. Serum PTH was measured using Roche autoanalyzers (Roche Diagnostics, Indianapolis, Indiana).

In Female Cohort 1, 25-hydroxyvitamin D levels were measured using the Incstar assay (Incstar Corporation, Stillwater, Minnesota). Serum PTH was measured using an Allegro assay (Nichols

Institute, San Juan Capistrano, CA). Fasting insulin levels were measured using an in-house radioimmunoassay, with an intra-assay CV of 3.9%, and an inter-assay CV of 7.8%.

In Female Cohort 2, 25-hydroxyvitamin D levels were measured using a DiaSorin radioimmunoassay (DiaSorin, Stillwater, Minnesota). PTH levels were not determined in this cohort.

In the FGF23 Cohort, FGF23 was measured using an intact ELISA kit (Kainos Laboratories, Tokyo, Japan, CV 6%). Phosphate and PTH concentrations were assessed using a Cobas modular autoanalyzer (Roche Diagnostics).

4.2.3 Statistical analyses

Associations between serum phosphate and other baseline parameters were assessed using Pearson's correlation. Correlations were adjusted for age, PTH, and 25-hydroxyvitamin D using multivariate linear regression.

All data analysis was done with SAS v9.4 (SAS Institute, NC), and figures were created using Prism v6.0 (GraphPad Software Inc, 2013). The threshold for statistical significance was $p < 0.05$.

4.3 RESULTS

A total of 1979 individuals (1656 women, 323 men) from the three principal cohorts were studied. Their baseline characteristics are presented in Table 4.1.

Table 4.1 Baseline characteristics of three independent cohorts

	Male Cohort (n=323)	**Female Cohort 1** (n=185)	**Female Cohort 2** (n=1471)
Age (y)	56.5 (10.3)	62.8 (5.8)	74.2 (4.2)
Height (cm)	1.77 (0.07)	1.62 (0.05)	1.59 (0.06)
Weight (kg)	82.7 (12.1)	68.1 (11.4)	66.9 (11.3)
BMI (kg/m^2)	26.5 (3.3)	26.0 (4.2)	26.4 (4.2)
Dietary calcium intake (mg/d)	870 (450)	1000 (510)	860 (390)
Systolic BP (mmHg)	131 (13)	130 (14)	141 (23)
Diastolic BP (mmHg)	78 (8)	82 (9)	73 (11)
Phosphate (mmol/L)	1.0 (0.1)	1.2 (0.1)	1.2 (0.1)
Total calcium (mmol/L)	2.33 (0.09)	2.29 (0.08)	2.32 (0.09)
PTH (pmol/L)	4.06 (1.25)[1]	2.96 (1.24)	
eGFR (mL/min/1.73m^2)	73.3 (10.8)	66.1 (9.7)	57.3 (10.0)
25OH vitamin D (nmol/L)	92.5 (32.9)	55.0 (23.2)	53.5 (17.9)
Glucose (mmol/L)	5.04 (0.48)	5.03 (0.90)	5.11 (0.70)
Insulin		10.6 (10.7)	
Fat mass (kg)	19.4 (7.6)	27.8 (8.7)	27.1 (9.6)
Lean mass (kg)	59.4 (6.9)	36.7 (3.6)	36.2 (4.1)
Total body BMD (g/cm^2)	1.26 (0.09)	1.06 (0.08)	1.03 (0.09)
Lumbar spine BMD (g/cm^2)[2]	1.25 (0.16)	1.07 (0.16)	1.06 (0.18)
Femoral neck BMD (g/cm^2)	1.01 (0.14)	0.87 (0.13)	0.82 (0.12)
Total hip BMD (g/cm^2)	1.08 (0.14)		0.86 (0.13)

Data are mean (SD)
[1]151 men in this cohort had PTH levels measured
[2]Represents BMD at L2-L4 in Female Cohort 2, and L1-L4 in the other two cohorts
BMI = body mass index, BP = blood pressure, PTH = parathyroid hormone, eGFR = estimated glomerular filtration rate, BMD = bone mineral density

4.3.1 Bivariate correlations of serum phosphate

Bivariate correlations of serum phosphate are shown in Table 4.2. Significant inverse correlations of serum phosphate with weight, BMI, and fat mass were observed in each of the cohorts ($-0.13 > r > -0.31$, $0.02 > p > 0.0001$). The correlations with fat mass are shown in Figure 4.1, and suggest that the relationships are robust and not influenced by outlying data points. Serum phosphate was also inversely related to diastolic blood pressure, lean mass and BMD in Female Cohorts 1 and 2, and to PTH, glucose and insulin in Female Cohort 1. PTH and insulin were not measured in Female Cohort 2.

4.3.2 Serum phosphate and adiposity

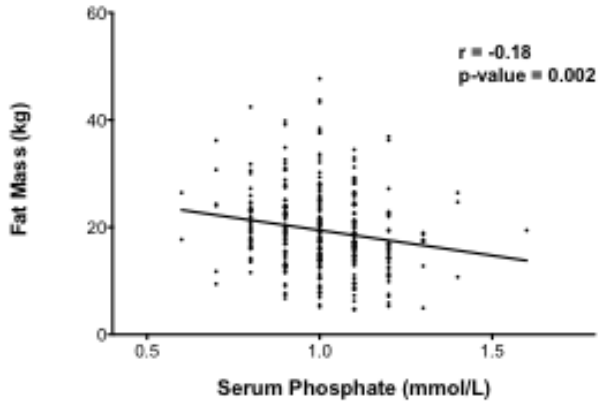
The inverse associations between serum phosphate and adiposity were further explored using multiple regression analysis to assess the possible role of PTH, which was measured in the Male Cohort and in Female Cohort 1. PTH was positively associated with BMI ($r=0.24$, $p=0.004$; $r=0.22$, $p=0.003$, in the respective cohorts) and fat mass ($r=0.23$, $p=0.005$; $r=0.28$, $p<0.0001$, respectively), and tended to be negatively related to serum phosphate ($r=-0.08$ $p=0.31$; $r=-0.24$, $p=0.001$, respectively). Multiple regression with phosphate as the dependent variable demonstrated that the relationship between phosphate and fat mass was independent of PTH, though PTH influenced phosphate in Female Cohort 1 (Table 4.3). When age and eGFR were added to the models, the results were little changed (Table 4.3).

Table 4.2 Correlation coefficients between serum phosphate and other parameters in three cohorts

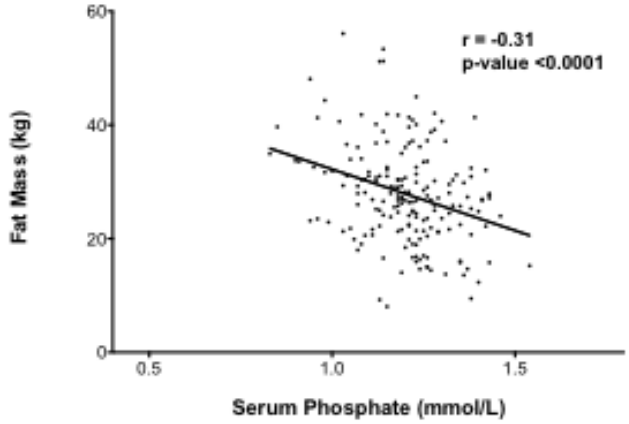
	Male Cohort (n=323)		**Female Cohort 1** (n=185)		**Female Cohort 2** (n=1471)	
	r	***p-value***	***r***	***p-value***	***r***	***p-value***
Age (y)	0.06	0.28	0.08	0.26	**0.08**	**0.004**
Height (cm)	-0.07	0.24	0.03	0.70	-0.04	0.16
Weight (kg)	**-0.15**	**0.007**	**-0.29**	**<0.0001**	**-0.19**	**<0.0001**
BMI (kg/m^2)	**-0.13**	**0.02**	**-0.31**	**<0.0001**	**-0.19**	**<0.0001**
Dietary calcium intake (mg/d)	**0.16**	**0.004**	0.12	0.12	-0.02	0.47
Systolic BP (mmHg)	-0.06	0.25	-0.12	0.18	-0.04	0.10
Diastolic BP (mmHg)	-0.06	0.30	**-0.21**	**0.02**	**-0.09**	**0.001**
Total calcium (mmol/L)	-0.02	0.75	-0.10	0.22	0.03	0.33
PTH (pmol/L)	-0.08	0.31	**-0.24**	**0.001**		
eGFR (mL/min/17.3m^2)	0.01	0.90	-0.05	0.48	0.00	0.95
25OH vitamin D (nmol/L)	0.03	0.61	0.11	0.24	0.00	0.94
Glucose (mmol/L)	**-0.13**	**0.02**	**-0.28**	**0.0002**	-0.03	0.26
Insulin			**-0.24**	**0.02**		
Fat mass (kg)	**-0.18**	**0.002**	**-0.31**	**<0.0001**	**-0.18**	**<0.0001**
Lean mass (kg)	-0.06	0.29	-0.11	0.13	**-0.10**	**<0.0001**
Total body BMD (g/cm^2)	-0.07	0.22	**-0.21**	**0.005**	**-0.12**	**<0.0001**
Lumbar spine BMD (g/cm^2)	-0.02	0.77	**-0.19**	**0.009**	**-0.09**	**0.0004**
Femoral neck BMD (g/cm^2)	0.02	0.78	**-0.29**	**<0.0001**	**-0.08**	**0.002**
Total hip BMD (g/cm^2)	0.00	0.95			**-0.13**	**<0.0001**

Significant associations are bolded.
BMI = body mass index, BP = blood pressure, PTH = parathyroid hormone, eGFR = estimated glomerular filtration rate, BMD = bone mineral density
r = Pearson's correlation coefficient

a. Male Cohort



b. Female Cohort 1



c. Female Cohort 2

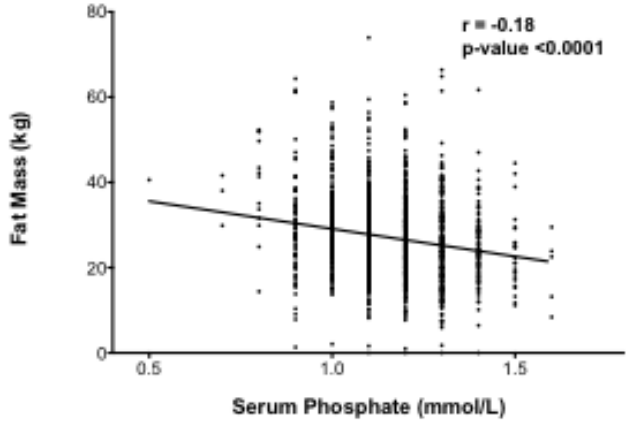


Figure 4.1 Relationship between serum phosphate and fat mass in three independent cohorts, (a) Male Cohort (n=323), (b) Female Cohort 1 (n=185), and (c) Female Cohort 2 (n=1471). r = Pearson's correlation coefficient

Table 4.3 Parathyroid hormone (PTH) and fat mass as predictors of serum phosphate in two cohorts before and after adjustment in a multivariable model

	Male Cohort		**Female Cohort 1**	
	β-coefficient	***p-value***	***β-coefficient***	***p-value***
Unadjusted Model				
Fat Mass	-0.18	0.002	-0.31	**<0.0001**
Model adjusted for PTH				
Fat Mass	-0.21	0.01	-0.27	**0.0004**
PTH	-0.04	0.67	-0.16	**0.03**
Model adjusted for PTH, age, eGFR				
Fat Mass	-0.16	0.06	-0.27	**0.0005**
PTH	-0.05	0.53	-0.17	**0.02**
Age	0.17	0.06	0.04	0.54
eGFR	0.21	0.02	-0.07	0.31

Significant associations are bolded.

Results are based on a multivariable model with serum phosphate as the dependent variable and the other parameters as predictor variables.

4.3.3 Serum phosphate and cardiometabolic parameters

To better understand the other bivariate correlations of serum phosphate shown in Table 4.2, adjustments for fat mass were made. In the Male Cohort, the association between serum phosphate and serum glucose was no longer significant after adjusting for fat mass. In Female Cohort 1, the associations of serum phosphate with diastolic blood pressure and insulin were no longer significant after adjustment for fat mass ($p=0.07$ for diastolic blood pressure and $p=0.10$ for glucose) but the relationship between phosphate and glucose remained significant ($p=0.0005$). In Female Cohort 2, the inverse association between serum phosphate and diastolic blood pressure remained statistically significant after adjusting for fat mass ($p=0.01$). Collectively, these findings suggest that fat mass is independently related to both phosphate and to these metabolic parameters, and that this co-dependence on fat mass mediates much of the apparent correlation between them.

4.3.4 Serum phosphate and skeletal parameters

Phosphate was negatively correlated with BMD at all sites in Female Cohorts 1 and 2, but not in the Male Cohort (Table 4.2). Because fat mass is a strong predictor of BMD, we adjusted these analyses for fat mass, in addition to age, eGFR, and 25-hydroxyvitamin D (Table 4.4). This was done using multivariable models in which phosphate was the dependent variable and the other parameters were predictor variables. Adjustment attenuated the relationships between phosphate and BMD, but they remained statistically significant at some skeletal sites (Table 4.4). Of the variables that were adjusted for, fat mass had the largest influence on the relationship between phosphate and BMD. The impact of adjustment was similar when BMI or weight were used in place of fat mass.

Table 4.4 Bone mineral density (BMD) as a predictor of serum phosphate in three cohorts before and after adjustment in multivariable models

BMD Site	**Male Cohort** (n=323)		**Female Cohort 1** (n=185)		**Female Cohort 2** (n=1471)	
	β-coefficient	***p-value***	***β-coefficient***	***p-value***	***β-coefficient***	***p-value***
Unadjusted Model						
Total body	-0.07	0.22	**-0.21**	**0.005**	**-0.12**	**<0.0001**
Lumbar spine	-0.02	0.77	**-0.19**	**0.009**	**-0.09**	**0.0004**
Femoral neck	0.02	0.78	**-0.29**	**<0.0001**	**-0.08**	**0.002**
Total hip	0.00	0.95			**-0.13**	**<0.0001**
Adjusted Model						
Total body	0.01	0.93	-0.12	0.27	**-0.06**	**0.03**
Lumbar spine	0.04	0.59	**-0.23**	**0.02**	-0.05	0.09
Femoral neck	0.13	0.14	**-0.26**	**0.02**	-0.04	0.14
Total hip	0.10	0.26			**-0.07**	**0.007**

Data are from a multivariate model in which serum phosphate is the dependent variable and BMD, parathyroid hormone (PTH), age, fat mass, estimated glomerular filtration rate (eGFR), and 25-hydroxyvitamin D are independent variables. Separate regression analyses were carried out for the four skeletal sites. PTH levels were not assessed in Female Cohort 2, so adjusted correlations reported for this cohort do not include adjustment for PTH.

4.3.5 Fibroblast growth factor 23 (FGF23) Cohort

The FGF23 Cohort consisted of 20 women, one of whom was excluded from analysis due to an outlying FGF23 level and subsequent diagnosis of primary hyperparathyroidism. For the remaining women (n=19), mean (SD) age was 67.3 (3.7) years, body weight 72.8 (11.7) kg, BMI 26.9 (4.1) kg/m^2, serum phosphate 1.14 (0.18) mmol/L, serum PTH 4.2 (1.0) pmol/L and FGF23 52.6 (12.4) pg/mL. FGF23 was significantly associated with both body weight (r=0.60, p=0.007) and BMI (r=0.49, p=0.03) (Figure 4.2). In this cohort, there were trends towards an inverse correlation between BMI and phosphate (r=-0.39, p=0.11), and a positive correlation between BMI and PTH (r=0.35, p=0.14), although these did not reach statistical significance because of the much smaller size of this cohort.

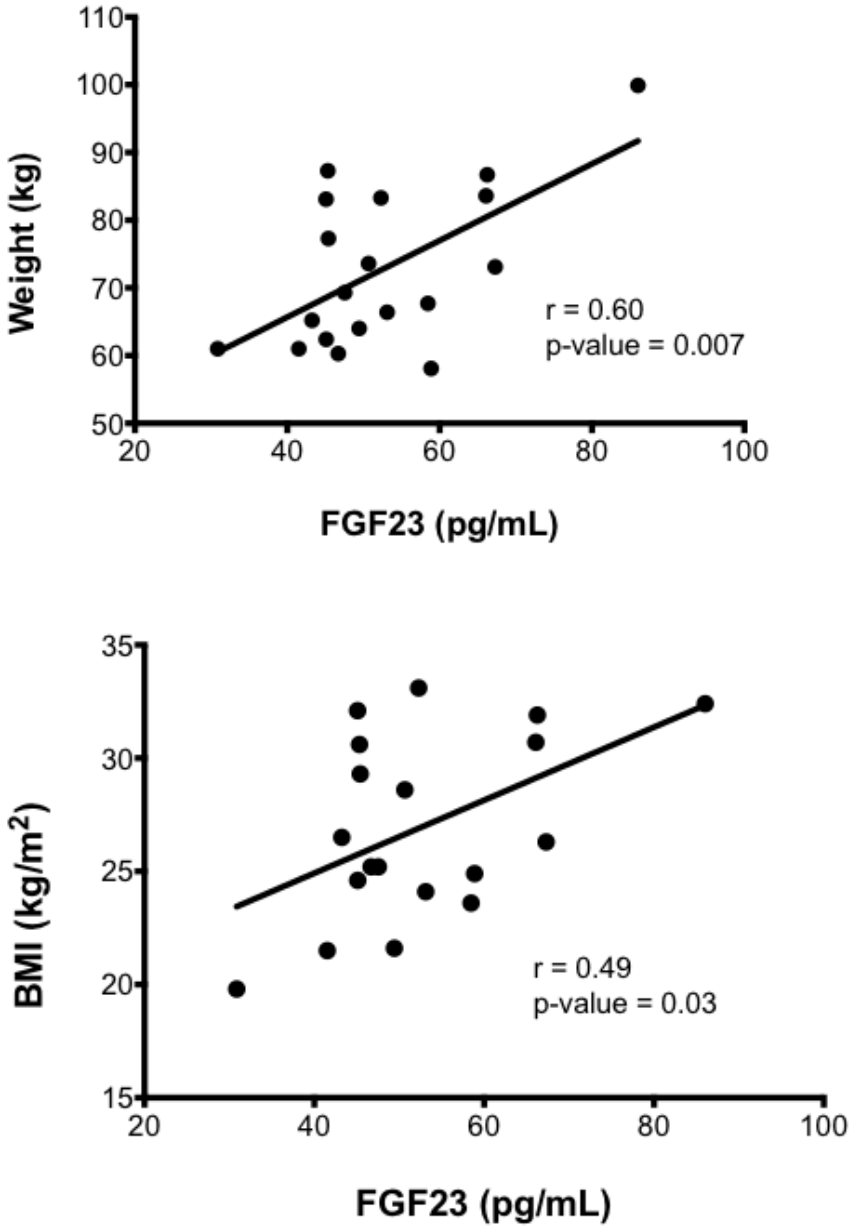


Figure 4.2 Relationship between fibroblast growth factor 23 (FGF23) and both weight (top) and body mass index (BMI, bottom) in a cohort of healthy postmenopausal women (n=19).
r = Pearson's correlation coefficient

4.4 DISCUSSION

The results presented in this chapter demonstrate an inverse association between serum phosphate concentrations and measures of adiposity in three cohorts of healthy individuals, and indicate that this relationship persists independently of circulating PTH concentrations. The strong correlation between FGF23 and BMI observed in the smaller fourth cohort indicates that this hormone may be a mediator of the relationship between serum phosphate and fat mass. I also observed an inverse relationship between phosphate and BMD in two female cohorts, which is small in magnitude but appears to persist, to some extent, independently of fat mass.

Though the relationship between serum phosphate concentration and measures of adiposity is not widely appreciated, there are some corroborative data in the literature, which have been largely overlooked since these were not the primary endpoints of those studies [83, 286, 401-403]. Lind and colleagues assessed 2183 50-year old males, selected from the general population, and observed inverse correlations between serum phosphate and both BMI ($r=-0.24$, $p<0.0008$) and waist-to-hip ratio ($r=-0.33$, $p<0.0001$). These relationships persisted after adjustment for age, sex and serum creatinine [286]. Haglin assessed 2265 adults (1272 women), most of whom were obese, and demonstrated an inverse correlation between serum phosphate and BMI in women ($r=-0.18$, $p=0.0001$) but not men ($r=-0.06$, $p=0.07$) [402]. Haap *et al* studied 881 healthy adults (540 women) with a family history of type 2 diabetes, and found serum phosphate to be negatively correlated with BMI, an association that remained significant after adjustment for age and gender ($r=-0.17$, $p <0.0001$) [401]. In an analysis of 46,798 healthy Korean adults (16,568 women), Park and colleagues reported a negative correlation between phosphate and both BMI and waist circumference, although the relationship with BMI was no longer statistically significant after

adjustment for age, sex, and serum calcium [83]. Individuals with metabolic syndrome have been reported to have lower phosphate levels than controls ($p < 0.0001$) [84]. Although the associations suggest modest effect sizes (r -0.18 to -0.31), indicating that only a small amount of variance in serum phosphate concentrations is related to adiposity, the findings of the present study provide confirmation of an inverse relationship between serum phosphate and adiposity in cohorts of both men and women, spanning a large range of ages.

Because PTH is correlated with fat mass [89] and because PTH directly influences serum phosphate concentrations, consideration of PTH status is important when assessing the relationship between phosphate and fat mass. In this study, I have shown that variance in PTH accounts for only part of the association between serum phosphate and fat mass. FGF23 is a second hormone that regulates serum phosphate. This was not assessed in the three primary cohorts, but data from the smaller FGF23 cohort demonstrates that circulating FGF23 levels are related to adiposity. As reviewed in Chapter 2, a number of pieces of evidence corroborate this finding. In the population-based Swedish MrOS cohort of 964 men, body weight was positively correlated with FGF23 after adjustment for age ($r=0.18$, $p<0.0001$) and after further adjustment for indices of mineral metabolism ($r=0.20$, $p<0.0001$) [85]. Similar relationships were seen with fat mass. In a community-based cohort of 946 70-year olds from Uppsala, correlations of FGF23 with body weight and fat mass were also found ($r=0.07$, $P<0.05$) [85]. In 2134 middle-aged men and women from the EPIC-Germany cohort, waist circumference and BMI increased across the quartiles of FGF23 [86]. In the Health ABC study, a similar significant relationship between quartiles of FGF23 and BMI was observed, mean BMI being 26.6 in FGF23 quartile 1 and 28.0 in quartile 4 [87]. Finally, in patients with primary hyperparathyroidism, FGF23 levels are

related to BMI ($p<0.001$) [88]. Obesity may be causative in these relationships, since leptin directly stimulates FGF23 expression in primary rat osteoblasts [404], and administration of leptin to ob/ob mice almost doubles serum FGF23 concentrations [375]. The causative role of obesity could also be inferred from the effects of weight loss on FGF23. This does not appear to have been comprehensively assessed, but there is a report of ten obese men who, after non-surgical weight loss of 20kg, showed a decline in FGF23 levels of about 20% [405]. I recently carried out an analysis of 21 obese adults (median BMI 41.0 kg/m^2) who underwent sleeve gastrectomy. FGF23 concentrations declined by a median (95% CI) of 12.4 (-19.5 to 2.0) pg/mL, or 19%, between baseline and one year post-op ($p=0.005$) (unpublished data, to be presented at 2017 American Society of Bone & Mineral Research Annual Meeting). PTH and FGF23 may act together to produce these effects since both are related to weight and both are phosphaturic. Their secretion may be inter-dependent, since FGF23 has been shown to promote proliferation of parathyroid cells [380], and reduction of PTH levels after parathyroidectomy reduces FGF23 levels by about 20% [88].

