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 and Deposit Licence.

Note: Masters Theses

The digital copy of a masters thesis is as submitted for examination and contains no corrections. The print copy, usually available in the University Library, may contain corrections made by hand, which have been requested by the supervisor.
VITAMIN D STATUS: DETERMINANTS, OPTIMAL LEVELS, AND SUPPLEMENTATION

Catherine Jane Bacon

A thesis presented in fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Auckland, 2009.
ABSTRACT

Vitamin D deficiency, indicated by the circulating metabolite 25-hydroxyvitamin D [25(OH)D], can lead to osteomalacia, increased fracture risk in the elderly, and may increase the risk of other medical conditions. However, optimal levels of 25(OH)D are uncertain, with some cross-sectional studies suggesting optimal levels of >75 nmol/L. This thesis assessed optimal levels of vitamin D and strategies for its supplementation.

In a trial of high-dose vitamin D$_3$ regimens in frail elderly, data suggest that 25(OH)D levels of 40 – 50 nmol/L may be sufficient. In the same study, calcium intake appeared to modify the relationship between 25(OH)D and PTH and subsequent estimates of optimal 25(OH)D based on these data may be lower when calcium intake is >1552 mg/day. It was also noted that large loading doses (500 000 IU) rapidly normalise 25(OH)D levels, whilst monthly 50 000 IU doses were also effective but took 3 – 5 months to reach plateau.

An analysis of adverse events recorded for a 5-year calcium trial in postmenopausal women showed that whilst season-adjusted baseline 25(OH)D levels <50 nmol/L increased the risk of stroke and a composite event (stroke, myocardial infarction or sudden death) compared to levels ≥50 nmol/L, these effects disappeared when adjustment for baseline confounders was made.

Data from two studies indicate that vitamin D deficiency or insufficiency is prevalent amongst urban Chinese women of childbearing age, and that in the Auckland region young Maori and Pacific women and children and adults of Middle Eastern, Southern Asian and African ethnicity are over-represented in the group of people identified as vitamin D deficient or insufficient. In a final study of middle-aged and older New Zealand men, more than half (55%) reported use of dietary supplements which may make a contribution to vitamin D status.

In conclusion, data here suggest that 25(OH)D levels of 50 nmol/L may be satisfactory for bone health, that large loading doses of vitamin D$_3$ are safe with respect to hypercalcaemia and effective, and that a number of non-elderly populations are at high risk of having insufficient vitamin D status.
ACKNOWLEDGEMENTS

This thesis would not have possible without the help and contributions of a number of people. First and foremost, I would like to express my sincere gratitude to my supervisor, Professor Ian Reid, for taking me on in the first instance, and then guiding me through the projects incorporated into this work. Professor Reid has made significant contributions to all chapters in this thesis.

Secondly I would like to thank Dr Mark Bolland for his assistance and feedback on various sections of this thesis. He provided helpful feedback on oral and written reports of data reported in Chapter Five. Dr Bolland also conceived the idea for a prospective investigation of the relationship between baseline 25(OH)D levels and adverse events in a large calcium intervention completed within this research group (Chapter Six) the idea of completing an additional analysis of dietary supplement use by men enrolled in another calcium intervention (Chapter Eight). For Chapter Six, I’d also like to acknowledge Dr Bolland’s help with the statistical analyses. For Chapter Seven, Dr Bolland has completed a parallel investigation using the same data set and incorporating some analysis, which was completed by myself, and is also included in this thesis.

My appreciation also goes to staff in the Clinical Bone Group at the University of Auckland, including Dr Anne Horne, Dr Andrew Grey, Ruth Ames, Barbara Mason and Diana Wattie who helped me with setting up and running the research reported in Chapter Five, provided feedback on oral reports of these data, and made me feel very welcome in our basement premises. Particular acknowledgement is extended to Dr Horne for her feedback on Chapter Five, and to Ruth Ames and Barbara Mason for the collection and management of data reported in Chapters Six and Eight. I would also especially like to thank our departmental statistician, Greg Gamble, for his advice and assistance with analyses for Chapters Three to Six.

Quite a number of other people have made specific intellectual and practical contributions to parts of this thesis and I’d like to acknowledge their input for each chapter.

