



<http://researchspace.auckland.ac.nz>

ResearchSpace@Auckland

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage.

<http://researchspace.auckland.ac.nz/feedback>

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.

**Studies of the Pathogenesis and Treatment
of Secondary Osteoporosis**

Mark Jonathan Bolland

**A thesis submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy,
Faculty of Medical and Health Sciences,
The University of Auckland, 2006.**

Abstract:

Background:

Osteoporosis commonly affects older men and women. Frequently, it is caused or exacerbated by a disease or treatment (termed secondary osteoporosis). The aim of this thesis is to explore aspects of the pathogenesis and treatment of three conditions that may cause or contribute to osteoporosis: human immunodeficiency virus (HIV) infection, primary hyperparathyroidism (PHPT), and vitamin D insufficiency. Each of these conditions is potentially linked to bone metabolism via associations with nutritional status, which in turn is a key regulator of bone mass and fracture risk.

Methods:

Eight studies were performed:

1. Cross-sectional comparison of bone mineral density (BMD) in HIV-infected men and age-matched controls.
2. Two-year randomised controlled trial of the effects of annual zoledronate on BMD in HIV-infected men.
3. Longitudinal comparison of the change in BMD over two years in HIV-infected men not receiving skeletal therapy and controls.
4. Meta-analysis of body weight in patients with PHPT.
5. Cross-sectional analysis of the relationship between parathyroid hormone and body weight in post-menopausal women.
6. Cross-sectional analysis of the determinants of 25-hydroxyvitamin D (25OHD) in post-menopausal women and middle-aged and older men.
7. Cross-sectional analysis of the effects of fat mass and seasonal variation on diagnosis of vitamin D sufficiency.
8. Cross-sectional analysis of the relationship between vitamin D binding protein and body weight in older men and women.

Results:

1. HIV-infected men were 6.3 kg lighter than controls, and after adjustment for this weight difference, did not have lower BMD than controls.
2. Annual intravenous zoledronate caused substantial increases in BMD in HIV-infected men over two years.
3. HIV-infected men did not have accelerated bone loss over time compared to controls.
4. Patients with PHPT are on average 3.1-3.3 kg heavier than age-matched controls.
5. Fat mass is an important determinant of parathyroid hormone levels.
6. The major determinants of 25OHD levels in men and women are surrogate measures of ultraviolet-B exposure, and fat mass, but men have higher 25OHD levels throughout the year than women.
7. Thresholds for diagnosis of vitamin D sufficiency vary by season and amount of fat mass.
8. Vitamin D binding protein does not mediate the relationships between 25OHD and age, gender or weight.

Conclusions:

Uncomplicated HIV infection is not associated with low BMD at baseline or accelerated bone loss over time. There are important relationships between body weight and PHPT, and among fat mass, parathyroid hormone and 25OHD that have significance both for the pathogenesis of PHPT and vitamin D sufficiency, and for the clinical diagnosis and treatment of these conditions.

Preface:

Osteoporosis affects a significant number of older men and women. Osteoporosis is defined by the WHO as “a systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures” [1]. In Caucasian populations, approximately 50% of post-menopausal women and 20% of men older than 50 years will experience at least one fragility fracture in their remaining lifetime [2]. In Caucasian populations, the lifetime risk for fragility fracture of the hip is 14% for women and 3% for men, and for fragility fracture of the spine is 28% for women and 12% for men [3]. In the majority of cases of osteoporosis, the bone loss is due to the normal ageing process, in which case it is termed primary or idiopathic osteoporosis. However, in up to 20-30% of post-menopausal women, 50% of pre-menopausal women, and more than 50% of men, osteoporosis is caused or exacerbated by a disease or treatment, in which case it is termed secondary osteoporosis [4]. Currently, there are more than 50 recognised causes of secondary osteoporosis [4]. Since there are very large numbers of people affected by osteoporosis and many of these cases of osteoporosis will be due to secondary causes, secondary osteoporosis is a significant health issue.

In 2000, Tebas et al. published the first report which suggested HIV or treatment of HIV with antiretroviral agents may cause osteoporosis [5]. In contrast, PHPT has been recognised to cause osteoporosis since the earliest descriptions of patients with osteitis fibrosa cystica [6], and vitamin D deficiency has also been known for many years to cause osteopenia and osteomalacia [7]. PTH and vitamin D are essential hormones in calcium homeostasis, and their roles in calcium metabolism are interlinked [8, 9]. Therefore, diseases with abnormal levels of serum PTH are likely to impact upon vitamin D metabolism, and, conversely, diseases with abnormal levels of serum vitamin D metabolites are likely to impact upon parathyroid gland function. Thus, in any consideration of PHPT or vitamin D insufficiency, it is important to consider the roles of PTH and vitamin D and its metabolites. Recent evidence has suggested that both PHPT and vitamin D insufficiency are associated with increased body weight. Since body weight is a major determinant of BMD and an important risk factor for osteoporotic fractures [10], and both PHPT and vitamin D insufficiency are important causes of secondary osteoporosis, it is important to explore the role of body weight in the pathogenesis of PHPT and vitamin D insufficiency.