There are other mechanisms which could contribute to the lower serum phosphate levels of adiposity. Overweight individuals tend to have a diet that is high in carbohydrates but low in protein, and therefore are more likely to have inadequate phosphate intake [402]. Intracellular phosphate shifts are promoted by glucose entry into cells, which is facilitated by insulin release. Many overweight and obese individuals develop hyperinsulinemia, which might contribute to lower extracellular phosphate concentrations. In keeping with this hypothesis, I identified negative correlations between serum phosphate and glucose levels in the Male Cohort, and in Female Cohort 1, as well as an inverse relationship between serum phosphate and insulin in

Female Cohort 1. As expected, these relationships were largely attenuated after adjustment for fat mass. Similarly, in their study of healthy Korean adults, Park *et al* identified an inverse association between serum phosphate and both fasting glucose ($r=-0.07$, $p<0.0001$) and fasting insulin ($r=-0.04$, $p<0.0001$), and these relationships were no longer present once analyses were adjusted for BMI [83].

Phosphate is an important component of bone mineral. Adequate local phosphate concentrations are required for bone mineralization, and very low phosphate levels are associated with osteomalacia [63]. Inverse associations between serum phosphate and BMD were identified in the two female cohorts that were assessed in this study, but not in the cohort of males. Following adjustment for age, PTH, fat mass, renal function, and 25-hydroxyvitamin D, associations between phosphate and BMD were attenuated. However, most associations remained statistically significant following these adjustments, suggesting that the relationship between phosphate and BMD may also be mediated by other factors. For instance, this relationship may reflect the activity of the phosphaturic hormone FGF23 and its co-factor, αKlotho. As outlined in Chapter 2, the presence of αKlotho is required for FGF23 to bind the fibroblast growth factor receptor and exert its phosphaturic effect [406]. Both FGF23 [407] and αKlotho [408] knock-out mice display a premature aging phenotype, which includes hyperphosphatemia and osteopenia.

Hyperphosphatemia has been suggested as a driver of premature aging, and a low phosphate diet has been shown to increase lifespan and reverse vascular calcification in mice lacking FGF23 [107] or αKlotho [409]. In humans, αKlotho polymorphisms have been associated with variations in BMD in some cohorts of postmenopausal women [410, 411]. These associations are

relatively weak, and appear to be limited to women who are at least ten years postmenopausal. To my knowledge, no human studies provide data relating to the effect of αKlotho polymorphisms on serum phosphate levels. While this area requires further study, it is possible that variations in FGF23 and/or αKlotho action are responsible for the relationship that was observed between serum phosphate and bone density in the present study.

In conclusion, this study suggests that serum phosphate is associated with markers of adiposity, adding substantially to the existing evidence for this. The biological significance of this phosphate-adiposity relationship remains to be determined, but it is relevant to any future studies that consider associations of serum phosphate, and may well represent an unrecognized confounder in some of the relationships that have already been described. The results reported in this chapter also raise the important question of the association between adiposity and FGF23 levels, which requires further exploration, but may explain the observed relationship between serum phosphate and fat mass. Finally, these results expand on the data presented in Chapter 3 by adding another facet to the already complicated relationship between bone metabolism and glucose-fat homeostasis.

CHAPTER 5:
THE EFFECT OF THIAZOLIDINEDIONES ON BONE MINERAL DENSITY AND BONE TURNOVER: A SYSTEMATIC REVIEW AND META-ANALYSIS

5.1 INTRODUCTION

TZD medications, such as rosiglitazone and pioglitazone, are agonists of the peroxisome-proliferator-activated receptor gamma isoform (PPAR-γ) that promote insulin sensitization [412]. They improve glycaemic control in patients with type 2 diabetes, and slow the development of diabetes in persons with impaired glucose tolerance (IGT) [413-416]. Pioglitazone has also been found to increase limb fat mass in persons with HIV-associated lipoatrophy [417].

However, as described in Chapter 2, TZD use has been associated with an increased risk of fracture [418, 419]. Post-hoc analyses of large randomized controlled trials have demonstrated a 1.5- to 2-fold increased risk of distal extremity fracture in women with diabetes receiving TZDs [174-176]. This finding has been confirmed in meta-analyses [418-420]. Observational studies also report increased risk of fractures in the axial skeleton with TZDs in both women and men [421, 422]. Diabetes itself is associated with increased skeletal fragility, and persons with diabetes are at increased risk of fracture despite having similar or higher bone mineral density (BMD) than healthy individuals [423]. The increased risk of fracture conferred by TZDs appears to be independent of the skeletal effects of diabetes [418, 419].

The mechanism by which TZDs increase fracture risk remains unclear. At a cellular level, PPAR-γ acts on mesenchymal stem cells to preferentially promote differentiation into adipogenic

cell lineages at the expense of osteoblastogenesis [44-47]. Preclinical data also points to a role for TZDs in stimulation of osteoblast apoptosis, and osteoclast differentiation [424, 425]. Some clinical studies report increased loss of BMD with TZDs [426-429], but effects on bone turnover markers have been inconsistent, with evidence for both reduced bone formation [389, 428, 430] and increased bone resorption [389, 426, 431].

It is possible to detect biologically significant effects in surrogate endpoints for fracture, such as BMD and bone turnover, in much smaller cohorts than are required for investigation of effects on fracture incidence. This chapter presents the results of a systematic review and meta-analysis of all randomized controlled trials that assessed the effects of TZDs on BMD and bone turnover markers to determine whether bone loss, due to an uncoupling of bone formation and resorption, may account for the increased risk of fracture in patients taking TZDs. I also investigated whether the effect of TZDs on BMD and bone turnover markers varies depending on patient characteristics (age, gender, hormonal status, comorbidities), or intervention characteristics (type of TZD, dose, treatment duration), and whether withdrawal of therapy results in reversal of any TZD-induced changes in BMD.

5.2 METHODS

In January 2014, I searched PubMed, EMBASE, and Cochrane CENTRAL from inception, without limits, for randomized controlled trials of TZDs with BMD and/or bone turnover markers as an endpoint. The complete search strategy is shown in the Supplementary Appendix. I also searched three clinical trials registries (www.clinicaltrials.gov, www.controlled-trials.com/mrct and www.anzctr.org.au) for ongoing trials, and hand-searched the reference lists of identified articles and recent review articles for relevant studies. An updated literature search of all databases was performed in January 2015.

Studies were included if they were randomized controlled trials carried out in adults aged ≥18 years that compared treatment with a TZD to a control group that received placebo, metformin, or a sulfonylurea and reported changes in BMD or bone turnover markers during the intervention period. I screened all titles and abstracts to identify potentially eligible studies. The full text of these studies was reviewed independently by myself and another investigator (Mark Bolland) to determine whether inclusion criteria were met. The flow of articles is shown in Figure 5.1.

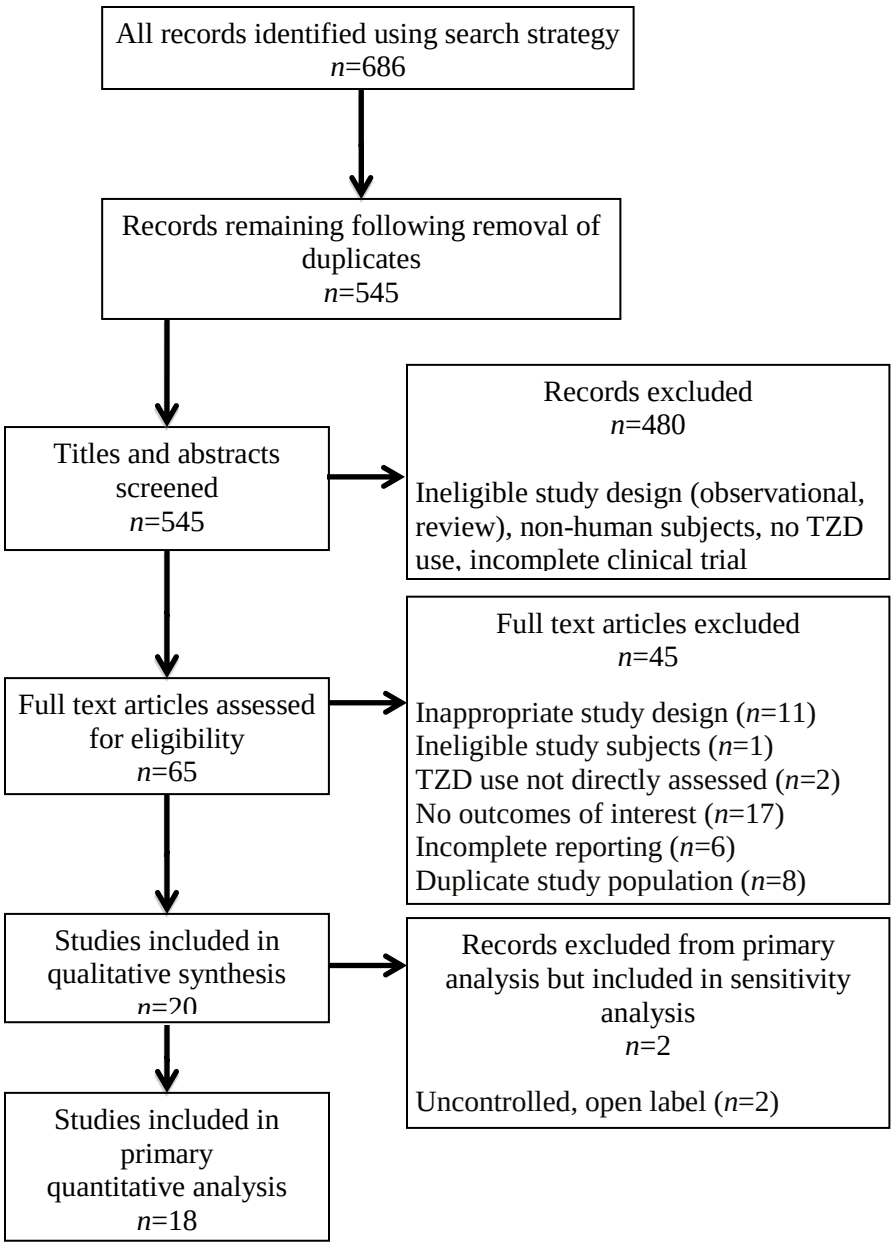


Figure 5.1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowsheet indicating process of selection of studies for inclusion in systematic review and meta-analysis. TZD = thiazolidinedione

Information extracted from each study included: participant characteristics, interventions, study design, outcome measures, funding source, and investigator conflicts of interest. Where necessary, data uncertainties were clarified with the study authors. I extracted data using a pre-specified extraction form, which was then checked by a second investigator (Mark Bolland). Any discrepancies were resolved through discussion. Risk of bias was assessed as recommended in the Cochrane Handbook and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [432]. The primary endpoints were the percentage change in BMD from baseline to the end of the intervention period at the lumbar spine, femoral neck, total hip, forearm, and total body. Secondary endpoints were percentage change in bone turnover markers (P1NP, CTX and bsALP) from baseline to the end of the intervention period, and percentage change in BMD and bone turnover markers following withdrawal of the intervention. Fractures were not assessed, as most studies designed to assess change in BMD were underpowered and of inadequate duration to assess fracture outcomes, and the risk of fractures with TZD use has been previously established in meta-analyses [418, 419]. Where data were presented in figures, we used digital callipers to extract data. For studies that presented absolute data rather than percentage change from baseline [427, 430, 433-436], we calculated the mean percentage change from the raw data and calculated the standard deviation (SD) of the percentage change using the approaches described in the Cochrane Handbook [437].

Data were pooled using random effects meta-analysis, and heterogeneity was assessed using the I^2 statistic (I^2 >50% was considered significant heterogeneity). Funnel plots and Egger's regression model were used to assess for the likelihood of publication bias. Sensitivity analyses were undertaken in pre-specified subgroups (gender, hormonal status, indication for TZD, type

of TZD, treatment dose, trial duration). All tests were two-tailed, and p-values <0.05 were considered statistically significant. Analyses were performed using Comprehensive Meta-Analysis (Version 2, Biostat, Englewood NJ).

5.3 RESULTS

5.3.1 Literature Search Results

Eighteen RCTs that met all the inclusion criteria were identified [389, 426-429, 431, 434-436, 438-446]. Two open-label studies, with a control group that received no treatment [430, 433], were included only in sensitivity analyses. In the initial search, I identified one completed study at www.ClinicalTrials.gov that met inclusion criteria, but no results had been reported [447]. Results were subsequently published on www.ClinicalTrials.gov, but neither BMD nor bone turnover marker outcomes were reported and these variables have been removed from the list of outcomes. Data was requested from the study contact, but they were unable to provide us with any study results. One conference abstract [448] was potentially eligible for inclusion but did not contain enough information for adequate data extraction and the study author was unable to be contacted to obtain further data.

5.3.2 Study Characteristics

The study design and selected baseline characteristics of the 18 RCTs in the primary analyses and the two open label studies included in a sensitivity analysis are summarized in Table 5.1. These 20 studies involved 3743 participants, of which 50% were female, and the mean age was

56 years. Baseline BMD, where reported, is shown in Table 5.2. Seven studies included only females and one only males; 12 studies involved persons with type 2 diabetes or IGT, one study patients with metabolic syndrome, four studies patients with HIV, one study women with polycystic ovarian syndrome, and two studies healthy postmenopausal women.

Rosiglitazone was studied in 12 trials and pioglitazone in 8. One multi-arm study administered pioglitazone in one arm and balaglitazone in the other two arms [442]. Results from these three TZD arms were pooled for the primary analyses. One study had a 2 x 2 factorial design, whereby one treatment arm consisted of rosiglitazone or placebo, and the other treatment arm consisted of metformin or placebo [440]. For the primary analyses, we compared the pooled 2 groups who received rosiglitazone with the pooled 2 groups who did not. The control group received placebo in 12 studies, metformin in four studies, metformin or placebo in one study, and metformin and glyburide in one study. Eight studies were ≤6 months, and 12 were >6 months. Three studies extended beyond 12 months, but none longer than 25 months [427, 430, 439]. Five studies included follow-up periods after withdrawal of TZD treatment, ranging from 24 to 52 weeks [426, 428, 429, 438, 441]. Fourteen studies reported BMD and 14 reported bone turnover marker outcomes.

Table 5.3 shows our assessment of the risk of bias- 16 trials were assessed as low risk of bias [389, 426-429, 435, 436, 438-446], two as moderate risk of bias [431, 434], and two as high risk of bias [430, 433]. Ten studies were conducted and/or funded by industry, and investigator conflicts of interest were reported for eight studies.

Table 5.1 Characteristics of randomized controlled trials assessing the effect of thiazolidinediones on bone mineral density and bone turnover markers in adults

Name	Trial length (wk)	Withdrawal duration (wk)	Location	Population	n	Mean age (y)	Sex (% F)	Mean BMI (kg/m^2)	Intervention (daily dose)	Control	Outcomes
Berberoglu[1], 2007	12	None	Turkey	DM2, PM	56	60	100	34	Rosi (4mg)	None	BTM
Grey, 2007	14	52	New Zealand	Healthy PM	50	68	100	27	Rosi (8mg)	Pl	BMD, BTM
Glintborg, 2008	16	None	Denmark	PCOS	30	33	100	34	Pio (30mg)	Pl	BMD, BTM
Schindler, 2009	26	None	Austria	HIV	44	46	8	23	Rosi (4mg)	Pl	BMD
Berberoglu[1], 2010	104	None	Turkey	DM2, PM	56	60	100	35	Rosi (4mg)	None	BMD, BTM
Gruntmanis, 2010	26	None	United States	DM2	150	56	41	34	Rosi (8mg)	Pl	BTM
Kanazawa,2010	52	None	Japan	DM2, PM	45	66	40	23	Pio (15-30mg)	Met	BMD, BTM
Tungsiripat, 2010	48	None	United States	HIV	78	50	17	26	Rosi (8mg)	Pl	BMD
Zinman, 2010	52	None	17 countries	DM2	1605	57	43	33	Rosi (8mg)	Met & G	BTM
Borges, 2011	80	None	9 countries	DM2	209	51	47	33	Rosi (8mg) & Met	Met	BMD, BTM
Harris, 2011	16	None	United States	HIV	70	44	39	28	Rosi (4mg)	Pl & Met	BMD, BTM
Harslof, 2011	14	26	Denmark	Healthy PM	57	65	100	27	Rosi (8mg)	Pl	BMD, BTM
Henriksen, 2011	26	None	Denmark, Sweden, Finland	DM2	409	61	38	34	Pio (45mg), Bala (10-20mg)	Pl	BMD, BTM
Ross, 2012	48	None	United States	HIV	71	50	17	26	Rosi (8mg)	Pl	BTM
van Lierop, 2012	24	None	Netherlands	DM2, men	71	56	0	29	Pio (30mg)	Met	BTM
Bilezikian, 2013	52	24	8 countries	DM2, PM	226	64	100	31	Rosi (8mg)	Met	BMD, BTM
Bone, 2013	52	26	United States	IFG, IGT, PM	156	60	100	16	Pio (45mg)	Pl	BMD, BTM
Bray, 2013	108[2]	None	United States	IGT	232	49	70	35	Pio (45mg)	Pl	BMD
Grey, 2014	52	52	New Zealand	DM2 or IGT	86	64	49	31	Pio (30mg)	Pl	BMD, BTM
Maalouf, 2014	52	None	United States	Met Synd.	42	53	64	33	Pio (45mg)	Pl	BMD

DM2 = type 2 diabetes, PM- post menopausal, IFG = impaired fasting glucose, IGT = impaired glucose tolerance, PCOS = polycystic ovarian syndrome, HIV = human immunodeficiency virus, Met Syndr= metabolic syndrome BMI = body mass index, Rosi= rosiglitazone, Pio= pioglitazone, Bala= balaglitazone, Met= metformin, Pl= Placebo, G= glyburide, BMD = bone mineral density, BTM = Bone turnover markers

[1]Open label studies in which the controls received no treatment; included in sensitivity analyses only

[2]Duration 108 weeks or until development of overt type 2 diabetes

Table 5.2 Baseline bone mineral density (BMD) of control and thiazolidinedione (TZD) groups

Study	Lumbar Spine (g/cm^2)		Femoral Neck (g/cm^2)		Total Hip (g/cm^2)		Forearm (g/cm^2)		Total Body (g/cm^2)	
	Control	**TZD**	**Control**	**TZD**	**Control**	**TZD**	**Control**	**TZD**	**Control**	**TZD**
Grey, 2007	1.13 (0.13)	1.18 (0.26)	-	-	0.96 (0.10)	0.99 (0.14)	-	-	-	-
Glintborg, 2008	1.13 (0.08)	1.14 (0.10)	0.92 (0.13)	0.97 (0.11)	1.04 (0.12)	1.08 (0.10)	-	-	-	-
Schindler, 2009	1.01 (0.10)	0.97 (0.12)	0.10 (0.80)	0.75 (0.10)	0.95 (0.09)	0.90 (0.13)	-	-	-	-
Berberoglu, 2010	0.95 (0.1)	1.01 (0.1)	0.81 (0.1)	0.84 (0.1)	0.97 (0.1)	0.10 (0.1)	-	-	-	-
Kanazawa, 2010	0.91 (0.14)	1.07 (0.19)	0.68 (0.11)	0.73 (0.12)	-	-	0.60 (0.09)	0.90 (0.13)	-	-
Tungsiripat, 2010	-	-	-	-	-	-	-	-	1.10 (0.10)	1.08 (0.11)
Borges, 2011	-	-	-	-	-	-	-	-	-	-
Harris, 2011	-	-	-	-	-	-	-	-	1.24 (0.12)	1.20 (0.07)
Harslof, 2011	0.96 (0.10)	0.99 (0.14)	0.74 (0.12)	0.76 (0.12)	0.88 (0.12	0.92 (0.14)	-	-	-	-
Henriksen, 2011	-	-	-	-	-	-	-	-	1.2 (0.13)	1.21 (0.13)
Bilezikian, 2013	-	-	-	-	-	-	-	-	-	-
Bone, 2013	1.04 (0.15)	1.01 (0.15)	0.80 (0.16)	0.81 (0.15)	0.92 (0.08)	0.93 (0.15)	0.66 (0.08)	0.66 (0.08)	0.98 (0.08)	0.96 (0.08)
Bray, 2013[1]	1.10 (1.54)	1.09 (1.32)	-	-	-	-	-	-	1.01 (0.83)	1.01 (0.84)
Grey, 2014	1.36 (0.24)	1.30 (0.18)	-	-	1.11 (0.12)	1.11 (0.15)	-	-	1.25 (0.10)	1.24 (0.11)
Maalouf, 2014	1.05 (0.14)	1.04 (0.15)	0.87 (0.12)	0.83 (0.09)	1.01 (0.11)	0.97 (0.11)	-	-	-	-

Data are reported as mean (SD)

[1]This study reported subtotal body BMD (head excluded)

Table 5.3 Risk of bias[1] in studies included in systematic review and meta-analysis

Study	Adequate Randomization	Appropriate Allocation Concealment	Blinding of participants	Blinding of outcome assessment	Incomplete Outcome Data	Significant Loss to Follow-up[2]	Possible Selective Reporting	Industry Funding	Investigator Conflict-of-Interest	Overall Risk of Bias
Berberoglu, 2007	NS	NS	N	N	N	NS	N	NS	NS	High
Grey, 2007	Y	Y	Y	Y	N	N	N	N	N	Low
Glintborg, 2008	NS	NS	Y	Y	N	NS	N	N	N	Low
Schindler, 2009	NS	NS	Y	Y	N	NS	N	Y	Y	Low
Berberoglu, 2010	NS	NS	N	N	N	Y	N	NS	NS	High
Gruntmanis, 2010	Y	NS	Y	Y	Y	Y	N	Y	Y	Moderate
Kanazawa, 2010	NS	NS	N	N	N	N	N	N	N	Moderate
Tungsiripat, 2010	Y	Y	Y	Y	N	N	N	Y	N	Low
Zinman, 2010	NS	NS	Y	Y	Y	NS	N	Y	Y	Low
Borges, 2011	NS	NS	Y	Y	N	NS	N	Y	Y	Low
Harris, 2011	NS	NS	Y	Y	N	NS	N	N	N	Low
Harslof, 2011	Y	Y	Y	Y	N	NS	N	N	N	Low
Henriksen, 2011	Y	Y	Y	Y	N	Y	N	N	Y	Low
Ross, 2012	Y	Y	Y	Y	N	N	N	Y	N	Low
van Lierop, 2012	Y	NS	Y	Y	N	N	N	Y	NS	Low
Bilezikian, 2013	Y	Y	Y	Y	N	Y	N	Y	Y	Low
Bone, 2013	Y	NS	Y	Y	N	Y	N	Y	Y	Low
Bray, 2013	NS	NS	Y	Y	N	NS	N	Y	N	Low
Grey, 2014	Y	Y	Y	Y	N	Y	N	N	N	Low
Maalouf, 2014	NS	NS	Y	Y	N	Y	N	N	Y	Low

Y = yes, N = no, NS = not stated

[1]Risk of bias was determined as recommended by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) and Cochrane Handbook for Systematic Reviews of Interventions

[2]Loss to follow-up considered significant if >20% of participants lost, or >10% discrepancy between control and intervention group

5.3.3 Changes in Bone Density

Fourteen studies (n=1734 patients) were included in the primary analyses [426-429, 434-436, 438-443, 445]. Figure 3.2 shows that TZDs decreased BMD compared with controls at the lumbar spine [difference -1.1% (95%CI: -1.6 to -0.7), p<0.0001], total hip [-1.0% (-1.4 to -0.6), p<0.0001], and forearm [-0.9% (-1.6 to -0.3), p=0.007]. There were statistically non-significant decreases in BMD at the femoral neck [-0.7%, (-1.3 to 0.0), p=0.06] and total body [-0.3%, (-0.5 to 0.0), p=0.07]. Inclusion of the open-label RCT [430] in a sensitivity analysis did not change the effect estimates although the femoral neck result became statistically significant (p=0.002). There was no evidence of publication bias for any of these outcomes based on visual inspection of funnel plots, and results from Egger's test.