For Chapters Three and Four Chapters I would like to acknowledge the researchers in Beijing and Hong Kong, some of whom I do not know, who collected data and managed other aspects of the original trial. In particular, I’d like to thank Drs Jean Woo, Christopher Lam, and Edith Lau, from Hong Kong who also liaised with the group in Beijing and answered my queries about some aspects of the methodology. I’d also like to thank Joanne Todd of Fonterra Brands Ltd. for
her input and Fonterra Brands Ltd. itself for sponsoring the trial from which these data were sourced. My own contributions to these chapters comprised of drafting analysis plans to address the research questions described therein, and then completing these analyses and writing the chapters.

For Chapter Five, I would like to acknowledge the help and advice of Dr Marilyn Scott and her team in Older People’s Health at Auckland District Health Board (A+) in setting up the data collection for this research. In addition, I’d like to thank API Consumer Brands Ltd. who provided cholecalciferol for the interventions. I would also like to acknowledge the help of the many residential home staff who helped ensure that the interventions and blood collections were applied at the right time and kept me informed about the changing addresses and medical conditions of the participants. I’d also like to thank the elderly participants in the trial itself, some of whom have now unfortunately passed away.

My own contribution to Chapter Six entailed formulating an analysis plan to address the research questions described therein, and then writing this chapter.

For Chapter Seven, I would like to acknowledge the assistance of Dr James Davidson, Dr Weldon Chiu and others in the team at A+ Department of Clinical Biochemistry, Labplus, Auckland Hospital who retrieved and prepared the data from their records. An analysis of these results was the subject of an abstract presented to The Endocrine Society, 86th Annual Meeting, June 16-19, New Orleans, 2004 [1] that took place prior to collaboration with this research group. My own contribution to this chapter entailed writing an analysis plan to address the research questions described therein, and then completing these analyses and writing the chapter.

For Chapter Eight, I would like to acknowledge Amanda Siu, who helped with data acquisition and categorisation. My own contribution to this chapter included designing the questionnaire to assess supplement use, developing a written procedure for the entry of nutritional data, formulating and completing the statistical analyses, and finally writing the thesis chapter.

Chapters Five, Six, and Eight would not have been possible without funding from the Health Research Council of New Zealand.

Finally, I’d like to thank my spouse for his patience and support in this endeavour, and my son, who was born in the midst of it and provided many unbidden challenges.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
<tr>
<td>CHAPTER 1: INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER 2: LITERATURE REVIEW</td>
<td>6</td>
</tr>
<tr>
<td>2.1 Physiology of Vitamin D and the Regulation of Calcium Homeostasis</td>
<td>6</td>
</tr>
<tr>
<td>2.1.1 Vitamin D, Parathyroid Hormone &amp; Calcium Homeostasis</td>
<td>6</td>
</tr>
<tr>
<td>2.1.2 Parathyroid Hormone Structure, Regulation &amp; Action</td>
<td>7</td>
</tr>
<tr>
<td>2.1.3 Vitamin D Synthesis &amp; Metabolism</td>
<td>9</td>
</tr>
<tr>
<td>2.1.4 Actions of Vitamin D and Its Metabolites</td>
<td>14</td>
</tr>
<tr>
<td>2.1.5 Regulation of Vitamin D Activity</td>
<td>15</td>
</tr>
<tr>
<td>2.2 Vitamin D Deficiency &amp; Insufficiency</td>
<td>18</td>
</tr>
<tr>
<td>2.2.1 Osteomalacia, Rickets &amp; Secondary Hyperparathyroidism</td>
<td>18</td>
</tr>
<tr>
<td>2.2.2 Optimal Level of 25-Hydroxyvitamin D</td>
<td>19</td>
</tr>
<tr>
<td>2.3 Seeking a 25-Hydroxyvitamin D Threshold From Investigations of Vitamin D-Health Outcome Relationships</td>
<td>22</td>
</tr>
<tr>
<td>2.3.1 Measurements of Calcium Absorption &amp; 1,25-Dihydroxyvitamin D</td>
<td>22</td>
</tr>
<tr>
<td>2.3.2 Muscle Functioning or Falls</td>
<td>25</td>
</tr>
<tr>
<td>2.3.3 Disease Risk Indicators</td>
<td>30</td>
</tr>
<tr>
<td>2.3.3.1 Cancer</td>
<td>30</td>
</tr>
<tr>
<td>2.3.3.2 Other Chronic Diseases</td>
<td>34</td>
</tr>
<tr>
<td>2.3.3.3 Overview</td>
<td>35</td>
</tr>
<tr>
<td>2.3.4 Skeletal Outcomes</td>
<td>39</td>
</tr>
<tr>
<td>2.3.4.1 Cranney et al. Report</td>
<td>39</td>
</tr>
<tr>
<td>2.3.4.2 Bone Mineral Content or Density</td>
<td>40</td>
</tr>
<tr>
<td>2.3.4.3 Bone Turnover Markers</td>
<td>51</td>
</tr>
<tr>
<td>2.3.4.4 Fracture Rates</td>
<td>52</td>
</tr>
<tr>
<td>2.3.5 Summary</td>
<td>54</td>
</tr>
<tr>
<td>2.3.6 Parathyroid Hormone &amp; Optimal Vitamin D Status</td>
<td>54</td>
</tr>
<tr>
<td>2.3.7 Factors Affecting the Relationship Between PTH &amp; 25(OH)D</td>
<td>55</td>
</tr>
<tr>
<td>2.3.7.1 Assays and Techniques Used to Estimate a Threshold for PTH</td>
<td>55</td>
</tr>
<tr>
<td>2.3.7.2 Seasonal Variation</td>
<td>56</td>
</tr>
<tr>
<td>2.3.7.3 Age, Sex &amp; Race</td>
<td>57</td>
</tr>
</tbody>
</table>
4.2 Introduction ........................................................................................................................ 87
4.3 Methods .............................................................................................................................. 88
4.4 Results ............................................................................................................................... 90
  4.4.1 Participant Characteristics .......................................................................................... 90
  4.4.2 Relationship Between 25(OH)D & PTH .................................................................... 90
  4.4.3 Effect of Calcium Intake & Adiposity ........................................................................ 92
  4.4.4 Determinants of 25(OH)D & PTH.............................................................................. 92
4.5 Discussion ........................................................................................................................... 93