The purpose of this thesis is to explore aspects of the pathogenesis and treatment of secondary osteoporosis in association with three conditions: HIV, PHPT and vitamin D insufficiency. In Chapters 1-4, I will review the association between HIV and osteoporosis, and then report and discuss the results of a cross-sectional study and a longitudinal study of BMD in HIV-infected men in comparison to age-matched healthy controls, and an intervention study on the effect of the potent bisphosphonate zoledronate on BMD in HIV-infected men. In Chapters 5-10, I will review the relationship between PTH, vitamin D, body weight and BMD, and then report and discuss the results of five studies that explore aspects of the relationships between PTH and body weight, vitamin D metabolites and body weight, and the determinants of PTH and vitamin D metabolites in a variety of population groups.

Acknowledgements:

I would like to thank Associate Professor Andrew Grey, my principal supervisor, and Professor Ian Reid, my associate supervisor, for firstly asking me to work as their research fellow in the Osteoporosis Research Group, and then encouraging me to undertake the PhD. Their continual enthusiastic support and encouragement, and prompt reading and revisions of the seemingly endless drafts of the various papers made it easy to complete what initially appeared an ambitious goal. I am extremely fortunate to have received their guidance and tuition, both in this PhD and the many other important research projects carried out during my time with the group.

I would also like to thank Greg Gamble, who acted as an advisor for this PhD, for teaching me about statistics and demystifying SAS. His accessibility, patience and support, as well as his knowledge and advice about statistics and science were invaluable.

I am lucky to have worked with so many other outstanding colleagues. Anne Horne co-ordinated the HIV trial, and her enthusiasm and professionalism ensured the study ran without any hitches. Our collaborators in the HIV trial were Rod Ellis-Pegler, Mark Thomas, Simon Briggs, and Andrew Woodhouse. Mark and Rod started the project by suggest HIV-related bone disease as a link between the specialities of Endocrinology and Infectious Diseases. The idea snowballed from there, and Mark, Rod and Andrew gave it their fullest support, and Simon managed to track down and recruit everyone possible.

Ruth Ames, Diana Wattie, Barbara Mason, and Anne Horne form the clinical arm of our group, and their kindness and professionalism make it a very enjoyable place to work, as well as ensuring that the studies run without hitches. Thanks especially to Barbara to always dealing cheerfully with my requests for yet more data. I would also like to thank Jill Cornish, Dorit Naot and the other members of the laboratory arm of our group for putting up with me in their work space, and for their enthusiastic support of these and our other shared projects.

I would like to acknowledge the generous financial support I have received from the Australia and New Zealand Bone and Mineral Society in the form of a post-graduate scholarship to carry out this research, travel grants to attend their annual scientific meetings and present data from this research, and prizes from those meetings.

Finally, I would like to thank my wife and family for their support which allows me to do a job I love.

Table of Contents:

Chapter 1: HIV and Osteoporosis	1
Background history of HIV and AIDS:	1
Osteoporosis prior to the use of HAART:	2
Osteoporosis in the HAART era:	3
Cross-sectional studies:	3
Longitudinal studies:	14
Bone turnover markers and calcitropic hormones:	16
Pathogenesis of bone loss in HIV infection:	19
Clinical fractures in HIV infection:	23
Treatment of osteoporosis in HIV infection:	25
Conclusion:	27
Chapter 2: A cross-sectional analysis of bone mineral density in HIV-infected men treated with HAART	28
Introduction:	28
Methods:	28
Subjects:	28
Measurements:	29
Statistics:	30
Results:	30
Discussion:	36
Chapter 3: Annual zoledronate increases bone mineral density in HAART-treated HIV-infected men: a randomised controlled trial	40
Introduction:	40
Methods:	41
Participants:	41
Protocol:	41
Measurements:	42
Statistics:	43
Results:	43
Discussion:	47
Chapter 4: Bone mineral density remains stable in HAART-treated HIV-infected men over two years	50
Introduction:	50
Methods:	50
Subjects:	50
Measurements:	51
Statistics:	51