Pre-specified sensitivity analyses in subgroups were performed to determine whether restricting the pooled analysis to studies with certain patient or intervention characteristics would alter our findings. The results of analyses restricted to women, postmenopausal women, cohorts with diabetes mellitus or IGT, RCTs of rosiglitazone, RCTs of pioglitazone, RCTs of low dose TZDs (average daily dose of ≤4mg rosiglitazone or ≤30mg pioglitazone), RCTs lasting ≤26 weeks, RCTs lasting >26 weeks, RCTs with metformin as a control, and RCTs of placebo as a control were all similar to the results for the primary analyses (results summarized in Tables 5.4 and 5.5). For this reason, and because most subgroups contained less than five studies, we did not formally

test for interaction between subgroups. There were insufficient studies to assess effects in men, or in cohorts with HIV.

Five studies reported on changes in BMD following withdrawal of TZD treatment for 24 to 52 weeks [426, 428, 429, 438, 441]. Figure 5.3 shows that there were no statistically significant differences in percentage change in BMD between the TZD group and controls following treatment withdrawal.

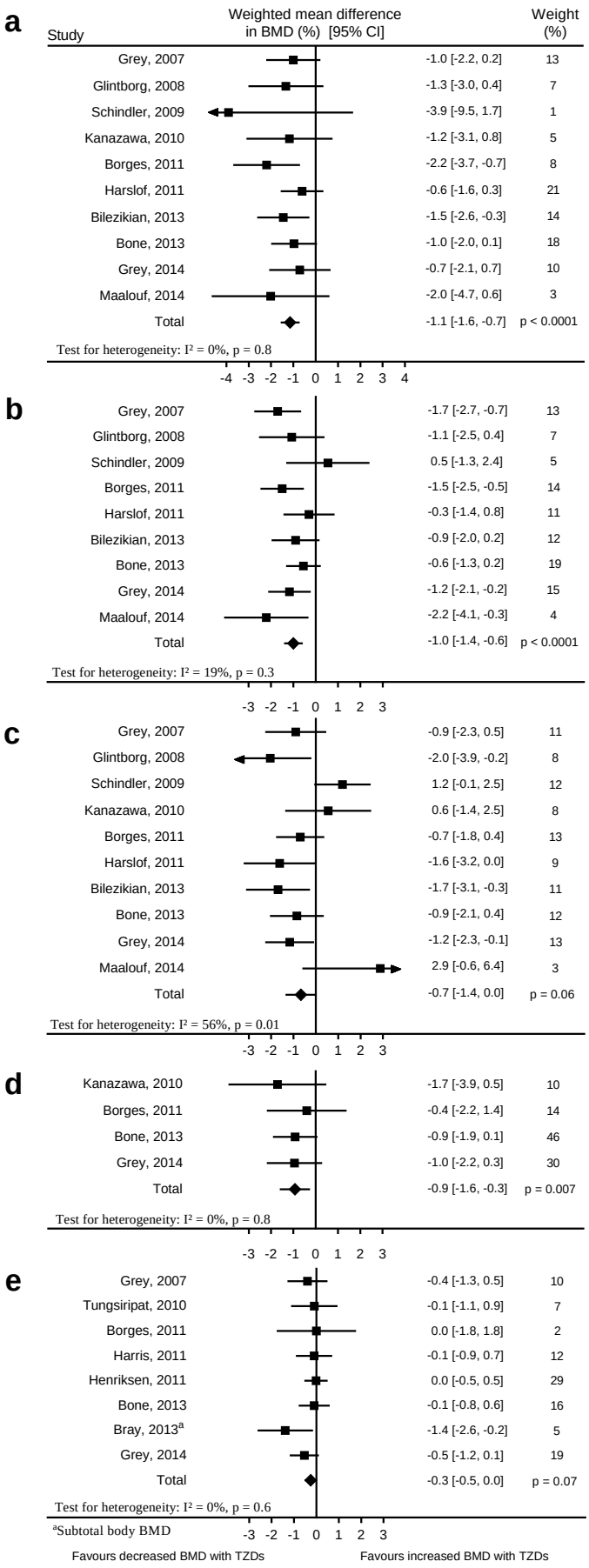


Figure 5.2 Meta-analyses of the effects of thiazolidinediones (TZDs) on the percentage change in bone mineral density (BMD) from baseline at five skeletal sites. (a) lumbar spine (b) total hip (c) femoral neck (d) forearm (e) total body

Table 5.4 Subgroup analysis of the effect of thiazolidinedione therapy on bone mineral density at five skeletal sites, stratified by patient characteristics

Site	% change in BMD [95% CI]					
				Postmenopausal		
	n	Women	n	Women	n	DM or IGT
Lumbar Spine	6	-1.1 [-1.6 to -0.7]	5	-1.1 [-1.6 to -0.6]	5	-1.3 [-1.8 to -0.7]
Total Hip	6	-0.9 [-1.4 to -0.5]	5	-0.8 [-1.3 to -0.4]	4	-1.0 [-1.4 to -0.5]
Femoral Neck	6	-1.2 [-1.8 to -0.6]	5	-1.2 [-1.8 to -0.5]	5	-0.9 [-1.5 to -0.3]
Forearm	2	-0.8 [-1.8 to 0.1]	2	-0.9 [-1.8 to 0.1]	4	-0.9 [-1.6 to -0.3]
Total Body	4	-0.5 [-1.2 to 0.2]	3	-0.2 [-0.8 to 0.3]	5	-0.3 [-0.6 to 0.1]

No significant heterogeneity between studies was present
DM = diabetes mellitus, IGT = impaired glucose tolerance

Table 5.5 Subgroup analysis of the effect of thiazolidinedione therapy on bone mineral density at five skeletal sites, stratified by study characteristics

Site	% change [95% CI]		% change [95% CI]		% change [95% CI]	
	n	Rosiglitazone	n	Pioglitazone	n	Low Dose[2]
Lumbar Spine	6	-1.2 [-1.7, -0.6]	4	-1.0 [-1.8, -0.3]	4	-1.0 [-2.0, -0.2]
Total Hip	5	-1.0 [-1.6, -0.3]	4	-0.9 [-1.5, -0.4]	3	-0.8 [-1.7, 0.1]
Femoral Neck	6	-0.5 [-1.5, 0.4][1]	4	-0.9 [-2.0, 0.3]	4	-0.3 [-1.8, 1.1][1]
Forearm	2	-0.9 [-2.3, 0.5]	2	-0.9 [-1.7, -0.2]	2	-1.1 [-2.2, -0.1]
Total Body	5	-0.2 [-0.6, 0.3]	4	-0.4 [-0.8, 0.0]	2	-0.4 [-0.-9, 0.1]
		Metformin Control		**Placebo Control**		
Lumbar Spine	3	-1.6 [-2.5, -0.8]	7	-0.9 [-1.4, -0.4]		
Total Hip	2	-1.2 [-1.9, -0.5]	7	-0.9 [-1.5, -0.4]		
Femoral Neck	3	-0.7 [-1.8, 0.3]	7	-0.6 [-1.6, 0.3][1]		
Forearm	2	-0.9 [-2.3, 0.5]	2	-0.9 [-1.7, -0.2]		
Total Body	2	-0.6 [-1.3, 0.0]	7	-0.3 [-0.6, 0.0]		
		Duration ≤26 weeks		**Duration >26 weeks**		
Lumbar Spine	4	-0.9 [-1.6, -0.2]	6	-1.3 [-1.9, -0.7]		
Total Hip	4	-0.8 [-1.7, 0.1]	5	-1.0 [-1.5, -0.6]		
Femoral Neck	4	-0.8 [-2.2, 0.7][1]	6	-0.8 [-1.4, -0.3]		
Forearm		-	4	-0.9 [-1.6, -0.3]		
Total Body	3	-0.1 [-0.5, 0.3]	5	-0.4 [-0.8, 0.0]		

[1]Significant heterogeneity between studies was present
[2]Low dose = average daily dose of ≤4mg rosiglitazone or ≤30mg pioglitazone in the treatment arm

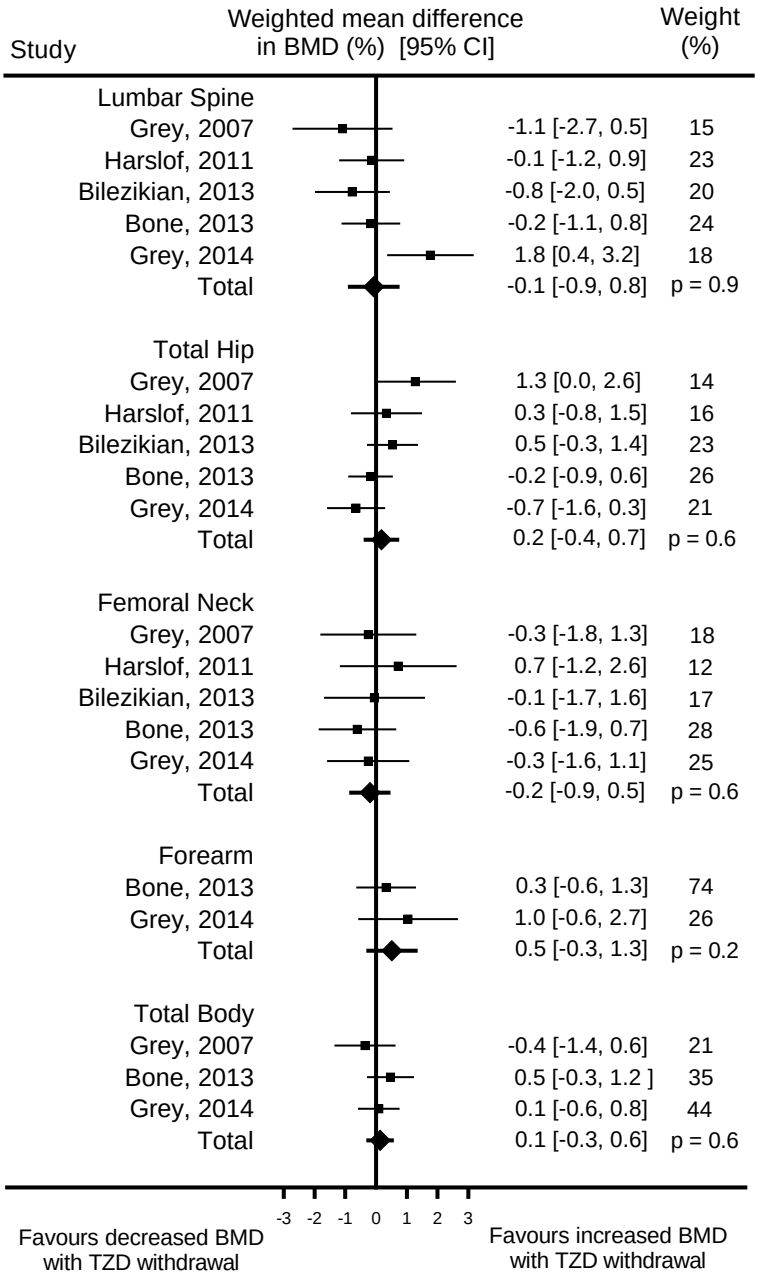


Figure 5.3 Meta-analysis of the effects of thiazolidinedione (TZD) cessation on the percentage change in bone mineral density (BMD) at five skeletal sites from the time of TZD withdrawal until the end of the follow-up period. No statistically significant heterogeneity was identified at any site.

5.3.4 Changes in Bone Turnover

Change in bone turnover markers is presented in Figure 5.4. Changes varied considerably between individual studies, and significant heterogeneity was observed for all turnover markers except osteocalcin. The pooled summary statistics [CTX difference 11.0% (95%CI 0.5, to 21.5), p= 0.04; bsALP 1.0% (-5.6 to 7.6), p= 0.8; P1NP 3.7% (-5.1 to 12.5), p= 0.4; osteocalcin -0.8% (-5.2 to 3.6), p= 0.7] may therefore not be generalizable. Of ten studies reporting on CTX, five reported significant increases with TZDs [389, 426, 431, 441, 446], and five showed no effect [427-429, 438, 444]. Of the five studies reporting on bsALP, one reported a significant increase with TZDs [426], and no effect was seen in four [389, 427, 438, 441]. Of the 11 studies reporting on P1NP, three reported significant increases with TZDs [426, 431, 446], two significant decreases [440, 444], and six no effect [389, 427-429, 438, 441]. Of the six studies that reported on osteocalcin, one demonstrated a significant decrease with TZDs [428], with five showing no effect [434, 436, 438, 441, 444]. Inclusion of the results of an open-label RCT [433] in a sensitivity analysis did not alter the findings. While statistically significant interactions between pre-specified subgroups were not formally assessed, heterogeneity between studies did not appear to be related to patient characteristics, type of TZD, TZD dose, type of control, or study duration.

Only two studies reported withdrawal data for markers of bone turnover [426, 438]. Pooling of these results did not identify significant changes in turnover markers following cessation of treatment with TZDs.

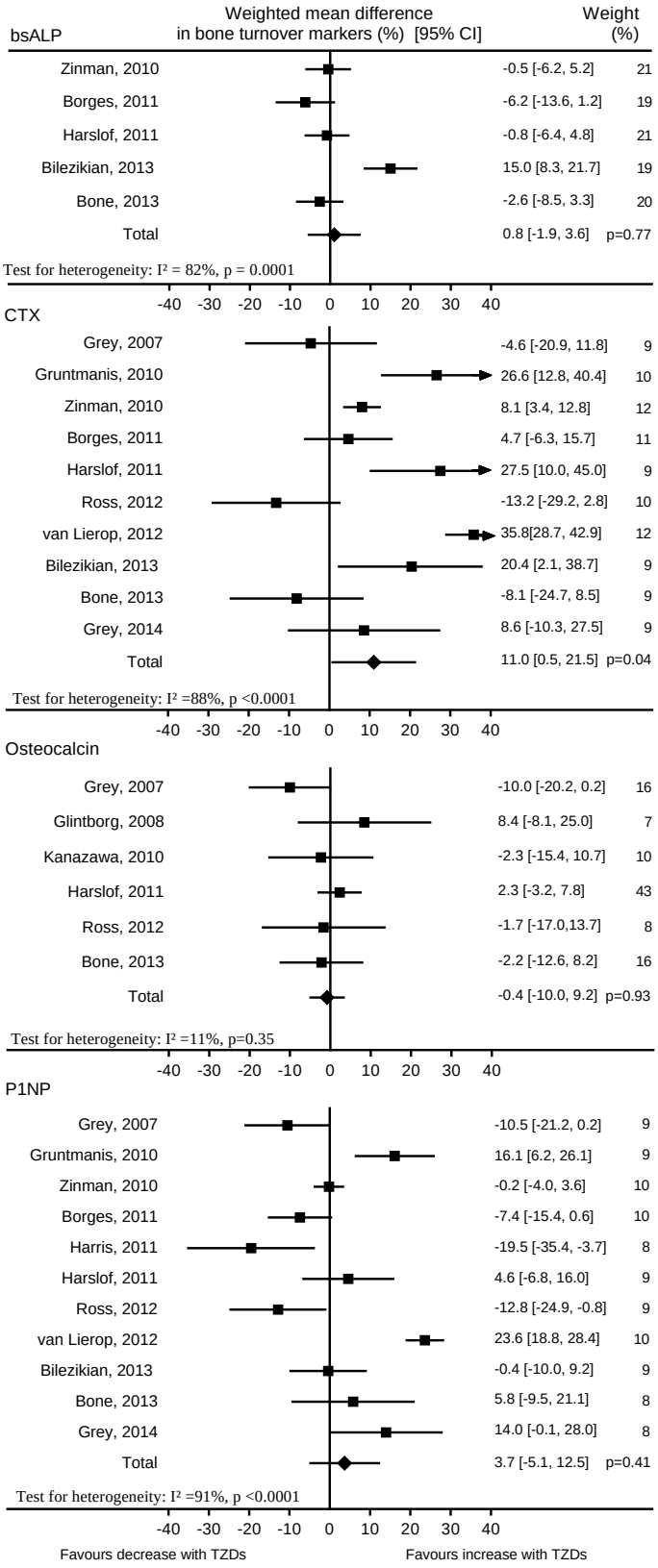


Figure 5.4 Meta-analyses of the effects of thiazolidinediones (TZDs) on the percentage change in bone turnover markers from baseline. bsALP = bone-specific alkaline phosphatase, CTX = β-C-terminal telopeptide of type I collagen, P1NP = procollagen type-I N-terminal propeptide.

5.4 DISCUSSION

In the meta-analyses presented in this chapter, treatment with TZDs for 3 to 24 months was found to reduce BMD at the lumbar spine, proximal femur, and forearm by 0.7-1.1% compared to placebo or metformin. After cessation of TZD therapy, there was no further loss of BMD, but there was also no regain of the earlier BMD loss. The decreases in BMD were consistent in different RCTs, across different patient populations, in RCTs of different TZD agents and doses, in RCTs with metformin or placebo as a control, and in trials lasting ≤6 or >6 months. By contrast, there was marked heterogeneity in changes of most bone turnover markers with TZDs.

The present analyses demonstrate that TZDs cause modest bone loss, an effect that is apparent by six months of treatment. An important clinical question is whether this bone loss persists with ongoing treatment. If there is little further BMD loss after one year of TZD treatment, the decreases in BMD of 0.7-1.1% observed in the meta-analyses are likely to be clinically insignificant, as they are much smaller than the normal variation of BMD in the population, and are equivalent to the average loss of BMD over one year in post-menopausal women. However, if BMD loss was cumulative at a rate of 1%/year, such decreases would be clinically important after five to ten years of treatment. Existing clinical trials are unable to provide a definitive answer to this question. While bone loss appeared similar in studies of ≤6 months and >6 months duration, the meta-analyses did not have sufficient power to detect small differences in BMD changes between these subgroups of trials, as only three studies extended beyond one year [427, 430, 439]. In

one of these extended studies, BMD loss at the lumbar spine was greater after 80 weeks than 56 weeks, arguing against a plateau [427]. Results of an observational cohort study by Schwartz and colleagues suggest that long-term treatment with TZDs may result in progressive bone loss. They found that persons who used TZDs for at least two years demonstrated progressive annual bone loss of -1.6% at the total hip and -1.1% at the whole body lumbar spine sub-region. In contrast, annual change in BMD amongst non-TZD users was -0.4% at the hip and +1.1% at the lumbar sub-region. However, this was not a controlled trial, and only 15 patients received more than two years of therapy, making these findings difficult to generalize [449]. Given the nature of type 2 diabetes as a chronic, life-long disease, many patients will be prescribed TZDs with the expectation of continuing treatment for at least five to ten years. Therefore, determining the long-term effects of TZDs on BMD is an important safety question that needs to be addressed with high priority.

The results of this study raise the possibility that TZDs may increase skeletal fragility by mechanisms other than decreasing BMD. The majority of included studies were underpowered and of inadequate duration to assess fracture outcomes, precluding assessment of the association between fracture risk and bone loss. However, results of the ADOPT trial suggest that the two-fold increased risk of fracture does not become evident until almost two years of treatment [176], and extrapolating the results from the present meta-analyses would suggest a 2% decrease in BMD by this time. This is unlikely to fully explain the changes in fracture risk seen in ADOPT and other RCTs [174-176]. The majority of fractures in patients taking TZDs in randomized trials occur at cortical sites

(humerus, distal forearm, tibia) [418, 419], but we did not observe a greater magnitude of bone loss at cortical sites than trabecular sites. Studies in both rodents and humans indicate that TZD exposure is associated with changes in cortical microarchitecture, which will not necessarily be evident on DXA. Animal studies have also demonstrated that TZD exposure results in increased cortical porosity and decreased cortical thickness, in the absence of significant changes in volumetric BMD [450, 451]. In women using TZDs, reduction in polar strength strain index at highly cortical sites has been observed [452]. Therefore, while TZD-mediated declines in BMD may result in an increased propensity for fracture, particularly if bone loss is sustained with long-term treatment, the excess distal extremity fractures observed in patients taking TZDs may be better explained by changes in microarchitecture at cortical sites.

Assessment of the effect of TZDs on bone turnover markers did not help to elucidate the mechanism by which these medications increase fracture risk. Change in bone turnover markers varied considerably between individual studies. While HIV, anti-retroviral treatment, diabetes, fluctuating glucose levels, and the postmenopausal state are all known to influence concentrations of turnover markers [212, 453, 454], no consistent changes in these markers were observed within the different study populations incorporated in this meta-analysis. These findings corroborate the results of animal studies, which have not demonstrated a consistent effect of TZDs on turnover markers [44]. In addition to being heterogeneous, changes in bone turnover markers did not correspond with the observed changes in BMD in this meta-analysis. Furthermore, several included studies that assessed both turnover markers and BMD did not observe a

correlation between the two [426, 427, 436, 442]. This is not particularly surprising, as the assessment of turnover marker concentrations is associated with considerable within-subject variability [212], and they do not always correspond with small changes in BMD [455]. Within the subgroup of the ADOPT trial in whom bone turnover markers were measured, Zinman *et al* did not observe differences amongst those who fractured and those who did not [389]. Therefore, turnover markers appear to have a limited role in the assessment of skeletal response to TZD therapy.

To my knowledge, there are no prior meta-analyses assessing the effect of TZD discontinuation on BMD. In the present analyses, BMD did not change significantly at any site following TZD withdrawal, suggesting that the BMD loss with TZD therapy is not reversible in the year following discontinuation. Although the mechanism by which TZDs act on bone is complex and not yet fully understood, these medications appear to have a direct effect upon differentiation of both osteoblasts and osteoclasts [46, 47, 424, 456]. PPAR-γ has been shown in preclinical studies to promote differentiation of mesenchymal stem cells into adipocytes rather than osteoblasts [45-47], and to promote osteoclast differentiation and increase osteoclast numbers [424, 456]. In addition, exposure to TZDs appears to induce osteoblast and osteocyte apoptosis [425, 457, 458]. A reduction in the number of osteocytes and osteoblasts has the potential for prolonged negative effects on bone, given the relatively long differentiation periods and lifespans of these cell types [32]. This may explain why there does not appear to be any regain of lost BMD following cessation of TZDs.

This meta-analysis has several limitations. Although 20 eligible RCTs were identified, there were not enough studies to carry out detailed subgroup analyses, and there were only three studies with outcome data beyond one year. There was marked heterogeneity amongst studies of bone turnover markers, and we were unable to draw conclusions regarding the effect of TZDs on turnover markers. Despite its limitations, this study has several strengths. It is the largest meta-analysis to evaluate the effect of TZDs on bone density and bone turnover markers, and the only one to assess the effect of TZD withdrawal on these parameters. Two previous meta-analyses have assessed the effect of TZDs on BMD [418, 459]. The earlier analysis identified two small RCTs published prior to June 2008 involving 95 participants [418]. The more recent analysis included seven studies [459]. Our meta-analysis provides important additional information by including more studies, previously unpublished data from some studies, more BMD sites, data from withdrawal studies, and data from bone turnover markers.

For physicians who treat patients with TZDs, these results raise an important safety flag. Longer-term studies assessing the effects of both TZD treatment and withdrawal on BMD are needed, together with a better understanding of the mechanism by which TZDs affect fracture risk. Until this information is available, it may be prudent for clinicians to periodically monitor BMD in older patients taking TZDs, and to consider avoiding these agents altogether in individuals at high risk of fracture, such as postmenopausal women with clinical risk factors for fracture.