CHAPTER 5: PHARMACODYNAMICS OF HIGH-DOSE REGIMENS OF ORAL VITAMIN D3 IN FRAIL ELDERLY ............................................................................................................. 97
  5.1 Abstract ............................................................................................................................. 97
  5.2 Introduction ....................................................................................................................... 97
  5.3 Methods ............................................................................................................................ 98
    5.3.1 Participants ................................................................................................................ 98
    5.3.2 Treatment .................................................................................................................. 99
    5.3.3 Assays ......................................................................................................................... 99
    5.3.4 Clinical Assessment .................................................................................................... 99
    5.3.5 Statistical Analysis .................................................................................................... 100
  5.4 Results .............................................................................................................................. 100
    5.4.1 Participants ................................................................................................................ 100
    5.4.2 25(OH)D, PTH & P1NP Relationships at Baseline .................................................. 102
    5.4.3 25-Hydroxyvitamin D Responses to Supplementation ............................................. 104
    5.4.4 PTH and P1NP Responses to Supplementation as Indicators of Optimal 25(OH)D Estimates ............................................................................................................................ 109
    5.4.6 Grip Strength ............................................................................................................. 112
  5.5 Discussion ......................................................................................................................... 112

CHAPTER 6: THE EFFECT OF 25-HYDROXYVITAMIN D LEVELS ON FIVE-YEAR HEALTH OUTCOMES IN POSTMENOPAUSAL WOMEN .................................................. 117
  6.1 Abstract ............................................................................................................................. 117
  6.2 Introduction ....................................................................................................................... 118
  6.3 Methods ............................................................................................................................ 120
    6.3.1 Participants ................................................................................................................ 120
    6.3.2 Measurements ........................................................................................................... 120
    6.3.3 Adverse Event Assessment ........................................................................................ 121
LIST OF TABLES