Results:	52
Discussion:.....	54
Conclusions to Chapters 1-4:	58
Chapter 5: The relationships between bone mineral density and parathyroid hormone, vitamin D, and body weight.....	
	60
Introduction:	60
PTH:.....	60
The effects of PTH on BMD and fractures:.....	61
Vitamin D:	63
The effects of vitamin D on bone turnover, BMD and fractures:.....	64
The relationship between PTH and Vitamin D:	67
The effects of body weight and body composition on bone turnover, BMD and fractures:	68
Conclusion:	70
Chapter 6: The relationship between primary hyperparathyroidism and body weight.....	
	71
Introduction:	71
Methods:	72
Study Selection:	72
Statistics:	72
Results:	73
Discussion:.....	77
Chapter 7: Fat mass is an important predictor of parathyroid hormone levels in post-menopausal women	
	82
Introduction:	82
Methods:	82
Results:	84
Discussion:.....	87
Chapter 8: Determinants of vitamin D status in middle-aged and older men and in older women	
	91
Introduction:	91
Methods:	92
Participants:.....	92
Measurements:	93
Statistics:	93
Results:	94
Women:.....	94
Men:	96

Comparisons between the women and men:.....	98
Discussion:.....	100
Chapter 9: The effects of seasonal variation of 25-hydroxyvitamin D and fat mass	
on diagnosis of vitamin D sufficiency.....	106
Introduction:	106
Methods:	106
Participants:.....	106
Measurements:	107
Statistics:	107
Results:	108
Discussion:.....	112
Chapter 10: Age-, gender-, and weight-related effects on levels of	
25-hydroxyvitamin D are not mediated by vitamin D binding protein.....	116
Introduction:	116
Methods:	117
Participants:.....	117
Measurements:	117
Statistics:	118
Results:.....	118
Discussion:.....	120
Conclusions to Chapters 5-10:	124
References:	127

List of Tables:

Table 1.1: Cross-sectional studies of BMD in HIV-infected patients prior to use of HAART ...	4
Table 1.2: Cross-sectional studies of BMD in HIV-infected adults in the HAART era.....	11
Table 1.3: Studies of HIV-infected adults that report bone turnover markers.....	18
Table 2.1: Baseline characteristics of HIV-infected group and control group.....	31
Table 2.2: Clinical characteristics of HIV-infected group	32
Table 2.3: Calcitropic hormones and bone-related laboratory parameters of HIV-infected group and control group	33
Table 2.4: Baseline characteristics of HIV-infected group in the presence or absence of lipodystrophy.....	36
Table 3.1: Baseline anthropometric, BMD, biochemical parameters and other characteristics of the groups.....	44
Table 3.2: HIV-related clinical characteristics of the groups.....	44
Table 4.1: Characteristics of the study groups at baseline and two years.....	52
Table 4.2: Clinical characteristics of HIV-infected group	53
Table 6.1: Studies of patients with PHPT and eucalcaemic controls presenting data on body weight.....	74
Table 6.2: Studies of patients with PHPT and eucalcaemic controls presenting data on BMI..	75
Table 7.1: Descriptive and biochemical characteristics of the study group.....	84
Table 7.2: Pearson’s correlations between vitamin D metabolites, PTH, and other variables ..	85
Table 7.3: Predictors of PTH and 25OHD in multivariate regression models.....	87
Table 8.1: Descriptive and biochemical characteristics of the study populations.....	94
Table 8.2: Mean levels of 25OHD by age range.....	95
Table 8.3: Frequency of low 25OHD levels by month of sampling	96
Table 8.4: Pearson’s correlations between 25OHD and other variables.....	97
Table 8.5: Predictors of 25OHD levels in multivariate regression models.....	97
Table 8.6: Studies from Europe and USA that report 25OHD levels in cohorts of men	101
Table 9.1: Baseline characteristics of the study populations	108
Table 9.2: The minimum 25OHD level required to have a predicted nadir 25OHD more than 50 nmol/L, by month of measurement	110
Table 9.3: The effect of fat mass on seasonal variation of 25OHD levels.....	111
Table 10.1: Biochemical and anthropometric characteristics of the study groups.....	119
Table 10.2: Pearson’s correlations between DBP, 25OHD, and other variables.....	120

List of Figures:

Figure 1.1: Factors that may contribute to low BMD in HIV-infection	23
Figure 2.1: Distribution of unadjusted BMD for HIV-infected group and control group at the lumbar spine, total hip, and total body.....	34
Figure 2.2: Mean BMD and 95% CI at the lumbar spine, total hip, and total body adjusted for body weight in HIV-infected group and controls	34
Figure 3.1: Flow of subjects through the study.....	42
Figure 3.2: The effects of 4 mg annual zoledronate or placebo on BMD at the lumbar spine, total hip, and total body in HIV-infected men.....	46
Figure 3.3: The effects of 4 mg annual zoledronate or placebo on bone turnover markers in HIV-infected men	47
Figure 4.1: The change in BMD from baseline at the lumbar spine, total hip, and total body in HIV-infected men and controls	54
Figure 6.1: Weighted mean difference in body weight in studies of PHPT and eucalcaemic controls that present data on body weight	75
Figure 6.2: Weighted mean difference in BMI in studies of PHPT and eucalcaemic controls that present data on BMI.....	76
Figure 6.3: Standard mean difference in studies of PHPT and eucalcaemic controls that present data on weight or BMI	76
Figure 7.1: Relationship between PTH and fat mass	85
Figure 8.1: Monthly variation of UV dose and 25OHD levels	95
Figure 8.2: The relationship between 25OHD and fat mass	99
Figure 9.1: Sine curve of best fit for 25OHD in the cohort of women (N=1606) with measured mean monthly 25OHD for comparison	109
Figure 9.2: Sine curve of best fit for 25OHD in the cohort of men (N=378) with measured mean monthly 25OHD for comparison	109
Figure 9.3: The effect of fat mass on seasonal variation of 25OHD levels in men and women.....	112
Figure 10.1: DBP levels in men and women.....	119

List of Publications:

Chapter 2:

Bolland MJ, Grey AB, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Woodhouse AF, Gamble GD, Reid IR. Bone mineral density is not reduced in HIV-infected Caucasian men treated with highly active antiretroviral therapy. *Clin Endocrinol (Oxf)*. 2006; 65:191-7

Chapter 3:

Bolland MJ, Grey AB, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Woodhouse AF, Gamble GD, Reid IR. Annual zoledronate increases bone density in highly active antiretroviral therapy-treated human immunodeficiency virus-infected men: a randomized controlled trial. *J Clin Endocrinol Metab*. 2007; 92:1283-8.

Chapter 4:

Bolland MJ, Grey AB, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Woodhouse AF, Gamble GD, Reid IR. Bone mineral density remains stable in HAART-treated HIV-infected men over 2 years. *Clin Endocrinol (Oxf)*. 2007; 67:270-5.

Chapter 6:

Bolland MJ, Grey AB, Gamble GD, Reid IR. Association between primary hyperparathyroidism and increased body weight: a meta-analysis. *J Clin Endocrinol Metab*. 2005; 90:1525-30

Chapter 7:

Bolland MJ, Grey AB, Ames RW, Horne AM, Gamble GD, Reid IR. Fat mass is an important predictor of parathyroid hormone levels in postmenopausal women. *Bone*. 2006; 38:317-21

Chapter 8:

Lucas JA, Bolland MJ, Grey AB, Ames RW, Mason BH, Horne AM, Gamble GD, Reid IR. Determinants of vitamin D status in older women living in a subtropical climate. *Osteoporos Int*. 2005; 16:1641-8

Bolland MJ, Grey AB, Ames RW, Mason BH, Horne AM, Gamble GD, Reid IR. Determinants of vitamin D status in older men living in a subtropical climate. *Osteoporos Int.* 2006; 17:1742-8

Chapter 9:

Bolland MJ, Grey AB, Ames RW, Mason BH, Horne AM, Gamble GD, Reid IR. The effects of seasonal variation of 25-hydroxyvitamin D and fat mass on diagnosis of vitamin D sufficiency. *Am J Clin Nutr.* 2007; 86:959-64.

Chapter 10:

Bolland MJ, Grey AB, Ames RW, Horne AM, Mason BH, Wattie DJ, Gamble GD, Bouillon R, Reid IR. Age-, gender-, and weight-related effects on levels of 25-hydroxyvitamin D are not mediated by vitamin D binding protein. *Clin Endocrinol (Oxf).* 2007; 67:259-64.

Abbreviations:

1,25(OH) ₂ D	1,25-dihydroxyvitamin D
25OHD	25-hydroxyvitamin D
AIDS	Acquired immunodeficiency syndrome
ALP	Alkaline phosphatase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
bsALP	Bone specific alkaline phosphatase
CI	Confidence interval
CTx	β-C-terminal telopeptide of type I collagen
CV	Coefficient of variation
DPD	Deoxypyridinoline
DXA	Dual energy x-ray absorptiometer
ELISA	Enzyme linked immunosorbent assay
GGT	γ-Glutamyl transferase
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
NIH	National Institutes of Health
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NTx	N-telopeptide of type I collagen
OC	Osteocalcin
OPG	Osteoprotegerin
PHPT	Primary hyperparathyroidism
PI	Protease inhibitor
PTH	Parathyroid hormone
PYD	Pyridinoline
RANK	Receptor activator of nuclear factor κβ
RANKL	Receptor activator of nuclear factor κβ ligand
RIA	Radioimmunoassay
SD	Standard deviation
SEM	Standard error of the mean
UV	Ultraviolet
WHO	World Health Organisation