CHAPTER 6:
COMPARISON OF FRAX AND GARVAN FRACTURE RISK CALCULATORS FOR MAKING TREATMENT DECISIONS IN OSTEOPOROSIS

6.1 INTRODUCTION

The primary clinical consequence of osteoporosis, fragility fracture, is associated with significant morbidity, mortality, and cost [2, 182]. Pharmacologic treatment of osteoporosis is associated with a 30-60% relative risk reduction in osteoporotic fracture rates, and is cost-effective when prescribed to individuals who have a high absolute fracture risk [182]. Treatment of individuals with a ten-year risk of hip fracture ≥3% is considered to reduce fracture incidence in a cost-effective manner [182]. Absolute fracture risk can be estimated using on-line calculators which integrate the effects of BMD and clinical risk factors. FRAX (http://www.shef.ac.uk/FRAX/) and Garvan (http://garvan.org.au/promotions/bone-fracture-risk/calculator/) calculators [13, 14, 202, 215, 226] are two of the most widely used, and are compared in Chapter 2 (see Table 2.1). They each use a different selection of risk factors, and clinicians using both sometimes find that hip fracture estimates are discrepant with respect to the suggested 3% treatment threshold.

Most prior comparisons of FRAX and Garvan have focused on average differences in risk estimates across patient groups [222, 225, 233], or on hypothetical patients [234, 235]. However, it is important for clinicians who use these calculators to understand how the differences between them apply to individual patients in a 'real world' context. Therefore, the research presented in this chapter aimed to determine which patient characteristics contribute to clinically relevant discrepancies between the two calculators

in a clinical setting. This was achieved by comparing hip fracture risk estimates with FRAX and Garvan in a cohort of postmenopausal women referred for fracture risk assessment.

6.2 METHODS

In this cross-sectional study, I reviewed the records of 122 consecutive women, aged 60 to 90 years, referred to our clinical service for BMD (GE-Lunar) assessments between July 1 and September 30, 2013. Height and weight were measured, and patients completed a questionnaire relating to clinical risk factors for fracture. The study was conducted in accordance with guidelines set out by the New Zealand Health and Disability Ethics Committee.

6.2.1 FRAX and Garvan Calculations

The ten-year estimated hip fracture risk for each patient was calculated using both FRAX and Garvan. The FRAX-UK was chosen to evaluate all Caucasian individuals, as it has been found to be better calibrated for the New Zealand population than FRAX-NZ [225]. In persons of Asian ethnicity who grew up in New Zealand, FRAX-US (Asian) was used. For those who had recently immigrated to New Zealand, the FRAX algorithm that best corresponded to their country of origin was used. Prior fractures were included in FRAX calculations if they had occurred in adult life (age ≥18) and resulted from trauma that would not have caused a fracture in a healthy person. Prior fractures were included in

Garvan calculations if they had occurred since the age 50 and did not result from major trauma (such as a car accident).

6.2.2 Comparison of Risk Estimates

The ten-year hip fracture risk estimates obtained with each calculator were compared. FRAX also estimates the ten-year risk of major osteoporotic fracture, while the Garvan calculator estimates the ten-year risk of fragility fracture at all skeletal sites, with the exception of the digits, skull, cervical spine and morphometric vertebral fractures. Since these outcomes do not include the same fractures, they were not compared.

6.2.3 Concordance Between FRAX and Garvan

Each woman was classified with respect to whether her ten-year hip fracture risk estimates were concordant or discordant around a threshold of 3%. Women were classified into the following groups: Concordant <3% (both Garvan and FRAX <3%), High Garvan (Garvan ≥3%, FRAX <3%), High FRAX (FRAX ≥3%, Garvan <3%), and Concordant ≥3% (both Garvan and FRAX ≥3%).

6.2.4 FRAX and Garvan Recalculations

To assess the effect of risk factors that are utilized by one calculator but not the other, I recalculated risk estimates without including calculator-specific risk factors. For example, if a woman had three falls within the past year, her Garvan risk was recalculated without including this risk factor. On the other hand, if a women had one fracture since age 50

(which is accounted for by both calculators), this risk factor was retained for the recalculations.

For women who initially had discordant risk estimates, I evaluated whether exclusion of calculator-specific risk factors resulted in estimates becoming concordant. If a women's ten-year hip fracture risk decreased from ≥3% to <3% following recalculations without a calculator-specific risk factor, then this risk factor was considered contributory to the woman's discordant result.

To determine whether discordance may be related to ethnicity, which is taken into account by FRAX but not Garvan, we identified all non-Caucasian women in the High Garvan group and recalculated their FRAX risk estimates using the FRAX-UK calculator in place of the ethnicity-specific FRAX calculator. If a woman's ten-year hip fracture risk with FRAX increased from <3% to ≥3% following this recalculation, then ethnicity was deemed contributory to her discordant result.

6.2.5 Statistical Analysis

Strength of association between FRAX and Garvan ten-year hip fracture risk estimates was determined using the Spearman correlation coefficients. Differences in median estimates between the two calculators were assessed using the paired Wilcoxon signed rank test. For comparison of patient characteristics between women with concordant and discordant results, Student's *t*-test was used for continuous variables and Fisher's exact test was used for binomial variables. Fisher's Exact test was used to assess whether

discordant results were more likely to occur in women with clinical risk factors for fracture, and Chi-squared tests were used to assess whether the proportion of women with discordant results varied by age group or femoral neck BMD strata. Trends across strata were assessed using Cochrane-Armitage tests.

Statistical analysis was done using SAS v9.4 (SAS Institute Inc, NC) and Prism v6.0 (GraphPad Software Inc, 2013). Figures were created with Prism v6.0. The threshold for statistical significance was set at a p-value <0.05.

6.3 RESULTS

Clinical characteristics of all 122 women are shown in Table 6.1. This group is typical of those referred for fracture risk assessment at our centre.

Table 6.1 Clinical Characteristics of all women assessed

Characteristic	**All Women** (n=122)
Age (y)	70.4 (7.4)
BMI (kg/m^2)	25.1 (4.6)
Caucasian	111 (90%)
Asian	11 (10%)
Fracture as an adult	49 (40%)
Any fracture ≥50y	41 (34%)
1 fracture ≥50y	23 (19%)
>1 fracture ≥50y	18 (15%)
2 fractures ≥50y	15 (12%)
≥3 fractures ≥50y	3 (23%)
Recent falls	23 (19%)
1 fall	13 (11%)
2 falls	6 (5%)
3 or more falls	4 (3%)
Parental hip fracture	13 (11%)
Current smoker	3 (3%)
Glucocorticoid use	10 (8%)
RA	1 (1%)
Secondary OP	19 (16%)
High alcohol intake	2 (2%)
FN BMD (g/cm^2)	0.82 (0.13)
LS BMD (g/cm^2)	1.05 (0.19)

Continuous variables reported as mean (standard deviation). Proportions reported as: number (%). BMI = body mass index, RA = rheumatoid arthritis, OP = osteoporosis, FN = femoral neck, LS = lumbar spine, BMD = bone mineral density

6.3.1 Comparison of Risk Estimates

Ten-year hip fracture estimates with FRAX and Garvan for each of the 122 women are shown in Figure 6.1. On average, Garvan estimates were higher than FRAX estimates, and the difference between the two calculators was greatest in women at the highest risk of fracture. Median (95% CI) estimated risk with Garvan was 4.7% (3.3-5.3) and 2.3% (1.9-2.9) with FRAX ($p<0.0001$ for difference). Correlation between the two calculators, done using Spearman correlation, was 0.87 ($p<0.0001$).

Median ten-year hip fracture risks with both Garvan and FRAX in women with calculator-specific risk factors for fracture are shown in Figure 6.2. Garvan estimates were three-fold higher in women with a history of recent falls ($p<0.0001$) and six-fold higher in women with more than one fracture since age 50 ($p<0.0001$). When women with FRAX-specific risk factors were considered, Garvan estimates generally remained higher than FRAX estimates.

Median ten-year hip fracture risk estimates with Garvan and FRAX, stratified by age and femoral neck T-score, are shown in Figure 6.3. Differences between the calculators were most pronounced at lower femoral neck BMD and higher age.

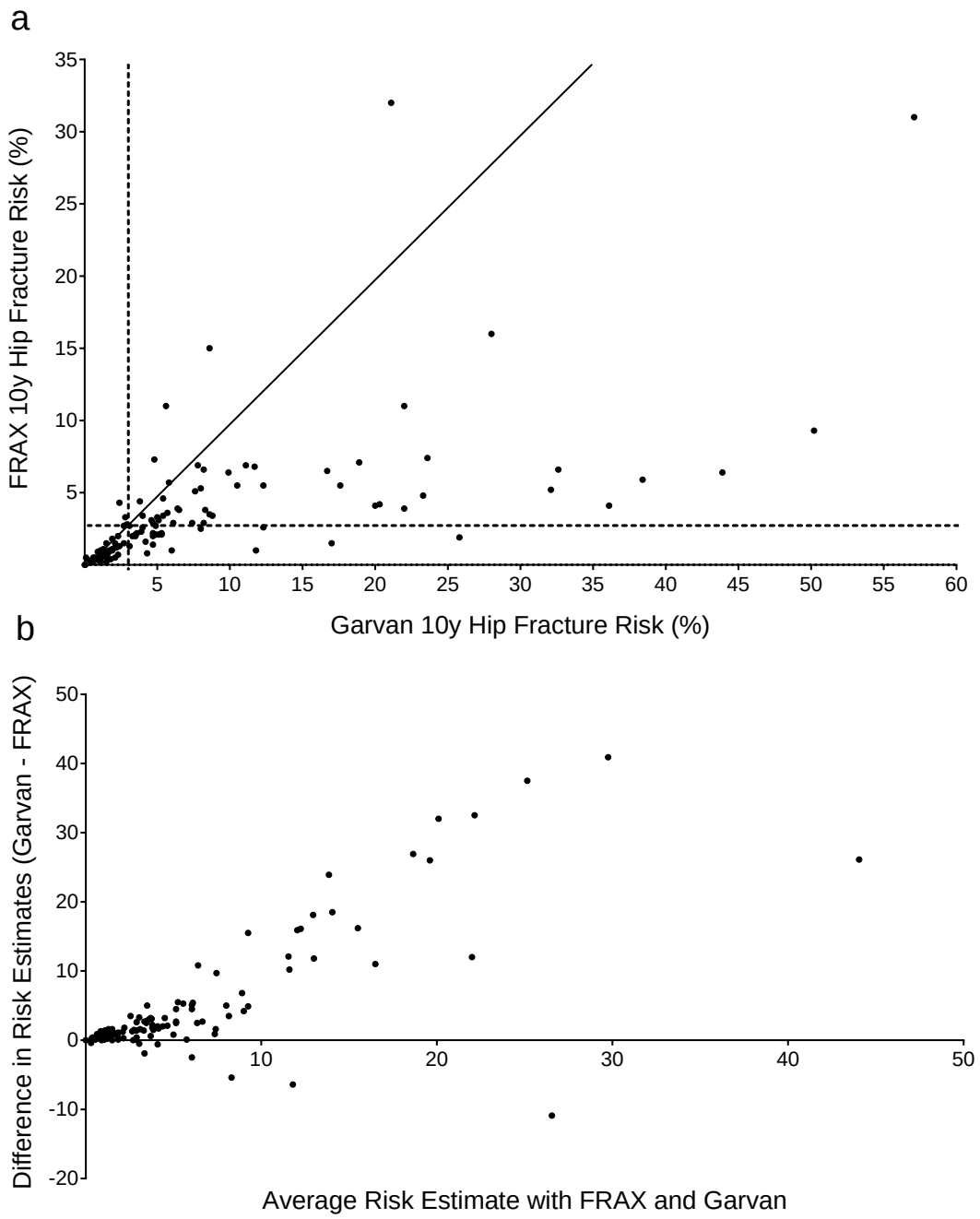


Figure 6.1 Comparison of estimated ten-year hip fracture risk with FRAX and Garvan for all women (n=122). In (a), FRAX estimates are plotted against Garvan estimates and the dotted lines represent the 3% treatment threshold, while the uninterrupted line is a line of equality (x=y). In (b), difference in risk estimates (Garvan - FRAX) is plotted against average risk estimate to create a Bland-Altman plot. In both (a) and (b), two concordant outliers (FRAX ≥3% and Garvan ≥3%) are outside the axis limits (Garvan=99.5%, FRAX=23%; Garvan=97.3%, FRAX=11%)

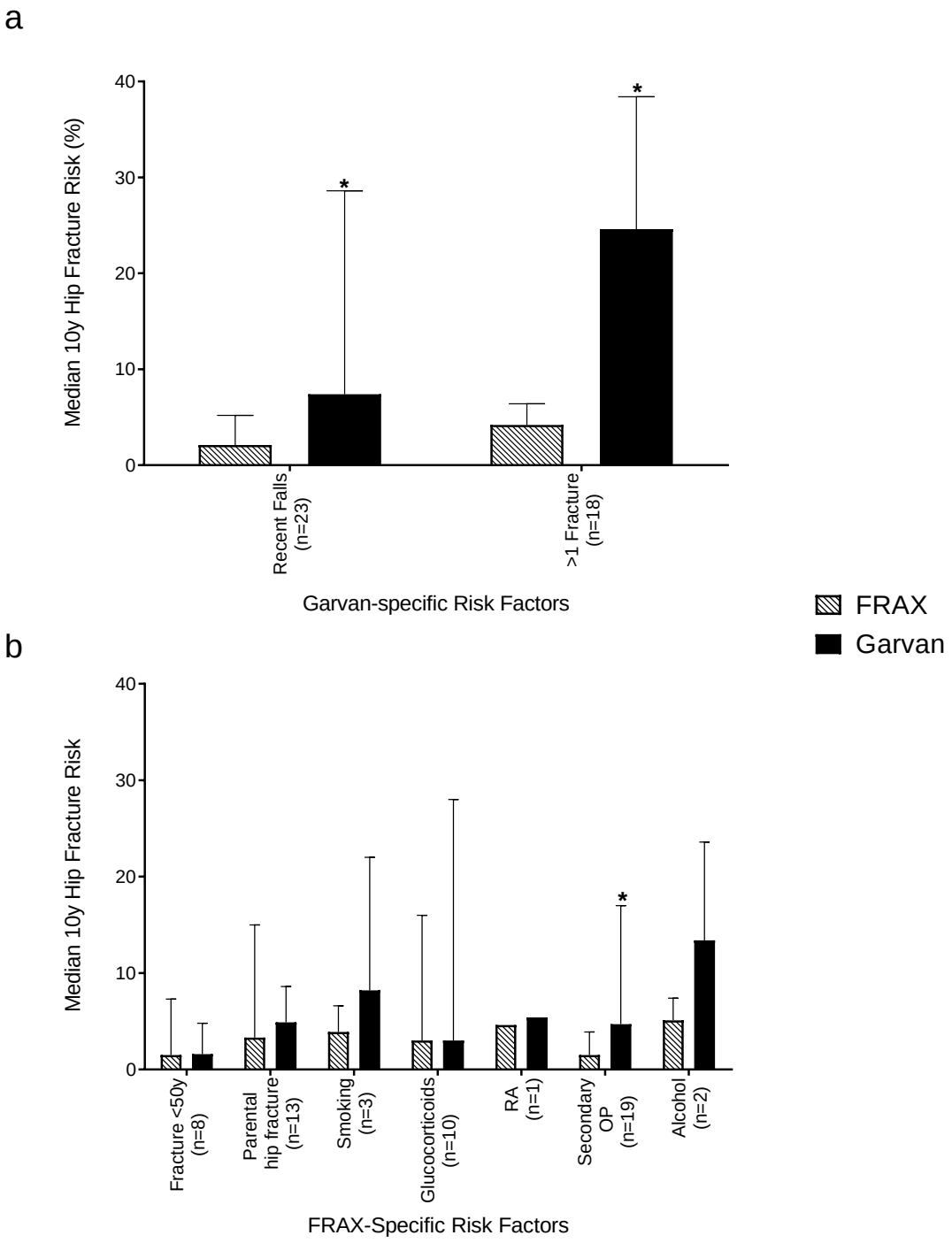


Figure 6.2 Median ten-year risk of hip fracture predicted by Garvan and FRAX, stratified by (a) presence of Garvan-specific risk factors for fracture, and (b) presence of FRAX-specific risk factors for fracture. Error bars represent upper 95% confidence intervals. *Denotes a significant difference in median estimates between the two calculators
RA = rheumatoid arthritis, OP = osteoporosis

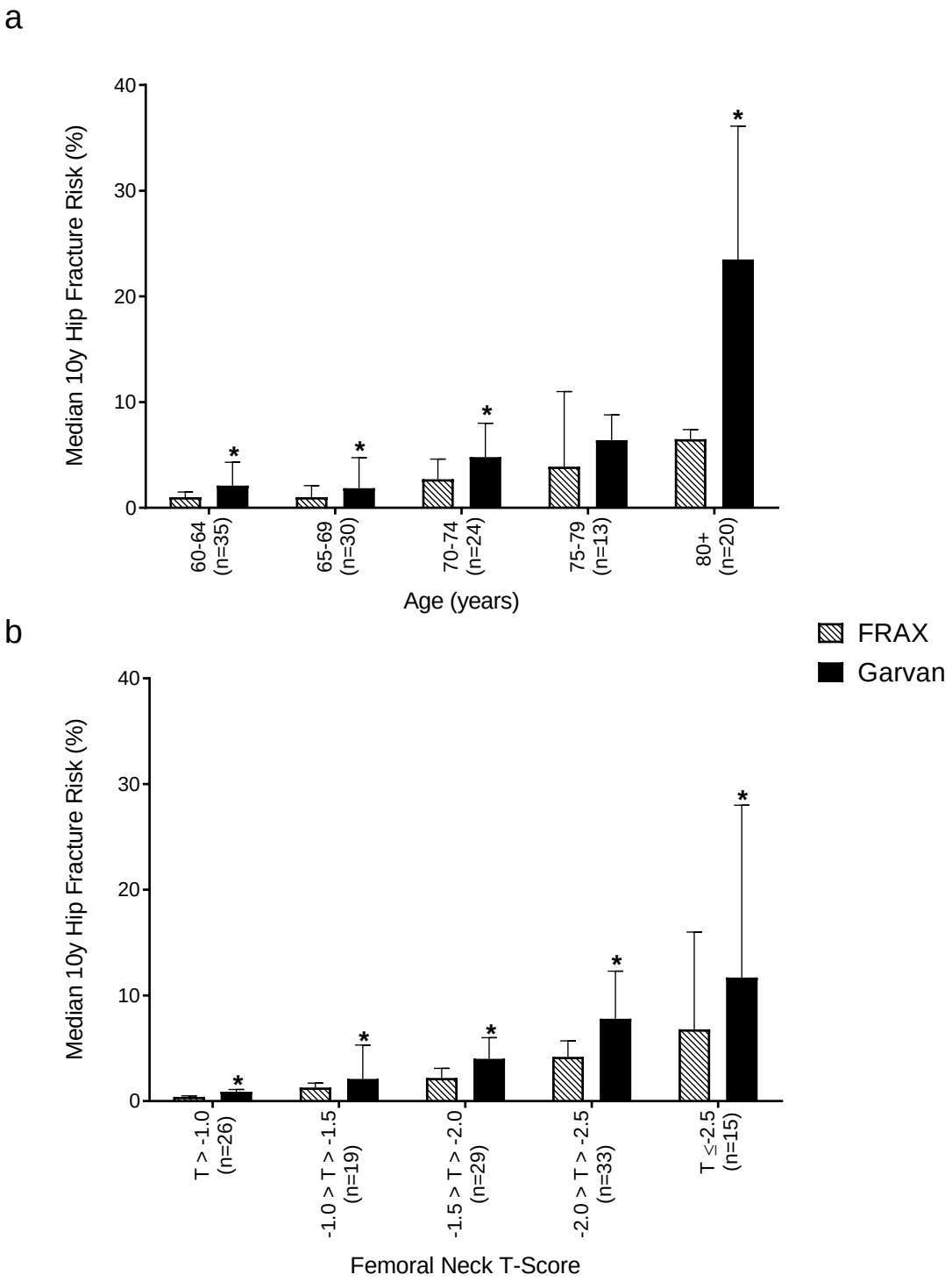


Figure 6.3 Median ten-year risk of hip fracture predicted by Garvan and FRAX, stratified by (a) age-group, and (b) femoral neck T-score. Error bars represent upper 95% confidence intervals.

*Denotes a significant difference in median estimates between the two calculators

6.3.2 Concordance between FRAX and Garvan

Concordant risk estimates were observed in 91 (75%) women (45 Concordant <3%; 46 Concordant ≥3%), and discordant risk estimates in 33 (25%) women (29 High Garvan; 2 High FRAX). Within the High Garvan group, median (95% CI) ten-year hip fracture risk was 4.7% (3.3-5.3) with Garvan and 2.1% (1.9-2.9) with FRAX. The two women in the High FRAX group had FRAX estimates of 4.3% and 3.3% and Garvan estimates of 2.4% and 2.8% respectively. Clinical characteristics of the women in each of these groups are presented in Table 6.2. The High Garvan group had more fractures and lower bone densities than those Concordant <3%, and they were younger than those Concordant ≥3%.

Discrepancies were very uncommon (4%) in women who had a Garvan estimate <3% or a FRAX estimate ≥3%, but occurred in 39% of the rest of the cohort. In most cases of discordance, the absolute difference in FRAX and Garvan estimates was small. In 18 women, the difference between estimates was ≤3% and in only six was it >6%.

Women with Garvan- or FRAX-specific clinical risk factors were no more likely to have discordant results than women who had no clinical risk factors. These data are summarized in Table 6.3. Twelve women of Asian ethnicity were included in the study. Discordant risk estimates were observed in 42% of Asian women and 24% of Caucasian women ($p = 0.06$).

The percentage of women with discordant results, stratified by age and femoral neck T-score, is shown in Figure 6.4. The highest proportion of discordant risk estimates was observed in women aged 70-74, and in women with femoral neck T-scores within the osteopenic range (-1.0 to -2.4). This is to be expected, as risk estimates for these groups (shown in Figure 6.3) tend to be near the intervention threshold.

Table 6.2 Comparison of women with concordant and discordant ten-year hip fracture risk estimates

Risk Factor	**Concordant <3%** (n=45)	**High Garvan[1]** (n=29)	**High FRAX[2]** (n=2)	**Concordant ≥3%** (n=46)
Age (y)	65.7 (4.2)	68.5 (5.0)[3,4]	70,72	76.2 (7.3)
BMI (kg/m^2)	25.8 (4.6)	24.3 (4.4)	19.2, 25.7	24.8 (4.6)
Caucasian	39 (87%)	24 (83%)[4]	2 (100%)	45 (98%)
Asian	6 (13%)	5 (17%)	0 (0%)	1 (2%)
Fracture as an adult	11 (24%)	12 (41%)	0 (0%)	26 (57%)
Any fracture ≥50y	4 (9%)	12 (41%)[3]	0 (0%)	25 (54%)
1 fracture ≥50y	3 (7%)	7 (24%)[3]	0 (0%)	13 (28%)
>1 fracture ≥50y	1 (2%)	5 (17%)[3]	0 (0%)	12 (26%)
2 fractures ≥50y	0 (0%)	4 (14%)[3]	0 (0%)	10 (22%)
≥3 fractures ≥50y	1 (2%)	1 (3%)	0 (0%)	2 (4%)
Recent falls	8 (18%)	7 (24%)	0 (0%)	8 (17%)
1 fall	5 (11%)	4 (14%)	0 (0%)	4 (9%)
2 falls	3 (7%)	2 (7%)	0 (0%)	1 (2%)
3 or more falls	0 (0%)	1 (3%)	0 (0%)	3 (7%)
Parental hip fracture	3 (7%)	3 (10%)	2 (100%)	5 (11%)
Current smoker	1 (2 %)	0 (0.0%)	0 (0%)	2 (4%)
Glucocorticoid use	5 (11%)	0 (0.0%)	0 (0%)	5 (11%)
RA	0 (0%)	0 (0.0%)	0 (0%)	1 (2%)
Secondary OP	8 (18%)	5 (17%)	1 (50%)	5 (11%)
High alcohol intake	0 (0%)	1 (3%)	0 (0%)	1 (2%)
FN BMD (g/cm^2)	0.90 (0.13)	0.78 (0.07)[3]	0.80, 0.84	0.72 (0.06)
LS BMD (g/cm^2)	1.13 (0.21)	0.98 (0.13)[3]	1.36, 1.18	1.02 (0.18)

[1]High Garvan group is those women with Garvan estimate ≥3% and FRAX estimate <3%
[2]High FRAX group is those women with FRAX estimate ≥3% and Garvan estimate <3%
[3]Significant difference between Concordant <3% group and High Garvan group ($p < 0.05$)
[4]Significant difference between Concordant ≥3% group and High Garvan group ($p < 0.05$)
Continuous variables reported as: mean (standard deviation). Proportions reported as: number (%). BMI = body mass index, RA = rheumatoid arthritis, OP = osteoporosis, FN = femoral neck, LS = lumbar spine, BMD = bone mineral density

Table 6.3 Prevalence of discordant risk estimates amongst women with and without clinical risk factors for fracture

Risk Factor	Number with risk factor	Number (%) with discordant estimates
No risk factors	50	12 (24%)
Garvan-specific risk factors		
Recent falls	23	7 (30%)
>1 fracture since age 50	18	5 (28%)
FRAX-specific risk factors		
Parental hip fracture	13	5 (38%)
Current smoker	3	0 (0%)
Glucocorticoid use	10	0 (0%)
RA	1	0 (0%)
Secondary OP	19	6 (32%)
High alcohol intake	2	1 (50%)
Fracture prior to age 50	8	0 (0%)

There were no statistically significant differences in proportions between the group of women with no risk factors and the groups of women with each calculator-specific risk factor
RA = rheumatoid arthritis, OP = osteoporosis, NS = non-significant

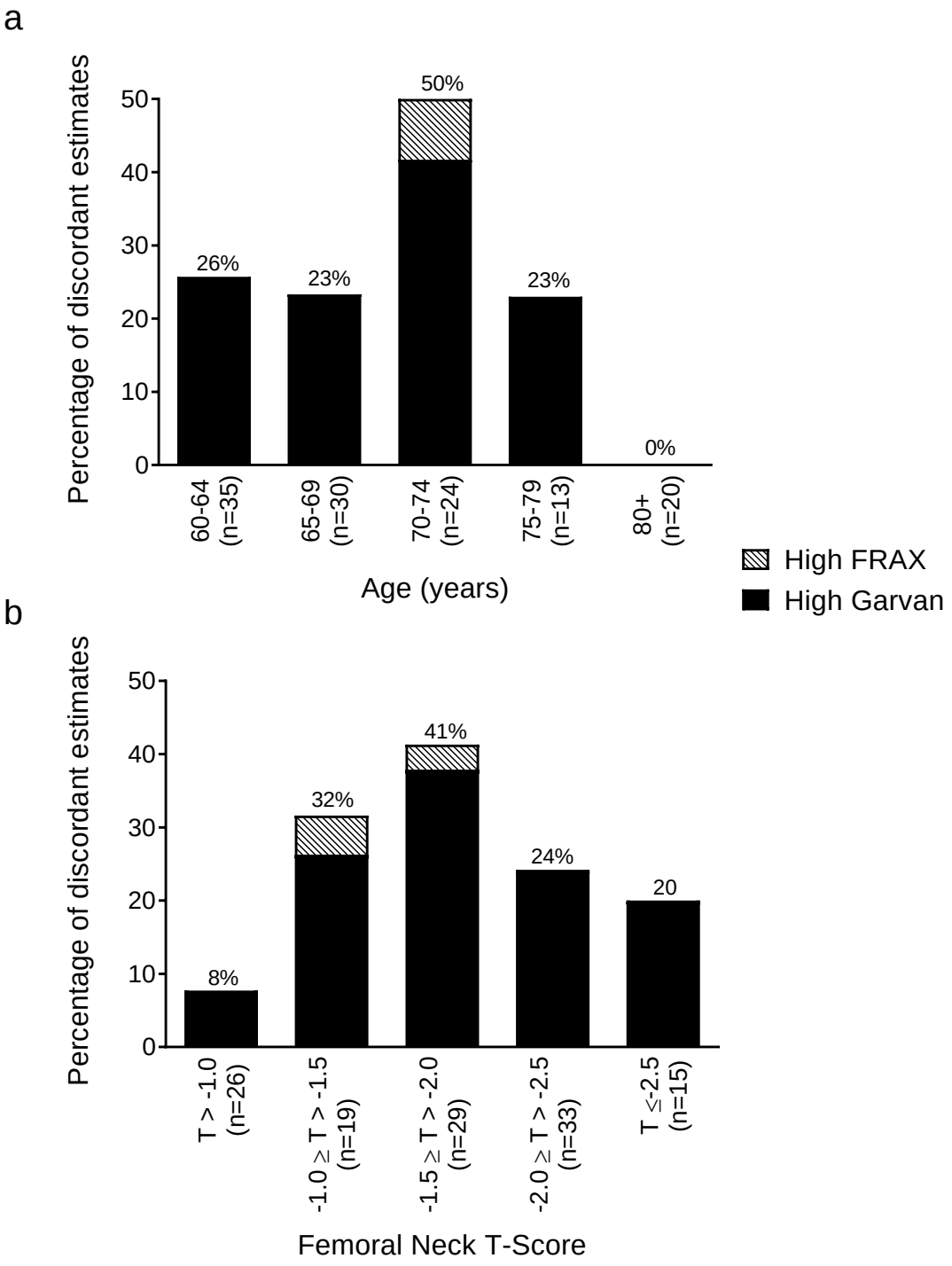


Figure 6.4 Frequency of discordant fracture risk estimates (Garvan ≥3% and FRAX <3%, or Garvan <3% and FRAX ≥3%) within each age group (a), or within each category of femoral neck T-score (b). The proportion of women with discordant estimates differed significantly across age groups (p=0.006) but not by T-score (p=0.07).

6.3.3 FRAX and Garvan Recalculations

Recalculation of ten-year hip fracture risk using the Garvan calculator without including Garvan-specific clinical risk factors (>1 fracture since age 50, falls within the past 12 months) resulted in a reduction in median (95% CI) calculated fracture risk for the entire cohort from 4.7% (3.5-5.3) to 4.0% (3.1-4.9) (Figure 4.5). In those who had Garvan-specific risk factors, the reduction was from 17.0% (4.9-23.6) to 5.0% (3.3-11.5). Similarly, removal of FRAX-specific risk factors from FRAX calculations resulted in a reduction in median (IQR) calculated fracture risk from 2.3% (1.9-2.9) to 2.1% (1.6-2.6) across the whole cohort (Figure 6.5), and from 2.7% (1.5-4.3) to 1.9% (1.1-2.7) in those who had these risk factors.

The effect of removing calculator-specific risk factors from calculations in women with discordant risk estimates is illustrated in Figure 6.6. Despite the substantial impact on fracture risk of removing Garvan-specific risk factors, these accounted for discrepancies around the 3% treatment threshold in only two of the 29 women in the High Garvan group. Ethnicity accounted for discrepancies in another two women in the High Garvan group. The FRAX-specific risk factor, history of parental hip fracture, accounted for the discrepancies in both women in the High FRAX group. Details of the women with discordant risk estimates are presented in Table 6.4.

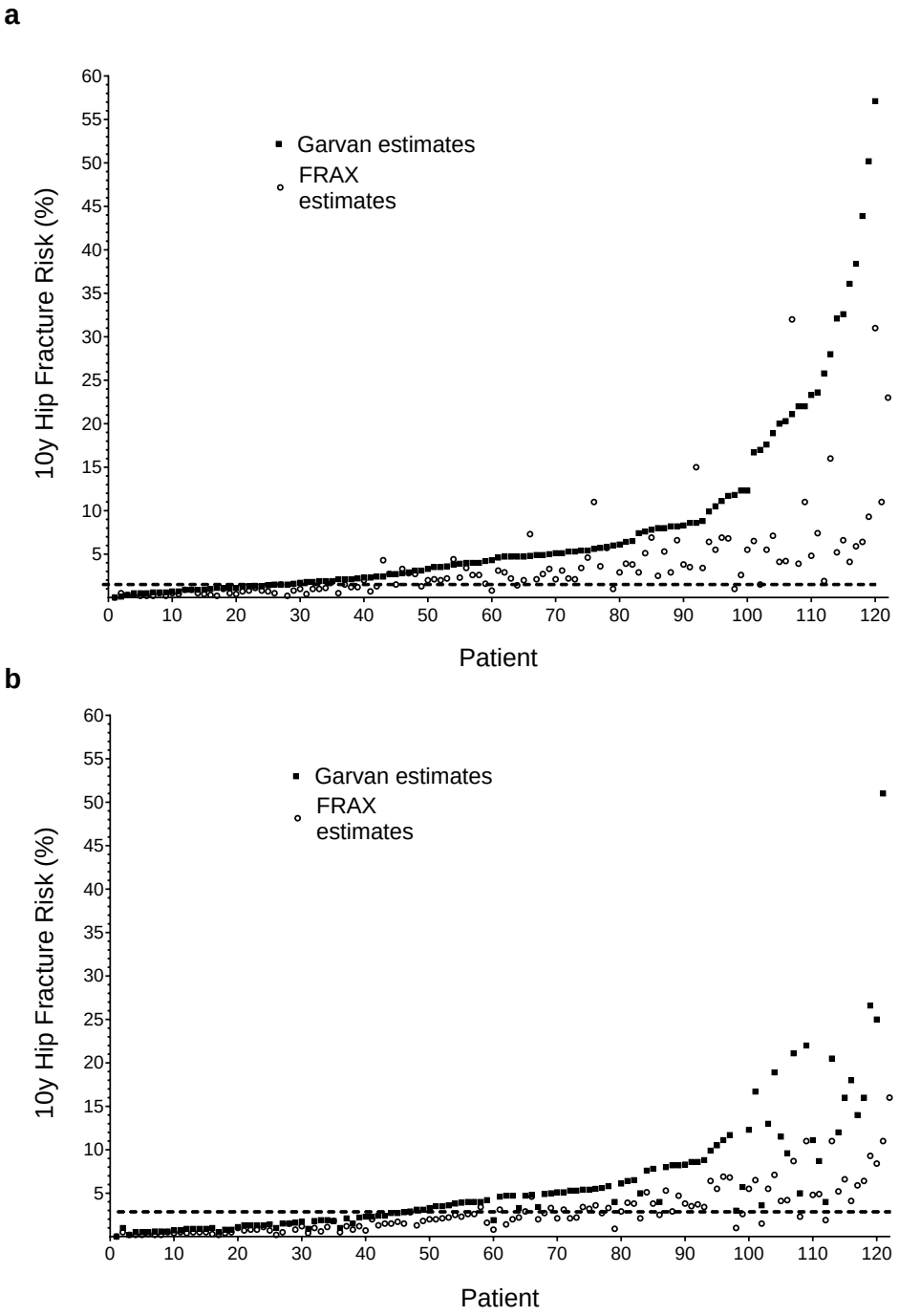


Figure 6.5 Ten-year risk of hip fracture estimated with both FRAX and Garvan in 122 postmenopausal women. Calculated risk estimates are shown in (a). Risk estimates recalculated with exclusion of calculator-specific risk factors are shown in (b). The interrupted line denotes the 3% treatment threshold. Two outliers are not shown (Outlier 1: Garvan=99.5%, FRAX=23% prior to recalculations, Garvan=65.3%, FRAX 16% after recalculations. Outlier 2: Garvan=97.3%, FRAX=11% prior to recalculations, Garvan=51.1%, FRAX 11% after recalculations)

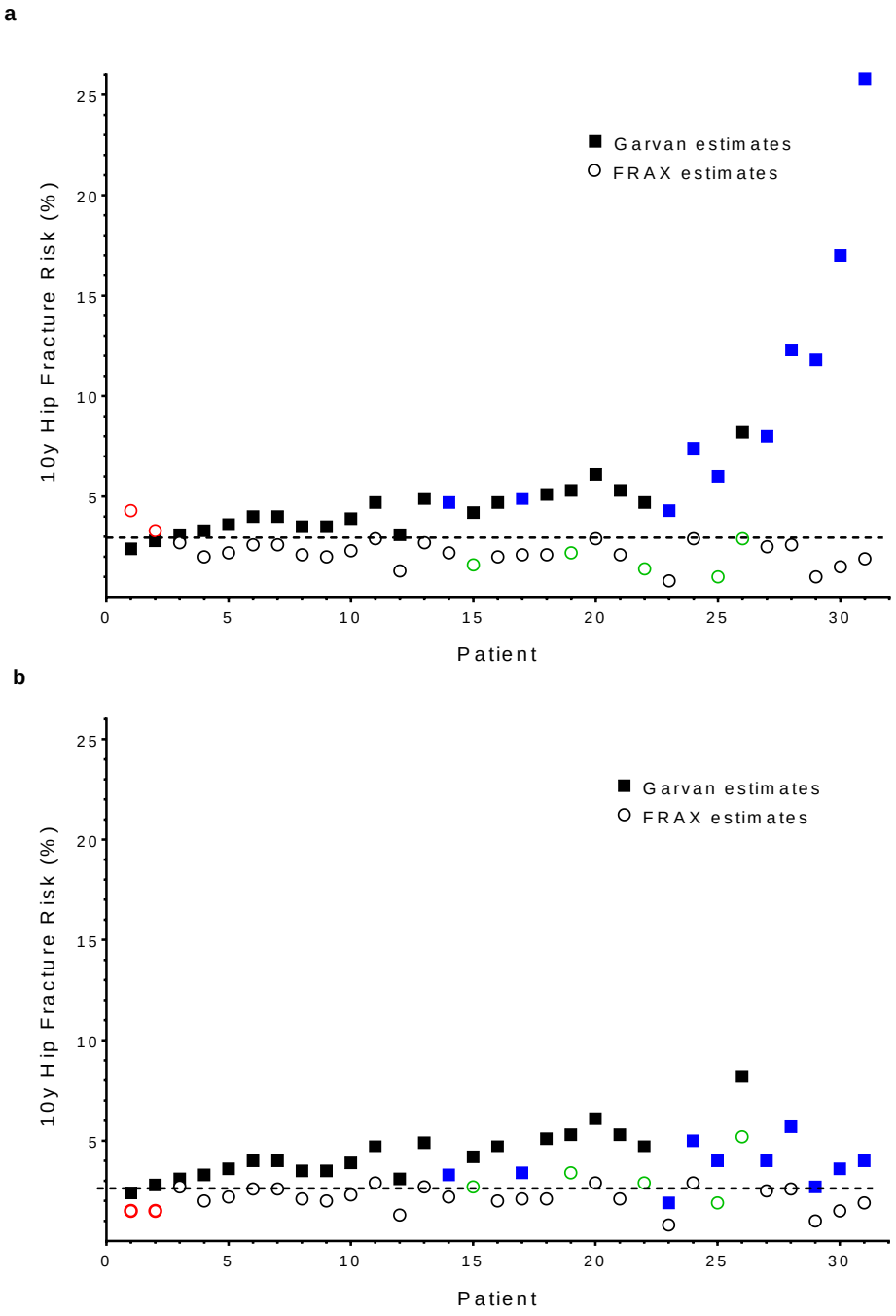


Figure 6.6 Ten-year risk of hip fracture estimated using both FRAX and Garvan in 31 women in whom these estimates were discordant. Calculated risk estimates are shown in (a). Risk estimates recalculated with exclusion of calculator-specific risk factors or the use of ethnicity-specific calculators are shown in (b). The interrupted line denotes the 3% treatment threshold. Women with FRAX-specific clinical risk factors are indicated in red, women with Garvan-specific risk factors are indicated in blue, and women of Asian ethnicity are indicated in green.

Table 6.4 Characteristics of women with discordant FRAX and Garvan ten-year hip fracture risk estimates

#	Age (y)	FN T-score	# ≥50y	FRAX risk (%)[1]	Garvan risk (%)[1]	Difference (Garvan – FRAX)	Garvan-specific RFs	Recalculated Garvan risk (%)[2]	Asian ethnicity	FRAX-UK risk (%)[3]	FRAX-specific RFs	Recalculated FRAX risk (%)[2]	Reason for discrepancy
1	72	-1.4		4.3	2.4	-1.9					Parental hip #, 2º OP	1.5	Parental hip #
2	70	-1.7		3.3	2.8	-0.5					Parental hip #, 2º OP	1.5	Parental hip #
3	71	-1.7		2.7	3.1	0.4					High alcohol intake	1.8	
4	71	-1.8		2.0	3.3	1.3							
5	70	-2		2.2	3.6	1.4							
6	68	-2.2		2.6	4.0	1.4							
7	76	-1.7		2.6	4.0	1.4							
8	73	-1.8		2.1	3.5	1.4							
9	66	-2.1		2.0	3.5	1.5							
10	64	-2.3		2.3	3.9	1.6							
11	63	-2.6		2.9	4.7	1.8					2º OP	2.9	
12	63	-1.3	Y	1.3	3.1	1.8							
13	70	-2.3		2.7	4.9	2.2							
14	73	-1.7		2.2	4.7	2.5	1 fall	3.3			2º OP	2.2	
15	71	-2.1		1.6	4.2	2.6			Y	2.7			
16	60	-1.9	Y	2.0	4.7	2.7							
17	62	-2.3		2.1	4.9	2.8	1 fall	3.4			Parental hip #	2.0	
18	65	-1.7	Y	2.1	5.1	3							
19	78	-1.9		2.2	5.3	3.1			Y	3.4			Ethnicity
20	76	-1.4	Y	2.9	6.1	3.2							
21	72	-1.4	Y	2.1	5.3	3.2					2º OP	2.1	
22	62	-2.6		1.4	4.7	3.3			Y	2.9			
23	64	-0.8	Y	0.8	4.3	3.5	2 #s	1.9					2 #s
24	67	-1.7	Y	2.9	7.4	4.5	1 fall	5.0			Parental hip #	2.1	
25	62	-1.7	Y	1.0	6.0	5	1 fall	4.0	Y	1.9	Parental hip #, 2º OP	0.9	
26	68	-2.9		2.9	8.2	5.3			Y	5.2			Ethnicity
27	73	-1.9		2.5	8.0	5.5	2 falls	4.0					
28	62	-2	Y	2.6	12.3	9.7	2 #s	5.7					
29	69	-0.8	Y	1.0	11.8	10.8	2 #s, 2 falls	2.7					2 #s, 2 falls
30	65	-1.4	Y	1.5	17.0	15.5	≥3 #s	3.6					
31	74	-1.1	Y	1.9	25.8	23.9	2 #s, ≥3 falls	4.0					

[1]Data are ten-year risk of hip fracture, [2]Risk estimate recalculated with the removal of any calculator-specific risk factors; [3]Risk estimate recalculated with FRAX-UK instead of FRAX-US(Asian). #=fracture, FN = femoral neck, RF = risk factor, 2º OP = secondary osteoporosis

6.4 DISCUSSION

In the present study, ten-year hip fracture risk estimates between FRAX and Garvan calculators were compared in a group of postmenopausal women, focusing on discrepancies around a treatment threshold of 3%. Garvan estimates tended to be higher than FRAX estimates. A history of falls or of multiple fractures increased risk calculated by Garvan 3- to 6-fold, but did not account for all of the difference between the calculators. Differences in fracture risk estimates were only clinically relevant in a quarter of women. Almost all women with discordant risk estimates had a Garvan estimate exceeding 3% and a FRAX estimate below 3%, but Garvan-specific risk factors only accounted for a small number of these discrepancies. Rather, most discrepancies occurred in women at borderline risk of fracture: those aged 70 to 74 years, those with osteopenic bone density, and those without calculator-specific risk factors for fracture. These findings provide reassurance that while FRAX and Garvan incorporate different sets of clinical risk factors, in the majority of women the recommendation relating to treatment will be the same. The two calculators often agree around the 3% treatment threshold, and the majority of differences which straddle this threshold are of small magnitude.

Similar to previous comparisons of FRAX and Garvan, these results indicate that Garvan risk estimates are higher than FRAX risk estimates, with absolute differences between the calculators being most marked in older women and in those at highest risk of fracture [222, 225, 233]. In terms of clinically relevant differences between the two calculators, FRAX and Garvan produced concordant risk estimates in 75% (95% CI: 66-81%) of women, comparable to the 80% that Pluskiewitcz *et al* have reported in a Polish population [233]. In the current study, discordance was very uncommon in women with Garvan estimates <3% or FRAX estimates ≥3%, occurring in only 4% (95% CI: 1-15%). However, discordance was much

more likely in women with Garvan estimates ≥3% or FRAX estimates <3%, occurring in 39% (95% CI: 28-50%). Given that Garvan tends to overestimate fracture risk while FRAX often underestimates it [222, 225, 460], this finding is unsurprising.

As anticipated, it was observed that Garvan ten-year hip fracture risk estimates were several-fold higher than FRAX estimates in women with Garvan-specific clinical risk factors. However, when the treatment threshold of ≥3% was considered, women with Garvan-specific clinical risk factors were no more likely to have discordant risk estimates than women who did not have these risk factors. Furthermore, while the removal of Garvan-specific risk factors from Garvan calculations resulted in a considerable decrease in Garvan estimates in women with one or more of these risk factors, this only accounted for discordance in two of the 29 women who had Garvan estimates ≥3% and FRAX estimates <3%. Therefore, while a history of recent falls or numerous prior fractures can significantly alter an individual's Garvan risk estimate, these changes are not often clinically relevant, since most women with these risk factors will have a high risk of fracture regardless of whether these risk factors are taken into account.

This study also considered the relationship between FRAX-specific clinical risk factors and differences in FRAX and Garvan risk estimates. Calculations in hypothetical patients have demonstrated that addition of FRAX-specific clinical risk factors to FRAX calculations results in more modest changes in estimated fracture risk than the addition of Garvan-specific risk factors to Garvan calculations [234]. In keeping with this, I found that Garvan estimates tended to be higher than FRAX estimates, even in women with FRAX-specific risk factors and without Garvan-specific risk factors. However, one FRAX-specific risk factor was found to have clinical relevance within our study population: history of parental hip fracture. The

two women with FRAX estimates ≥3% and Garvan estimates <3% each had a history of parental hip fracture. Removal of this risk factor from FRAX calculations dropped risk estimates from 4.3 to 1.5% in one woman and 3.3 to 1.5% in the other, indicating that this risk factor is influential. A history of parental hip fracture has a larger impact on FRAX risk estimates in women of older age [461].

The presence of country- and ethnicity-specific FRAX calculators was also considered as a possible contributor to discordant risk estimates. FRAX adjusts risk estimates to account for variation in life-expectancy and fracture rates in different countries. In the FRAX-US algorithms, ethnicity can also be factored in, and women of Asian ethnicity are assigned lower risk estimates than Caucasian women [461]. Garvan does not account for geographic location or ethnicity, and although this calculator performs well in Canadian, New Zealand and Norwegian populations [224, 225, 232], it has not yet been validated in a non-Caucasian population. In the present study, two of five Asian women with discordant results were reclassified as having concordant results when FRAX-UK was used to estimate ten-year hip fracture risk instead of FRAX-US(Asian). This indicates that differential handling of geographic location and ethnicity by the two calculators can account for discordant risk estimates, in some cases.

Twenty-five women had discordant risk estimates that were not explained by calculator-specific risk factors or ethnicity. The majority of these women had hip fracture risk estimates that were close to the intervention threshold (i.e. between 2% and 5%) with both calculators. Not surprisingly, discordant risk estimates were most frequent in populations likely to have borderline fracture risk: women aged 70 to 74 years, and women with osteopenia at the femoral neck. Older individuals tended to have more pronounced differences in Garvan and

FRAX estimates, possibly as a result of incorporation of competing mortality by the FRAX calculator [221, 233], but both estimates exceeded 3% in most cases and so these differences were rarely clinically relevant. The same was true for individuals with femoral neck T-scores in the osteoporotic range. In younger individuals and those with normal bone density, FRAX and Garvan estimates were usually both below the treatment threshold.

Certain limitations should be considered when interpreting the results of this study. Our sample included only one woman with rheumatoid arthritis, two with high alcohol consumption, and three current smokers. As a result of these small numbers, it is possible that we have underestimated the contribution of these FRAX-specific clinical risk factors towards discordance between FRAX and Garvan. However, a strength of this sample size is that it allowed us to dissect the contributions of individual risk factors to risk estimates, which is not feasible in larger studies. Classifying patients around an intervention threshold does not take into account the imprecision of the fracture risk estimates. For example, estimates of 2.8% and 3.2% are unlikely to be meaningfully different from one another though they do lie on opposite sides of the threshold used here. However, this arbitrariness is part of the use of thresholds in clinical practice, so reflects the problems in the routine use of these tools. As the purpose of the present study was to explore the reasons for discrepancies between FRAX and Garvan, rather than to validate the two calculators, prospective follow-up was not undertaken. Therefore, we are unable to conclude which calculator is more accurate at predicting fracture in individuals with discrepant risk estimates. Previous validation studies indicate that FRAX tends to underestimate hip fracture risk, while Garvan tends to overestimate it [222, 225, 460]. However, these studies did not specifically focus on fracture outcomes in individuals with discrepant risk estimates, or in persons with calculator-specific risk factors. Further research is required to determine which calculator is most accurate at predicting fracture risk in these individuals."

In summary, the results of this study provide reassurance that although Garvan ten-year hip fracture risk estimates usually exceed those of FRAX, the majority of these differences do not impact on treatment recommendations when the 3% treatment threshold is considered. In particular, discrepancies are rare when the FRAX estimate is ≥3% or the Garvan estimate is <3%. The majority of women with discordant risk estimates will be at borderline risk of fracture by both calculators, and will not have calculator-specific risk factors for fracture. However, falls, multiple fractures, ethnicity and a history of parental hip fracture in an older person are sometimes influential. In patients classified at borderline risk with one calculator (particularly if FRAX <3% or Garvan ≥3%), and in those with calculator-specific risk factors, calculation of fracture risk with the alternate calculator should be considered. Concordant estimates ≥3% strengthen the argument for treatment, while concordant estimates <3% support an observational approach. Discordant estimates might serve two important purposes. They may serve to highlight the significance of a calculator-specific risk factor, particularly when the difference between estimates is large, or they may suggest that the rationale for therapy is equivocal, particularly when both estimates sit close to the intervention threshold. Finally, the major impact of falls on fracture risk that is demonstrated by the Garvan calculator is a reminder that this risk factor requires a separate evaluation and set of interventions from standard osteoporosis treatments.

CHAPTER 7:
THE EFFECTS OF CALCIUM SUPPLEMENTS ON BLOOD PRESSURE: A RANDOMIZED CROSSOVER TRIAL

7.1 INTRODUCTION

Calcium supplements have been widely recommended for the prevention and treatment of osteoporosis for the past several decades. However, as I reviewed in Chapter 2, it is now evident that calcium supplements have only small and inconsistent effects on bone density and fracture risk [260, 263], and are associated with several important adverse effects. In addition to gastrointestinal intolerance [272] and nephrolithiasis [271], calcium supplements have been associated with an increased risk of myocardial infarction and stroke. This was first suggested in the Auckland Calcium Study [274], and has been corroborated in meta-analyses of randomized controlled trials of calcium monotherapy [273] and calcium co-administered with vitamin D [275]. Evidence from observational studies of adverse cardiovascular effects of calcium supplements has been more mixed with some [277-279], but not other [280-282], studies reporting an association.

While the mechanism(s) by which calcium supplements affect the cardiovascular system is not yet known, it has been postulated that this relates to the acute increase in serum calcium that accompanies ingestion of a supplement. As indicated in Chapter 2, serum calcium levels increase by approximately one standard deviation or 0.1 mmol/L within two hours of ingestion of a 500-1000mg calcium supplement and remain increased for up to 12 hours [283-285]. A one standard deviation increase in serum calcium has been associated with an 8 to 20% increased risk of cardiovascular disease and death in observational studies [101], indicating that changes of this magnitude are likely to be clinically relevant.

One way by which excursions of serum calcium may increase the risk of cardiovascular events is through an effect on blood pressure. In observational studies, serum calcium concentrations correlate positively with blood pressure [81, 286, 287]. Correspondingly, the acute increases in serum calcium concentration that follow intravenous calcium infusion are accompanied by elevations of systolic blood pressure by about 10 mmHg [94, 95].

Members of our research group recently conducted a secondary analysis of a randomized controlled trial comparing the acute effects of a 1000mg calcium supplement with placebo on blood pressure in postmenopausal women. They found that blood pressure decreased over 8 hours in both the treatment and placebo groups [462], reflecting its diurnal rhythm. However, this blood pressure reduction was significantly attenuated after calcium, compared to placebo. The present study follows up this observation in a new randomized controlled crossover trial of calcium supplements in postmenopausal women, with blood pressure as a primary endpoint.

7.2 METHODS

7.2.1 Participants

Participants were 40 women who were at least five years postmenopausal. Women who had volunteered to participate in previous studies at the University of Auckland were contacted by letter and invited to take part. Women were excluded if they were using any of the following medications: calcium supplements (>100mg/day), glucocorticoids, antihypertensives, aspirin, warfarin, vitamin D (>50µg/day), or other bone-active

medications. Women who had used hormone replacement therapy within 12 months or bisphosphonate medications within two years were also ineligible to participate, as were current smokers, women with a major systemic illness, and women with a disease known to impact on bone. Written informed consent was obtained from all participants. This study was conducted in accordance with the Declaration of Helsinki and was approved by the New Zealand Health and Disability Ethics Committee. The study was registered with the Australia New Zealand Clinical Trials Registry (ACTRN12614000865617).

7.2.2 Study Design

This study is a randomized, double-blind, crossover trial, comparing the acute effects on blood pressure of 1000mg of calcium as citrate with placebo. Participants attended our research clinic at the University of Auckland on two occasions, separated by a washout period of at least seven days. The maximum time between visits was 41 days. The order of receiving calcium or placebo was randomized using a computer-generated variable-block schedule. Both calcium and placebo were administered as identical encapsulated powders. Participants and study staff were blinded for the duration of the study.

At the first visit, participants attended following an overnight fast. Baseline blood pressure was measured between 08:00 and 09:30 hours. Immediately following blood pressure measurement, a venous cannula was inserted and baseline blood samples were collected (the '0 hour' sampling point). The first treatment (calcium or placebo) was administered with a glass of water. Participants were provided with a light breakfast (toast with jam, marmalade or honey, tinned peaches in juice and decaffeinated coffee or tea) immediately following the

intervention. Blood pressure was measured at 2, 4 and 6 hours after the intervention. At each of these time points, blood samples were collected following blood pressure measurement. A light lunch (identical to breakfast) was provided after the 4 hour time-point. The second study visit was identical to the first, with the exception of the treatment (calcium or placebo) that was administered. Water consumption was *ad libitum* for the first visit, and participants were asked to replicate this intake at the second visit.

7.2.3 Measurements

Height was measured using a Harpenden stadiometer. Weight was measured using electronic scales. Ionised calcium was measured on anaerobically handled blood samples within 60 minutes of collection using an ABLFLEX 800 blood gas analyser (Radiometer, Bronshoj). Total calcium was batch-analysed at the end of each six hour session using a Cobas modular analyser (Roche Diagnostics, Basel). Blood pressure measurements were obtained with a Dinamap automatic monitor (Johnson & Johnson, Tampa). The blood pressure cuff was positioned on the right arm with the participant in a sitting position. Following a five minute rest interval, the device took three blood pressure measurements, each three minutes apart. We find that the first blood pressure measurement at each time point is higher than the subsequent two readings, so the mean of the second and third readings was used for analysis.

7.2.4 Statistical Analyses

Sample size was such that differences in blood pressure of at least 5mmHg systolic and 3mmHg diastolic could be detected with 80% power at the 5% significance level [462]. Analysis of the primary endpoint was done using mixed model repeated measures analysis of

covariance (ANCOVA), using the baseline level of blood pressure was as a covariate and unstructured covariance to construct an appropriate error term for each of the comparisons between treatment and control at each time point. As these comparisons were pre-planned, no adjustment for multiplicity was necessary. Analyses were performed in SAS (v9.4 SAS Institute Inc). All tests were two tailed and $P < 0.05$ was considered significant.

7.3 RESULTS

Flow of the participants through the study is presented in Figure 7.1. Three participants were excluded from the crossover analyses. The first was found to have primary hyperparathyroidism following the first study visit, and was excluded from further participation in the study. The second participant became acutely unwell when venous cannulation was attempted at the second visit and chose not complete the study. A third participant failed to fast for the second visit and was excluded. The baseline clinical and biochemical characteristics of participants are presented in Table 7.1.

Changes in serum ionized calcium and total calcium are presented in Figure 7.2. Ionized and total calcium progressively increased after calcium to 4 hours, and then remained stable to 6 hours. The changes were significantly greater after calcium compared with placebo ($p<0.0001$).

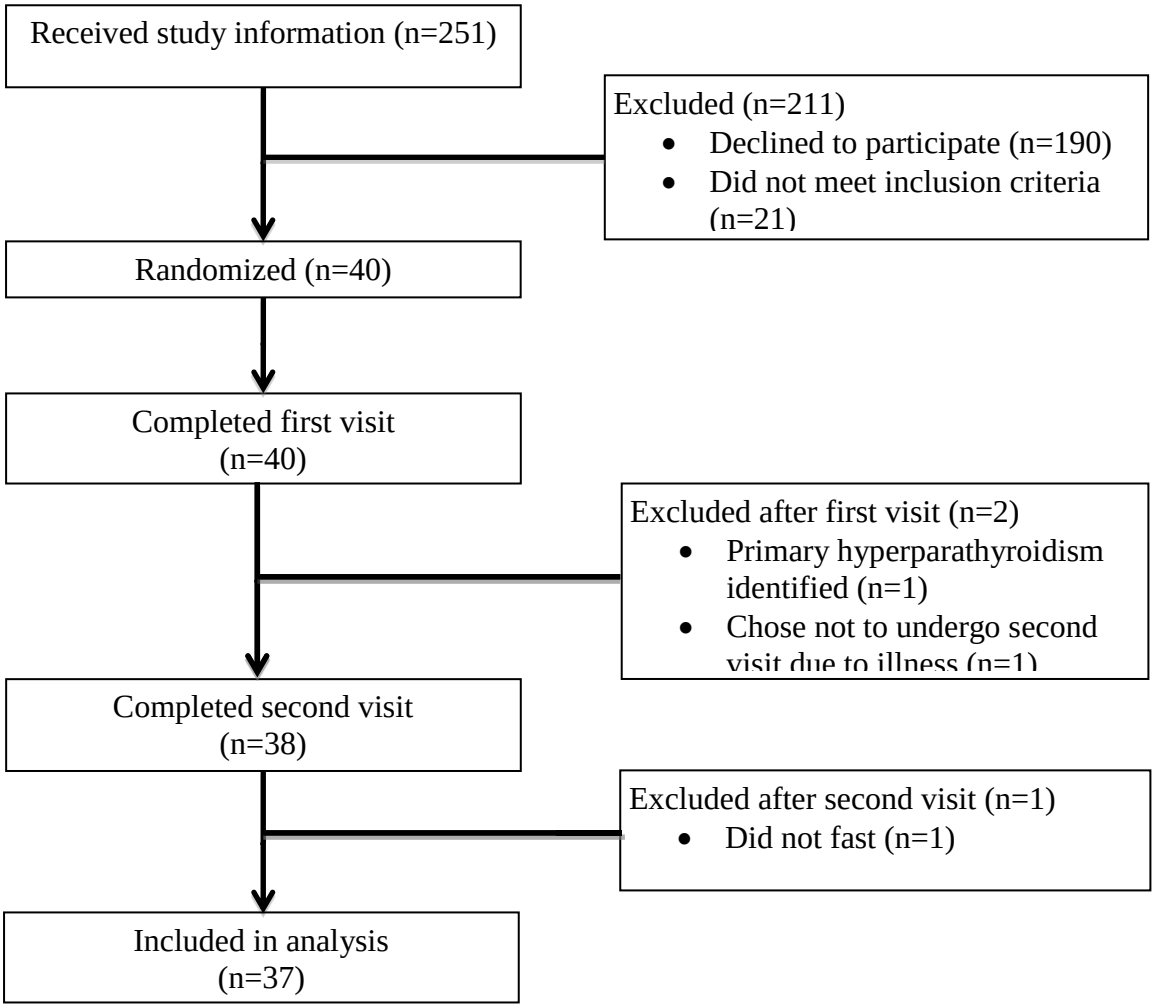


Figure 7.1 Flow of participants through the study.

Table 7.1 Baseline characteristics of participants

Characteristic	**Mean (SD)**
Age	71 (4)
Height (cm)	161 (6)
Weight (kg)	70.5 (12.2)
Body mass index (kg/m^2)	27.2 (5.0)
Dietary calcium (mg/day)	900 (400)
Serum ionised calcium (mmol/l)	1.23 (0.04)
Serum total calcium (mmol/l)	2.32 (0.08)
Systolic blood pressure (mmHg)	127 (19)
Diastolic blood pressure (mmHg)	70 (7)

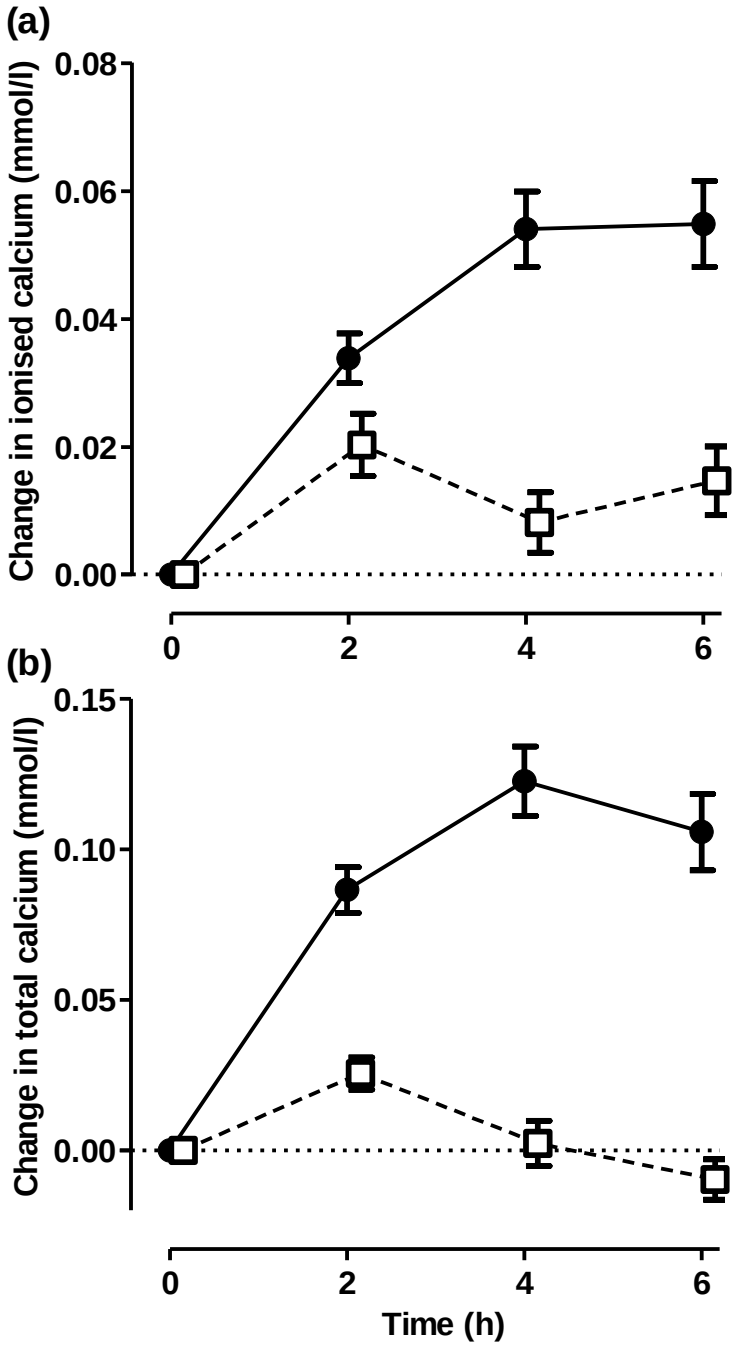


Figure 7.2 Changes in serum (a) ionized and (b) total calcium in postmenopausal women over 6 hours after the ingestion of 1000mg of calcium as citrate (•) or a placebo (□). Differences between the changes in ionized and total calcium over 6 hours after calcium and placebo were significant (ANOVA, $p<0.0001$). When individual time-points were examined, differences were significant at all time-points between 2 and 6 hours for ionized (all $p<0.007$) and total (all $p<0.0001$) calcium.

Changes in blood pressure are shown in Figure 7.3. Blood pressures declined after both calcium and placebo, particularly after the meals given at 0 and 4 hours, but these falls tended to be attenuated after calcium. Systolic blood pressure was lower than baseline between 2 and 6 hours after placebo ($p<0.0001$), and at 2 and 6 hours after calcium ($p<0.02$). The reduction in systolic blood pressure was smaller after calcium compared with placebo, and the difference between the interventions was significant (ANOVA, $p=0.016$).

Diastolic blood pressure followed a similar pattern, being lower than baseline between 2 and 6 hours after both placebo (all $p <0.0001$) and calcium (all $p <0.03$), with nadirs at 2 and 6 hours. While the reduction in diastolic blood pressure from baseline appeared to be less after calcium compared with placebo, differences between the treatments were smaller than for systolic pressure (1 to 2mmHg) and not significant (ANOVA, $p=0.33$).

There was no evidence of a carry-over effect for any parameter (all $p>0.05$).

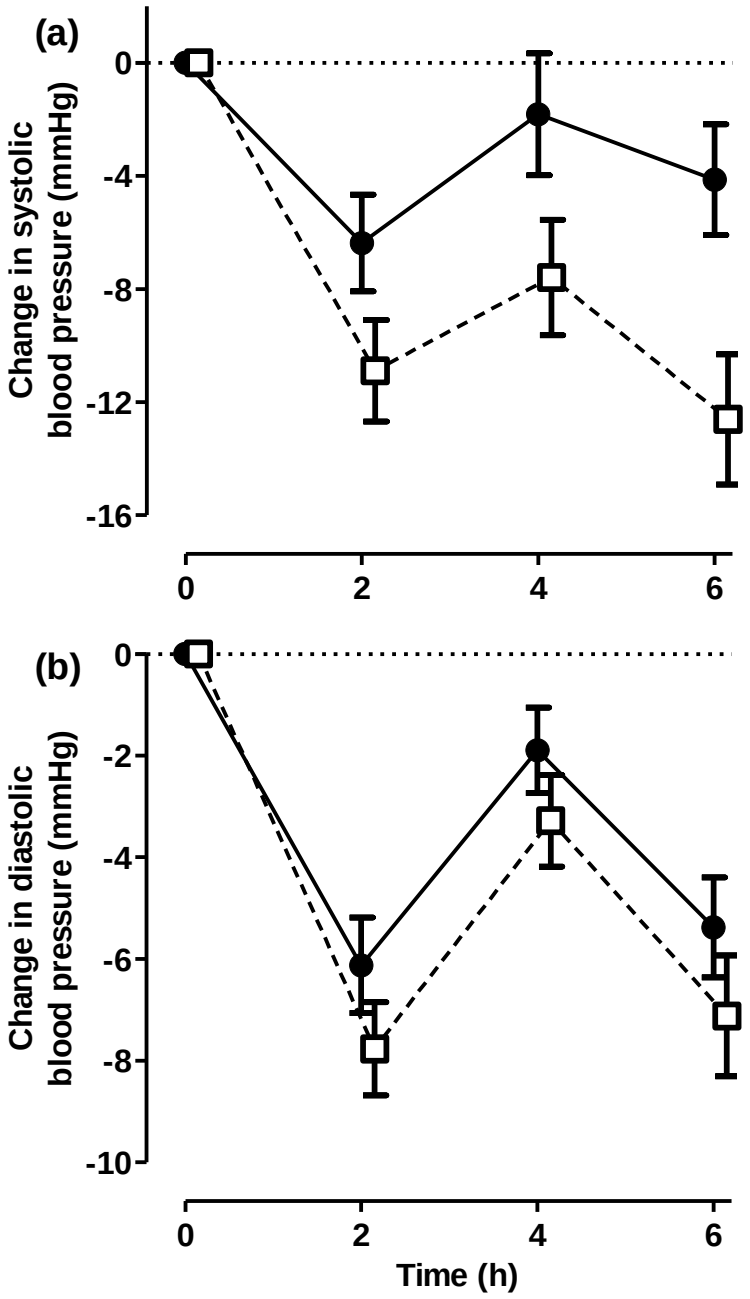


Figure 7.3 Changes in systolic (a) and diastolic (b) blood pressure in postmenopausal women over 6 hours after the ingestion of 1000mg of calcium as citrate (•) or a placebo (□). The difference between the changes in systolic blood pressure after calcium and placebo was significant (ANOVA, p=0.016). When individual time-points were examined, differences in systolic blood pressure were significant at 4 hours (between-groups difference 6mmHg, p=0.036) and 6 hours (9mmHg, p=0.009). The difference between the changes in diastolic blood pressure after calcium and placebo was not significant (p=0.34).

7.4 DISCUSSION

The present study demonstrates that, in a group of postmenopausal women, blood pressure declines in the hours after a fasting morning reading, and this reduction is attenuated following the ingestion of a calcium supplement. These changes mirror the increases in in serum calcium following ingestion of a supplement. These findings suggest that a smaller fall in systolic blood pressure throughout the day could contribute to the increase in cardiovascular risk associated with supplement use.

In healthy individuals, blood pressure shows a diurnal fluctuation. There is an early morning surge followed by a gradual decline throughout the day, and a further drop during sleep [463]. In older adults, blood pressure also dips following meals [464]. The present study is consistent with this, blood pressure declining throughout each study period, particularly at the two time-points following the meals (2 and 6 hours). Following the ingestion of a calcium supplement, the reduction in systolic blood pressure was smaller. This corroborates previous research, which demonstrated that the ingestion of a calcium supplement was followed by an attenuated diurnal rhythm in blood pressure over the subsequent 6 hours, resulting in calcium supplemented women having pressures 5-10mmHg higher than controls [462]. Again, the differences were more marked for systolic pressure. Intravenous infusion of calcium in humans has been shown to acutely increase systolic blood pressure by up to 10mmHg [94, 95]. Conversely, in two small uncontrolled trials, the administration of a calcium supplement was reported as not changing blood pressure at two [465] or three [466] hours. However, without a control group, neither of these studies could account for the diurnal variation in blood pressure, so those results are not inconsistent with the present findings and they do not accurately characterize the effect of calcium supplements on blood pressure.

In the present study, reduction in systolic blood pressure was 6 to 9mmHg less following calcium supplementation than placebo. These differences are large enough to be clinically important. Commonly used antihypertensives, such as thiazides and angiotensin converting enzyme inhibitors, have blood pressure lowering effects of about this magnitude [467]. In clinical trials, differences in cardiovascular endpoints often become evident with differences in blood pressure between the treatment and placebo groups of 3 to 10mmHg systolic [468]. Blood pressure was only assessed for 6 hours in the present study, although there is evidence suggesting that alterations in normal blood pressure patterns throughout only a part of the diurnal cycle can have adverse effects. In normal individuals, blood pressure declines by 10-20% during sleep [469]. Failure of the blood pressure to dip appropriately overnight is associated with an increased risk of cardiovascular events [470], and it has been estimated that each 5% attenuation in the decline of nocturnal BP confers a 20% rise in cardiovascular mortality [471]. Thus, it is possible that attenuation of the diurnal blood pressure drop on a daily basis, as seen following ingestion of a calcium supplement, might increase an individual's cardiovascular risk.

A number of studies have assessed the longer term (≥3 weeks) impact of calcium supplementation on blood pressure, and have shown either no change or a small decline in blood pressure of 0.5–2 mmHg [396, 472-474]. In these studies, follow-up blood pressure was measured in the morning, at least 12 hours following the last calcium dose, whereas the acute effect of calcium on blood pressure demonstrated here appears to wane after 6 hours [462]. Together, these studies suggest a bi-phasic effect of a calcium supplement on blood pressure, with the largest part of this being an increase in pressures in the 6 hours after dosing.

There are a number of potential mechanisms by which circulating calcium concentrations could influence blood pressure, most of which involve an increase in systemic vascular resistance [475]. Increases in extracellular calcium may promote intracellular calcium influx, a process which increases vascular tone and can be reversed by calcium channel blockers [98]. Increases in extracellular calcium could also increase vascular tone by binding to calcium-sensing receptors expressed throughout the vasculature [96]. Studies in mice have shown that administration of a calcimimetic results in an acute rise in blood pressure [476, 477]. Higher levels of circulating calcium may indirectly contribute to increased peripheral vascular resistance via stimulation of adrenal catecholamine secretion, since increases in circulating epinephrine have been observed in healthy volunteers following an intravenous calcium infusion [475]. A drop in blood pressure following a meal is common in older adults [464], and it is possible that the effect of calcium on blood pressure that was observed in the present study is related to postprandial blood pressure control. For instance, postprandial blood pressure regulation has been related to the release of vasoactive gut hormones, such as glucagon-like peptide-1 [478], which may be influenced by the calcium content of the meal [479]. Consistent with the present findings, the administration of a calcium supplement with a meal has been shown to prevent the post-meal fall in vascular tone [480].

The present study assessed healthy postmenopausal white women, and these results cannot necessarily be generalized to men, persons of other ethnicities, or to individuals taking antihypertensives. However, in a previous study carried out by our research group, 25% of participants were taking antihypertensive medications, and a similar effect of calcium on blood pressure was observed [462]. It is not known whether the effects of calcium on blood

pressure are maintained when calcium supplements are taken long-term. However, it has recently been shown by members of our research group that the acute elevation in serum calcium following calcium persists after three months of supplementation [283], so it is likely that the blood pressure response would continue to occur.

A major strength of this study is its crossover design, which reduces the risk of confounding from inter-individual variation. No evidence of carry-over was detected, indicating that the washout period was adequate. The study is also strengthened by the measurement of blood pressure at several time points, and the standardization of meals, allowing the normal diurnal blood pressure variation to be accounted for.

In summary, the results of this study demonstrate that the ingestion of a calcium supplement results a smaller reduction in systolic blood pressures by approximately 6 to 9mmHg than after placebo over 6 hours after dosing. This finding corroborates those of a recent similar study. The size of the change in blood pressure is large enough to be clinically important. This effect could potentially contribute to the increased cardiovascular risk associated with calcium supplements, but further research is required to determine if these acute effects are maintained long term.

CHAPTER 8:
THE EFFECT OF SINGLE-DOSE DEXAMETHASONE ON ACUTE PHASE REPONSE FOLLOWING ZOLEDRONIC ACID: A RANDOMIZED CONTROLLED TRIAL

8.1 INTRODUCTION

The potent bisphosphonate zoledronic acid is an effective treatment for osteoporosis, as well as Paget's disease and hypercalcemia of malignancy [295, 481, 482]. A single intravenous infusion of zoledronic acid results in an antiresorptive effect on bone that lasts several years [314, 315] and, unlike oral bisphosphonates, the effectiveness of this medication is not limited by issues with absorption and adherence [483]. However, as outlined in Chapter 2, treatment with aminobisphosphonates, zoledronic acid in particular, can provoke an inflammatory reaction in some patients, known as the acute phase response (APR) [325]. Aminobisphosphonate medications block the enzyme farnesyl pyrophosphate synthase, which acts within the mevalonate pathway. This leads to accumulation of upstream metabolites which activate gamma-delta T-cells, resulting in release of proinflammatory cytokines such as interferon-γ, TNF-α and IL-6 [321-324].

While the clinical manifestations of the APR can be variable, symptoms such as fever, arthralgia, nausea, headache and fatigue are most common [325]. APRs typically occur within 72 hours of infusion, often beginning in the first 24 hours [322]. They are almost always self-limited, resolving within several days [322, 325]. Estimates of APR incidence range from 42-76%, with most studies defining APR as an increase in temperature and/or the development of typical APR symptoms [322, 325-329] [330].

Although APRs are common, preventative strategies are not well established. Two large randomized controlled trials of bisphosphonate-naïve postmenopausal women have

demonstrated that paracetamol/acetaminophen and ibuprofen can reduce the incidence of APR by 35-42% [327, 330]. Glucocorticoids downregulate the transcription of proinflammatory cytokines [484], and a potential preventative role for these medications has been considered. In a recent abstract, Chen *et al* described 80 first-time zoledronic acid recipients who received hydrocortisone 100mg intravenously prior to bisphosphonate infusion. Patients then received prednisolone 5mg daily for three days following the infusion, as well as regularly scheduled acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs). Only one patient who received zoledronic acid in this setting developed an APR [485]. While this study was uncontrolled, and multiple interventions were utilized, the results suggest that the frequency of zoledronic acid-induced APRs may be reduced with concurrent administration of glucocorticoids and raise the possibility that the effect of glucocorticoids on the incidence of the APR may be greater than what has been previously reported with non-steroidal anti-inflammatory medications [327, 330].

The aim of the study reported in this chapter was to determine, using a double-blind, randomized, controlled trial, whether a single dose of glucocorticoid given at the time of zoledronic acid infusion reduced the incidence or severity of APR compared to placebo. Dexamethasone was selected as the treatment medication as it has high oral bioavailability [486, 487], strong anti-inflammatory potency, and a long elimination half-life [488, 489]. Single doses of up to 8mg are usually well-tolerated [490, 491].

8.2 METHODS

This study is a randomized, double-blind, controlled trial comparing the effects of dexamethasone 4mg and placebo on the development and severity of the APR following first exposure to zoledronic acid. Research was conducted at the University of Auckland, New

Zealand. The study was registered with the Australia New Zealand Clinical Trials Registry (ACTRN12615000794505).

8.2.1 Participants

Participants were 40 adults (age ≥20 years) who required an infusion of zoledronic acid for the treatment of osteoporosis (n=39) or bone pain (n=1). Exclusion criteria were prior treatment with zoledronic acid, diabetes mellitus, uncontrolled hypertension (BP >160/90), major active systemic illness, history of adverse reaction to glucocorticoids, treatment with glucocorticoids within the past week, or history of fever, infection, or influenza-like illness within the past week. Written informed consent was obtained from all participants. This study was conducted in accordance with the Declaration of Helsinki and was approved by the New Zealand Health and Disability Ethics Committee.

8.2.2 Study design

Participants were randomized 1:1 to receive either dexamethasone or placebo, using a computer generated balanced block design. Blocks were balanced for age, to ensure an equal number of participants ≥70 years in each group. Participants, study investigators, treating physicians and nurses were blinded as to allocation. Study medication was administered within the 15 minutes leading up to the zoledronic acid infusion. Participants allocated to the treatment group received a tablet of dexamethasone 4mg (Douglas Pharmaceuticals Ltd, Auckland, New Zealand). Participants allocated to the placebo group received a placebo tablet that was similar in appearance to the dexamethasone tablets. Study medication was provided to each participant in a sequentially numbered envelope. Envelopes were prepared by an individual who was not involved in study coordination or data collection.

8.2.3 Data Collection

At the time of study enrolment, data relating to medical history, current and past medication use, and lifestyle were collected via self-administered questionnaire. Height was measured using a Harpenden stadiometer, weight was measured using an electronic scale, and blood pressure was obtained using an automatic monitor.

Temperature

Oral temperature was measured by each participant, using a digital thermometer (Hangzhou Haan Medical, Hangzhou Zeijang, China; manufacturer-reported accuracy of +/- 0.1^{o}C). At baseline, three temperature measurements, each one minute apart, were taken by the participant under the supervision of a study investigator. Results for this time point were averaged. The standard deviation of these triplicates was calculated for each patient and the root mean square standard deviation for the group calculated to determine precision error and coefficient of variation. Participants were instructed to obtain a single oral temperature measurement at bedtime on the day of the infusion, and then three times per day (before breakfast, mid-afternoon, bedtime) for three days following the infusion. Participants were advised not to have any food or drink for 30 minutes prior to each temperature measurement.

Symptom scores

Symptoms of the APR were assessed using a self-administered questionnaire, based on the validated Generic Assessment of Side Effects questionnaire [492]. This questionnaire addresses four symptoms frequently associated with APRs (headache, nausea, muscle or joint pain, feverishness) and requires participants to rate the severity of each symptom on a four-point categorical scale (0=absent, 1=mild, 2=moderate, 3=severe). Total symptom scores can therefore range from 0 to 12. The questionnaire also permits participants to document

additional symptoms and rate their severity. Participants completed the APR symptom questionnaire at baseline and at bedtime that night and on the following three nights. Fifteen days after the study intervention, participants were contacted via telephone by a study investigator, and the APR symptom questionnaire was administered over the telephone.

Anti-inflammatory use

At baseline, participants were informed that they may take a rescue medication (paracetamol or an NSAID such as ibuprofen) if they experienced APR-related symptoms following their zoledronic acid infusion. They were asked to document the timing and dose of any rescue medications taken within three days following the infusion.

8.2.4 Statistical analysis

The pre-specified primary outcome was difference in temperature change from baseline between the dexamethasone and placebo groups over three days of follow-up. Secondary outcomes included between-group difference in APR symptom score change from baseline, and between-group differences in the proportion of patients with a significant increase in oral temperature (≥1^{o}C) or symptom score (≥3 points).

Based on a previous study showing a mean temperature increase of 0.85^{o}C following zoledronic acid infusion and a mean increase if 0.20^{o}C following placebo (difference: 0.65^{o}C) [330], it was determined that a sample size of 40 would provide >80% power to detect a temperature difference of at least 0.65^{o}C between treatment groups in the present study, with a significance threshold of 5% [493, 494].

Change in mean temperature from baseline was modelled using a mixed effect model approach to repeated measures. The main effects of treatment allocation, time, and their interaction were modelled in the analysis of covariance (ANCOVA), which included baseline mean temperature as a covariate. The same method was used to compare the secondary outcome measure change in symptom scores between the dexamethasone and placebo groups. Differences in the proportion of participants with a significant increase in oral temperature or symptom severity were assessed using Fisher's exact test. Confidence intervals for the percentage of patients experiencing pre-specified events were calculated using CIA (Confidence Interval Analysis Software, Version 2.2.0, Trevor Bryant, University of Southampton, UK). Difference in the proportion of patients requiring a rescue medication in the three days following intervention was assessed using Fisher's exact test. Analyses were intention-to-treat.

All data analysis was done using SAS v9.4 (SAS Institute, NC). All tests were two-tailed, and $p < 0.05$ was considered statistically significant.

8.3 RESULTS

Baseline characteristics of the study participants are shown in Table 8.1. A total of 40 individuals (72.6 years, 90% female) undergoing treatment with zoledronic acid were recruited between September 2015 and July 2016. One participant in the placebo group had received prior treatment with risedronate, but the remainder of the study population were bisphosphonate-naïve. The flow of participants through the study is outlined in Figure 8.1. All 40 participants received the study medication and were therefore included in analyses. Two participants (5%) did not record symptom scores on day 2 or day 3 post-infusion, and one participant (3%) did not perform a mid-afternoon temperature measurement on day 3

post-infusion. Nine participants (23%) could not be reached for completion of the APR symptom questionnaire on day 15 post-infusion.

Table 8.1 Baseline characteristics of adults receiving a first-time infusion of zoledronic acid 5mg randomized to receive dexamethasone 4mg or placebo at the time of infusion

Characteristic	**Dexamethasone** (n=20)	**Placebo** (n=20)
Age (y)	71.8 (7.0)	73.2 (12.2)
Male, n (%)	1 (5)	3 (15)
Weight (kg)	62.0 (12.7)	60.4 (10.0)
BMI (kg/m^2)	24.2 (4.4)	23.0 (3.9)
SBP (mmHg)	129 (17)	133 (16)
DBP (mmHg)	70 (10)	72 (9)
Current smoking, n (%)	3 (15)	0 (0)
Serum creatinine (µmol/L)	78 (11)	76 (16
Estimated GFR (mL/min/1.73m^2)[1]	66 (12)	70 (14
Recent anti-inflammatory use, n (%)[2]	7 (35)	4 (20)
Oral temperature (^{o}C)	36.2 (0.9)	36.2 (0.3)
Total symptom score (out of 12)[3]	0.6 (0.8)	0.6 (0.7)

Data are mean (SD) unless otherwise indicated.
[1]eGFR calculated using MDRD equation
[2]Use of acetaminophen or nonsteroidal anti-inflammatories within the week leading up to treatment.
[3]Symptoms of the acute phase response (APR) were assessed using a questionnaire that assesses the presence and severity of four symptoms frequently associated with APRs (headache, nausea, muscle or joint pain, feverishness).
BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate

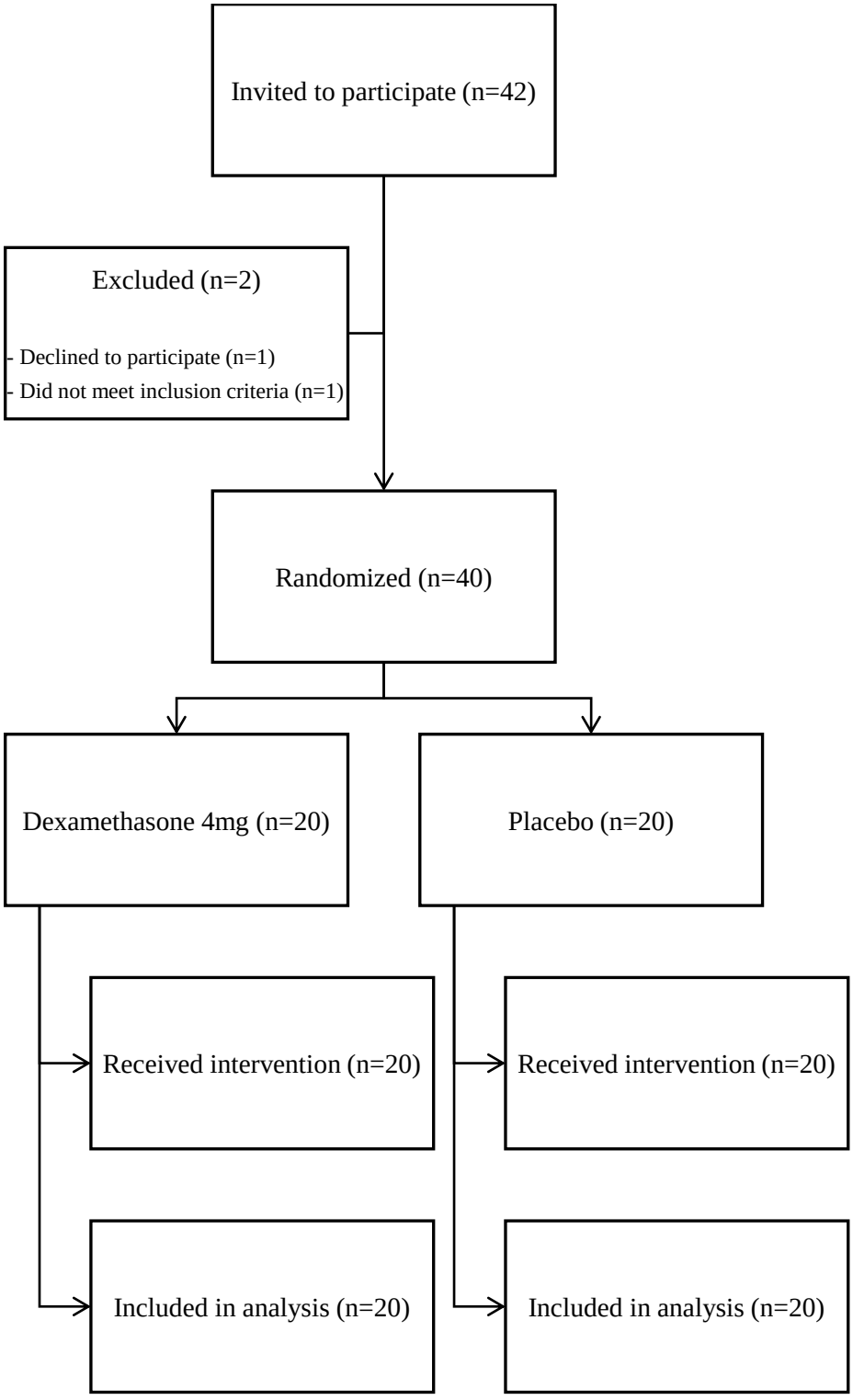


Figure 8.1 Flow of participants through the study.

Change in oral temperature in the three days following intervention is displayed in Figure 8.2. There was a significant change in temperature as a function of time (p=0.0001), but no significant interaction between treatment group and time (p=0.07). Maximal temperature increase from baseline occurred before bedtime on the first post-infusion day in both groups, with a mean temperature increase of 0.44 ºC (95% CI: -0.05 to 0.93) in the dexamethasone group and 0.58 ºC (95% CI: 0.20 to 0.95) in the placebo group. The between-group difference in temperature change at this time point was not statistically significant (0.14ºC, 95% CI: -0.74 to 0.47), and there were no significant between-group differences in temperature at any other time point (p=0.95). The measurement of three oral temperatures by each participant at baseline permitted an assessment of thermometer precision. The precision error for the thermometers was 0.22ºC.

Change in APR symptom score in the three days following intervention, and at day 15 post-intervention is shown in Figure 8.3. There was a significant change in symptom score as a function of time (p<0.0001), but no significant interaction between treatment group and time (p=0.38). Between-group difference in change in APR symptom score did not reach statistical significance at any time point, and this did not change when the 11 participants who had taken paracetamol or NSAIDs in the week prior to zoledronic acid infusion were excluded from analysis. Mean symptom scores were higher throughout the three days following infusion for participants <70 years than for participants ≥70 years (1.52 vs 0.36; p=0.0063).

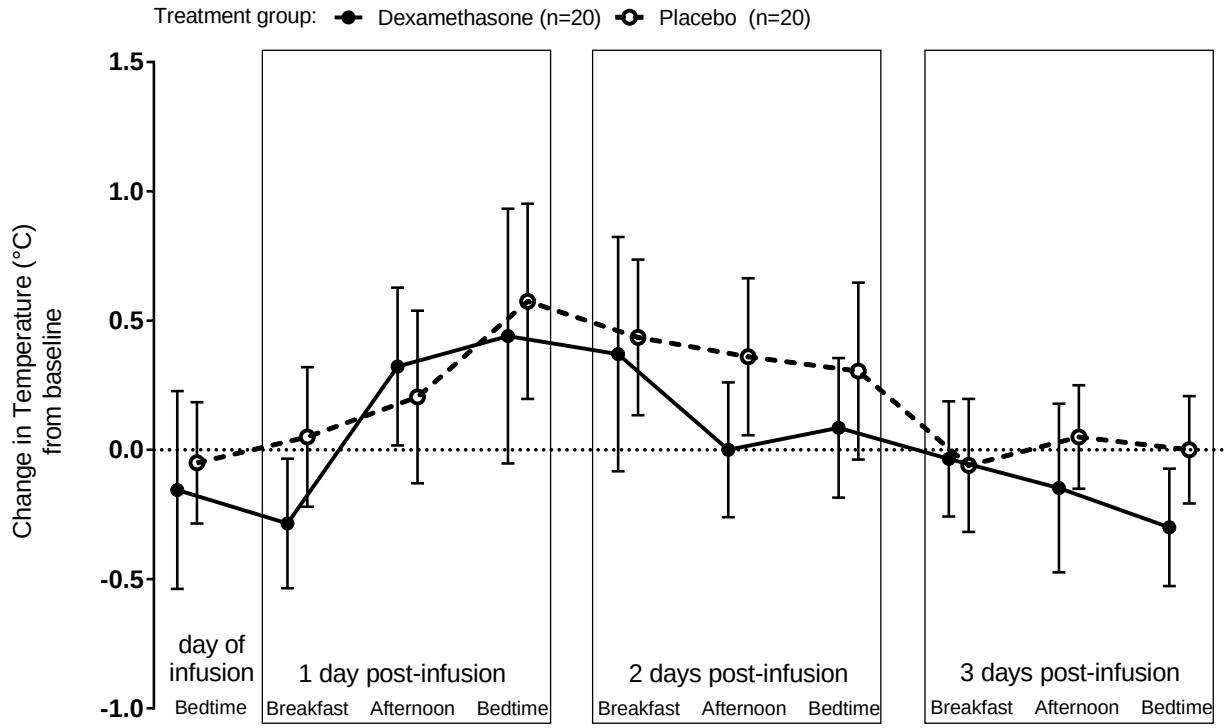


Figure 8.2 Oral temperature change following intravenous zoledronic acid infusion in participants randomized to receive concurrent treatment with either dexamethasone 4mg or placebo. Data are means and 95% confidence intervals. p=0.0001 for time effect and p=0.95 for between-group difference in temperature change.

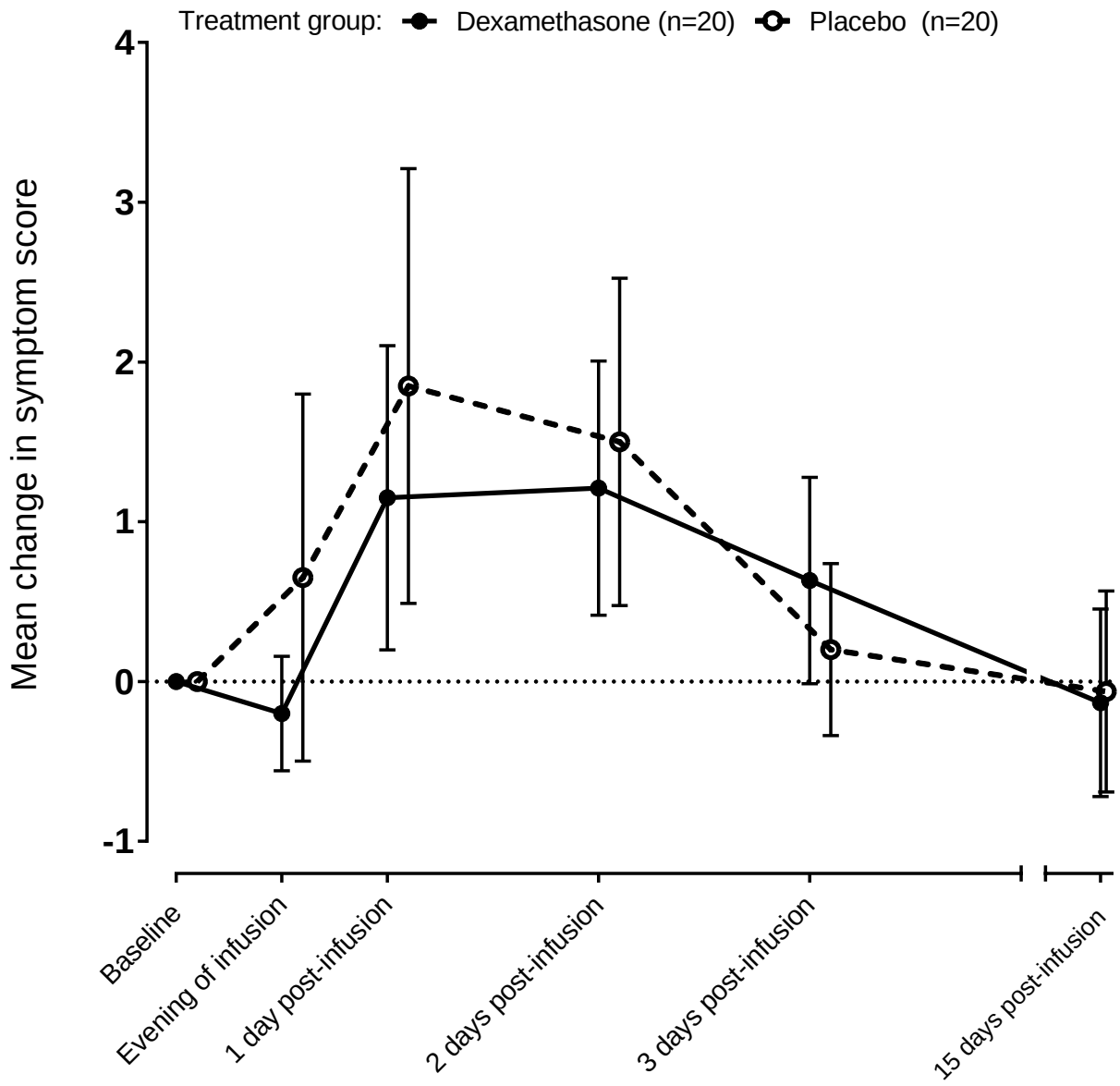


Figure 8.3 Change in acute phase response (APR) symptom score following intravenous zoledronic acid infusion in participants randomized to receive concurrent treatment with dexamethasone 4mg or placebo. Data are mean (95% confidence intervals). $p<0.0001$ for time effect and $p=0.42$ for difference in symptom score between dexamethasone and placebo groups. Symptoms of the APR were assessed using a self-administered questionnaire which assesses the presence and severity of four symptoms frequently associated with APRs (headache, nausea, muscle or joint pain, feverishness).

The proportion of participants in each group that experienced a temperature increase of ≥1°C, an increase in APR symptom score of ≥3 points, or required the use of rescue medications in the three days following intervention are shown in Figure 8.4. There was no difference in the proportion of participants with an increase in symptom score of ≥3 points at day 15 post-infusion (p=0.48). A total of 13 (65%) dexamethasone recipients and 12 (60%) placebo recipients required rescue medications within three days of the zoledronic acid infusion (p=0.99). Mean (SD) number of rescue medication tablets taken was 4.0 (4.9) in the dexamethasone group and 4.7 (4.6) in the placebo group (p=0.62). Of the 29 participants who had not taken paracetamol or NSAIDs in the week leading up to the infusion, 7 (54%) in the dexamethasone group and 8 (50%) in the placebo group required rescue medication in the three days following zoledronic acid (p=0.91). Among these 29 participants, mean (SD) number of rescue medication tablets taken was 2.8 (4.5) for dexamethasone recipients and 3.5 (4.2) for placebo recipients (p=0.73). When total number of paracetamol or ibuprofen tablets taken in the three days following infusion was included as a baseline covariate in the ANCOVA models, no significant between-group differences in temperature or symptom score change from baseline were observed.

Sensitivity analyses were undertaken following the exclusion of: 1) the participant who was treated with zoledronic acid for bone pain, and 2) the participant who had been previously treated with risedronate. Results of these analyses were unchanged from the primary analyses.

No participants in the dexamethasone or placebo groups reported adverse events related to dexamethasone use.

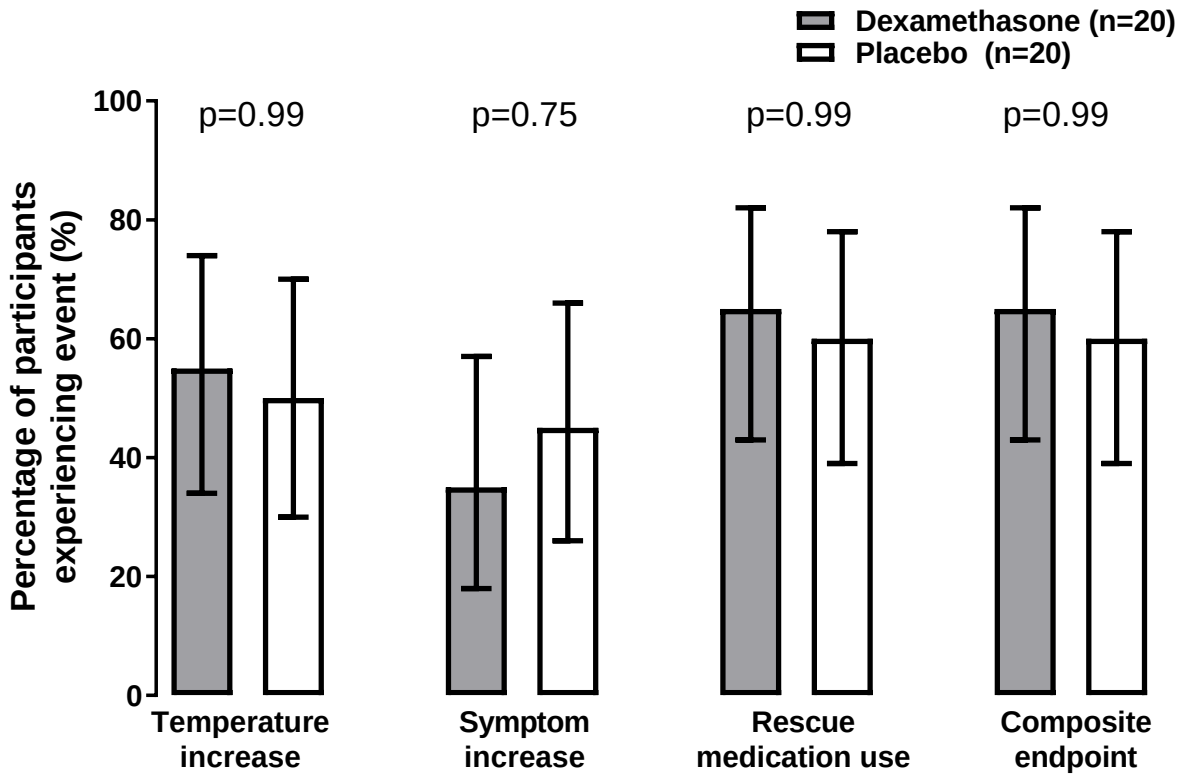


Figure 8.4 Comparison of the percentage of participants who had ≥1°C temperature increase from baseline, ≥3-point increase in acute phase response (APR) symptom score, or required use of a rescue medication in the three days following an intravenous infusion of zoledronic acid and concurrent treatment with dexamethasone 4mg or placebo. Composite endpoint includes any one of these outcomes. Error bars represent the 95% confidence intervals around the percentage of participants experiencing each even. Dexamethasone and placebo groups are compared using Fisher's exact method.

8.4 DISCUSSION

To my knowledge, the present study is the first randomized, controlled trial to assess the effect of glucocorticoid administration on the development of APR following intravenous administration of zoledronic acid. The results of this trial demonstrate that while a single oral dose of dexamethasone 4mg administered at the time of zoledronic acid infusion appears to be well-tolerated, this intervention does not reduce the change in temperature or development of APR symptoms in the three days following infusion.

The incidence and timing of APR observed in this study were similar to previous reports. Fifty-three percent of participants exhibited a temperature increase of ≥1^{o}C, 40% had a clinically relevant increase in APR symptoms, and 63% required rescue medications. In a post-hoc analysis of the HORIZON-Pivotal Fracture Trial, which evaluated the efficacy of zoledronic acid in postmenopausal osteoporosis, 42.4% of women receiving a first dose of zoledronic acid reported symptoms suggestive of an APR, compared to 11.7% of the placebo group [325]. In a bisphosphonate-naïve population of postmenopausal women, Wark and colleagues observed a significant increase in oral temperature (≥1^{o}C to a temperature above 37.5^{o}C) in 63.5%, although they reported symptoms suggestive of an APR in a larger proportion of patients (75.9%) [330]. Other estimates of APR incidence have ranged from 42-71% [322, 326-329]. In our study population, change in temperature and symptom score were greatest in the evening, one day following the zoledronic acid infusion, and returned to baseline levels within three days. This is in keeping with findings by Anastasilakis *et al*, who assessed the occurrence of APR in 51 postmenopausal women receiving zoledronic acid, and found that the APR developed at a mean (SE) of 17.9 (2.1) h after the infusion and lasted for

42.5 (2.6) h [322]. Post-hoc analyses of the HORIZON-PFT demonstrated peak onset of APR symptoms within one day, with resolution occurring over a median of three days [325].

There are a number of potential explanations for the negative findings of the present study. Although the bioavailability of oral dexamethasone is upwards of 70% [486, 487], and dexamethasone has potent anti-inflammatory effects compared with other glucocorticoid preparations [488, 489], it is possible that a 4mg dose was not sufficient to counteract the gamma-delta T cell activation that follows zoledronic acid infusion. The patients described by Chen and colleagues [485] received a hydrocortisone dose that is roughly equivalent to 4mg of dexamethasone [495], although the hydrocortisone was delivered intravenously, which is expected to improve bioavailability. The average time-to-peak serum concentration following oral dexamethasone administration is less than two hours, and the duration of action approaches 72 hours [489]. However, significant inter-individual variation in metabolism and clearance of dexamethasone has been reported [496], raising the possibility that a longer treatment course may be necessary to prevent the APR. The observation from the present study that maximal changes in temperature and symptom scores occurred more than 24 hours following administration of the study medication supports the hypothesis that more than one dose of dexamethasone might be required to prevent these changes. It is worth noting that the treatment protocol described by Chen *et al* included three days of prednisolone treatment following zoledronic acid infusion [485].

Assessment of the typical, clinically important manifestations of the APR in the setting of a controlled trial presents a challenge. Patient-reported symptoms are subjective, and oral temperature measurement can be subject to operational and reporting errors. These

limitations should be taken into consideration when interpreting the results of the present study. Furthermore, reliance on longitudinal patient-reported outcomes introduces the risk of recall bias and is susceptible to loss to follow-up. Almost all study participants provided complete follow-up data up to and including the third day following zoledronic acid administration. However, at day 15 post-intervention, 23% of participants could not be reached to ask about persistent APR symptoms, limiting our ability to draw conclusions about differences in symptoms between the dexamethasone and placebo groups at that time point.

Paracetamol/acetaminophen and NSAIDs are currently the mainstays of preventative therapy for the APR, although their effects are relatively modest. Wark and colleagues randomized 481 bisphosphonate-naïve postmenopausal women to receive either paracetamol/acetaminophen, ibuprofen, or placebo four hours following zoledronic acid infusion. Study medications were continued every six hours for three days. Compared to placebo, the incidence of a significant temperature increase (≥1ºC above 37.5ºC) was reduced 42% by ibuprofen and 41% by paracetamol/acetaminophen, and the incidence of a worsening symptom score was reduced 36% by ibuprofen and 39% by paracetamol/acetaminophen [330]. Silverman *et al* randomized postmenopausal women to receive acetaminophen (n=264) or placebo (n=267) 45 minutes prior to their first infusion of zoledronic acid. Study medications were administered every six hours for three days. APR was defined as an increase in oral temperature of ≥1ºC, or a temperature of ≥38.5ºC, or use of rescue medication during the three days following the infusion. Incidence of APR was 35% lower in the acetaminophen group than the placebo group [327]. There has also been interest in whether statin medications may prevent development of the APR. These medications inhibit the enzyme HMG-CoA reductase, which acts upstream of farnesyl pyrophosphate synthase in the mevalonate pathway [328]. However, controlled trials of these agents have had

disappointing results [327, 328]. Blood levels of 25-hydroxyvitamin D have been shown to fall following zoledronic acid infusion [322], and lower baseline levels appear to be associated with a greater inflammatory response [326]. However, while treatment with a bolus of vitamin D 300,000 international units prior to zoledronic acid infusion did reduce the severity of musculoskeletal pain and biochemical markers of inflammation, it did not alter the incidence of APR (defined as an axillary temperature >37ºC or the development or worsening of any symptom typical of the APR) in a randomized trial of 60 postmenopausal women [497]. APR is the major short-term complication of zoledronic acid treatment, and given that over 40% of first-time recipients develop a clinically relevant APR, further investigation into preventative therapies is necessary.

In summary, treatment with a single dose of dexamethasone 4mg at the time of zoledronic acid infusion did not significantly reduce the incidence or severity of the APR. Paracetamol/acetaminophen and NSAIDs are currently the only agents demonstrated to reduce the incidence of APR in controlled trials, and these medications afford a modest reduction in APR symptoms. Further research evaluating other strategies for managing APR are warranted.

CHAPTER 9:
CONCLUSIONS

Osteoporosis and fragility fracture affect a large proportion of the population. Our understanding of bone biology, our ability to identify individuals at high risk of fragility fracture, and our treatment options for osteoporosis have improved significantly over the past several decades. However, several areas of controversy remain. These persisting uncertainties limit the ability of clinicians to diagnose and manage osteoporosis and other disorders of bone metabolism. The body of research described in this thesis has addressed several of these outstanding questions.

PTH plays a crucial role in the regulation of blood calcium concentration. The presence of sustained elevations in circulating PTH results in increased bone resorption, permitting liberation of calcium from the skeleton at the expense of bone mass [42]. Accordingly, disorders of PTH excess, such as primary hyperparathyroidism, are associated with osteoporosis. It has also become evident that circulating concentrations of PTH and calcium are positively associated with markers of cardiometabolic risk [79, 81, 89, 94, 95, 119, 130], and cardiovascular events [101, 121, 128, 131, 132]. The direction of causality in these associations has been uncertain. It has also been unclear whether these relationships are linear or become evident only after a certain 'cut point'. In Chapter 3, I presented the relationships between serum PTH, BMD and markers of cardiometabolic risk in a cohort of healthy men. In accordance with previous reports of other cohorts, I demonstrated that PTH concentrations were positively associated with measures of adiposity, blood pressure, and cholesterol. My findings also indicated that PTH is positively associated with coronary artery calcium score. These associations were linear, arguing against the idea of a PTH 'cut point', above which risk increases significantly. No significant relationships between PTH and BMD were

observed, and in a proportion of the cohort who were followed up over 2 years, change in BMD did not vary with baseline PTH concentration. The results from Chapter 3 indicate that in the general population who are free from referral bias, those with the highest PTH concentrations are not necessarily at increased risk of osteoporosis or excess bone loss. However, these individuals do appear to be at increased cardiometabolic risk. This study, like others that have preceded it, was limited in that it could not determine the direction of causality. It is not known whether lowering of high-normal or mildly elevated PTH concentrations results in a reduction in cardiometabolic risk, or vice versa. However, my findings raise the possibility that metabolic factors, such as adiposity, might drive PTH production, perhaps representing an unrecognized cause of secondary hyperparathyroidism. As reflected in the Discussion of Chapter 3, I am now undertaking further research that addresses changes in PTH following weight loss, in order to clarify whether this is the case. Although results from small case series' and cohort studies have been conflicting, large population-based studies may be able to shed light on whether PTH is pathogenic in the development of adiposity and cardiovascular dysfunction or vice versa. For instance, the direction of effect of the association between PTH and blood pressure could be further evaluated by assessing changes PTH levels in hypertensive individuals who begin treatment with antihypertensive agents, and in persons with primary hyperaldosteronism who undergo adrenalectomy. Large studies comparing cardiometabolic outcomes in individuals with hyperparathyroidism who undergo parathyroidectomy (or treatment with a calcimimetic agent) and those who are managed observationally may also help to elucidate the direction of causality and will be addressed in my future research.

An incidental finding from my analysis of the cohort of healthy men described in Chapter 3 was an inverse association between serum phosphate concentrations and measures of

adiposity. Upon review of the literature, this finding had been previously reported, but not widely appreciated [83, 286, 401-403]. As associations observed in a single large database may be spurious, I undertook analyses of two additional independent cohorts of healthy women to determine whether this association was present. As PTH is one of the primary drivers of phosphate metabolism and is also related to adiposity, I also assessed whether PTH may mediate relationships between phosphate and fat mass. These analyses, reported in Chapter 4, confirmed a negative association between phosphate and measures of adiposity in both female cohorts in addition to the male cohort, a relationship that could not be fully accounted for by PTH. In a fourth cohort of postmenopausal women in whom concentrations of FGF23 had been previously determined, I observed strong positive correlations between FGF23 and both body weight and BMI. The finding that FGF23 levels were tightly correlated with body weight and BMI supports the possibility that FGF23 might mediate the relationship between serum phosphate and adiposity. However, the analyses described in Chapter 4 were limited in that measures of FGF23 were only available in one small cohort, and so correlates of FGF23 could not be further interrogated in the three larger cohorts. In addition, the data were cross-sectional and did not allow for determination of the direction of causality. The Discussion of Chapter 4 references further research that I am undertaking to assess the effects of weight loss on lowering of FGF23 and PTH concentrations. Additional studies evaluating the effects of the adipokine leptin on levels of these hormones are also required, and may help to clarify the mechanisms underpinning the relationships between phosphate and adiposity.

Several secondary causes of osteoporosis exist, beyond disorders of PTH excess. In many cases of secondary osteoporosis, the mechanism is not known. Post hoc analyses of RCTs have demonstrated that insulin-sensitizing TZD medications, used to treat people with type 2

diabetes, increase the risk of fracture [174-176]. In the meta-analysis presented in Chapter 5, I demonstrated that TZD use for 3 to 24 months is associated with reductions in BMD of 0.7-1.1% when compared with placebo. This is likely to explain some, but not all, of the increased risk of fracture associated with TZD use. It remains unknown whether bone loss continues beyond 24 months of TZD use. Of concern, the results of this meta-analysis also demonstrated that the effects of TZD medications on bone density do not appear to be reversed in the year following discontinuation. These findings suggest that TZD use should be avoided in people with type 2 diabetes at increased risk of fracture, such as postmenopausal women. Studies evaluating changes in bone histomorphometry that occur as the result of TZD therapy may clarify the effects of these agents on osteoclast and osteoblast activity and provide further insight on the mechanism responsible for TZD-related bone loss.

The goal of osteoporosis screening and diagnosis is to identify the individuals at highest risk of fracture, in whom therapy is most likely to be beneficial. In Chapter 6, I presented the results of a comparison of risk estimates between two clinical prediction tools designed to estimate absolute fracture risk: FRAX and Garvan. Using a treatment threshold of a ten-year risk of hip fracture of 3% or higher, I demonstrated that, in a group of postmenopausal women, Garvan risk estimates are higher than FRAX risk estimates on average, but the results lie on the same side of the treatment threshold in 75% of cases. Most cases of discordant risk estimates lie quite close to the threshold with both calculators. The presence of risk factors that are incorporated by one calculator and not the other (such as falls and multiple fractures incorporated by Garvan, and history of parental hip fracture incorporated by FRAX) sometimes result in discordant risk estimates. These results serve as an important reminder to clinicians who see patients with osteoporosis that different fracture risk calculators may produce different results, depending on the patient's risk profile. However,

the results also provide some reassurance that the two calculators are almost always concordant when a person's risk is above the threshold with FRAX or below the threshold with Garvan. This study did not assess ten-year fracture outcomes, although assessing fracture incidence in persons with discordant FRAX and Garvan estimates would help to guide practice in this group of patients.

Higher serum calcium concentrations are associated with an increased risk of cardiovascular events [101], and concern has been raised that the use of calcium supplements, widely recommended for the treatment of osteoporosis, may increase the risk of myocardial infarction [273, 274]. It has been postulated that calcium supplements may increase this risk via effects on blood pressure [462]. In Chapter 7, I presented the results of a randomized crossover trial assessing the acute effects of a 1000mg calcium supplementation on blood pressure in postmenopausal women, compared to placebo. While blood pressure displayed expected diurnal fluctuations in the 6 hours after administration of calcium or placebo, decreasing throughout the day in both instances, the blood pressure decrease was attenuated by 6 to 9mmHg following ingestion of a calcium supplement. Therefore, alteration of the normal diurnal blood pressure variation may be a mechanism by which calcium supplementation increases cardiovascular risk. Assessing the effects of a once- or twice-daily calcium supplement on blood pressure over a 24-hour time period is a priority for upcoming research, as is determining whether the blood pressure effects persist with long-term calcium supplementation.

Treatment of patients at high risk of fragility fracture is limited by concerns from patients and physicians about the short-term and long-term side effects of available pharmacologic therapies. Uptake of pharmacologic therapy is limited, even in patients at the highest risk of

fracture [498]. Zoledronic acid is shown to be effective at reducing the risk of fracture, and its intravenous dosing affords high bioavailability and an infrequent dosing schedule. However, development of the inflammatory APR is common following the first infusion of zoledronic acid and may reduce the likelihood of adherence. In Chapter 8, I presented results from an RCT assessing the effect of a single dose of oral dexamethasone on the incidence and severity of the APR following first infusion of zoledronic acid. These results demonstrated that a single dose of dexamethasone did not reduce the incidence or severity of APR. The lack of response to dexamethasone may reflect an inadequate dose or duration of therapy, and further research planned by our group will assess whether a three-day course of oral dexamethasone can ameliorate the APR. In the interim, use of acetaminophen and NSAIDs remain the mainstay of therapy, with these agents shown to reduce the incidence of APR by 30-40%.

The research described herein has endeavoured to clarify a number of uncertainties that have emerged in in the field of osteoporosis over the past decade, and my findings have several implications for the management of patients with osteoporosis and cardiometabolic dysfunction. For instance, adiposity and cardiometabolic risk factors should be considered potential causes of secondary hyperparathyroidism. By the same token, individuals with high and high-normal PTH levels may benefit from screening for cardiovascular risk factors. It may be prudent to avoid TZD use in individuals who are at increased risk of fracture, such as postmenopausal women. In most postmenopausal women, the FRAX and Garvan fracture risk calculators produce congruent results. However, clinicians should be aware that these calculators incorporate different risk factors for fracture, which may sometimes result in discrepancies. Calcium supplementation appears to attenuate normal diurnal variations in blood pressure, and may work via this mechanism to increase cardiovascular risk.

Development of a clinically significant APR is common following the first infusion of zoledronic acid and is not ameliorated by giving a single dose of 4mg dexamethasone at the time of zoledronic acid infusion. It is anticipated that research carried out in the coming decade will build upon these findings to provide a more complete understanding of the crosstalk between fat and bone, further improvement in our ability to predict fracture risk, and the ability to ameliorate or avoid completely the side effects of osteoporosis treatment.

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