Table 2.1 Calciferol Intervention Studies Assessing Changes in Muscle Function or Falls ..........28
Table 2.2 Studies Showing Beneficial Associations of Vitamin D Supplementation or Status With Chronic Disease Risk ........................................................................................................................................36
Table 2.3 Studies Investigating Cross-Sectional Associations Between Vitamin D Status & Bone Mineral Content or Density ........................................................................................................................................44
Table 2.4 Studies Reporting Cross-Sectional & Longitudinal Data Showing Opposing Seasonal Changes in 25-Hydroxyvitamin D & Bone Mineral Content or Density ........................................................................................................................................47
Table 2.5 Studies Investigating Other Longitudinal Associations Between Vitamin D Status and Change in Bone Mineral Content or Density ........................................................................................................................................48
Table 2.6 Vitamin D Interventions Reporting 25-Hydroxyvitamin D & Bone Mineral Density or Content Outcomes ................................................................................................................................................49
Table 2.7 Response of 25(OH)D to Oral Doses of Vitamin D₃ ........................................................................................................................................61
Table 3.1 Baseline Characteristics of Study Participants ........................................................................................................................................79
Table 3.2 Correlates of Baseline 25-Hydroxyvitamin D and Parathyroid Hormone ..................80
Table 3.3 Three-Month Changes in 25-Hydroxyvitamin D and Parathyroid Hormone .............81
Table 4.1 Characteristics of Study Participants ........................................................................90
Table 4.2 Correlates of Baseline 25-Hydroxyvitamin D and Parathyroid Hormone in the Beijing and in the pooled Beijing and Hong Kong cohorts. ........................................................................................................................................93
Table 5.1 Baseline Characteristics of Study Participants ..........................................................102
Table 5.2 Correlations Between Baseline Variables ................................................................104
Table 5.3 Serum Albumin-Adjusted Calcium Values Throughout the Study ..........................108
Table 6.1 Baseline Characteristics of Study Participants by Vitamin D Status ..........................123
Table 6.2 Incident Events in Postmenopausal Women by Treatment Allocation and Vitamin D Status ........................................................................................................................................126
Table 6.3 Changes from Baseline at Five Years for Continuous Variables in Postmenopausal Women by Treatment Allocation and Vitamin D Status .................................................................128
Table 6.4 Incident Events in Postmenopausal Women by Tertile of Seasonally Adjusted 25-Hydroxyvitamin D ........................................................................................................................................130
Table 6.5 Changes from Baseline at Five Years for Continuous Variables in Postmenopausal Women by Tertile of Seasonally Adjusted 25-Hydroxyvitamin D ........................................................................................................................................131
Table 7.1 Age, Gender and Ethnicity Distribution of the Cohort ..............................................145
Table 7.2 Distribution of Ethnic Groups Compared to New Zealand Census, 2006 Data from the Auckland Region ........................................................................................................................................146
Table 7.3 Levels of 25-Hydroxyvitamin D in Children by Gender, Age and Ethnicity Compared with Means from the 2002 National Children’s Nutritional Survey (CNS02) for Each Category

Table 7.4 Proportions (%) of Children Identified as Insufficient [25(OH)D < 37.5 nmol/L] Compared with Those from the 2002 National Children’s Nutrition Survey (CNS02)

Table 7.5 Mean levels of 25-Hydroxyvitamin D in Adults by Gender, Age and Ethnicity Compared With Data From the 1997 National Nutritional Survey (NNS97)

Table 7.6 Proportions (%) of adults identified as insufficient [25(OH)D < 50 nmol/L] compared with those from the 1997 National Nutritional Survey (NNS97)

Table 7.7 Parameters of the Fitted Sine Curves for 25-Hydroxyvitamin D Versus Day of the Year by Age Group

Table 7.8 Distribution of Individuals Predicted to be Vitamin D Deficient or Insufficient at Some Time During the Year According to Age Group, Sex & Referring Doctor Type

Table 7.9 Frequency of Individuals Predicted to be Vitamin D Deficient or Insufficient at Some Time During the Year by Ethnicity, Sex & Referring Doctor Type

Table 8.1 General Characteristics of Respondents at Baseline

Table 8.2 Age Group Comparisons of Supplement Use in Middle-Aged Men

Table 8.3 Importance or Sources of Information in Supplement-Taking Decisions

Table 8.4 Daily Vitamin Intakes from Nutritional Supplements in Men Who Took Any Supplement

Table 8.5 Daily Vitamin Intakes from Food and Nutritional Supplements Combined in Men Who Took Any Supplement
LIST OF FIGURES

Figure 2.1 The Characteristic Tetracyclic Skeleton of Cyclopental[α]phenanthrene That All Steroids Possess or From Which They are Derived ................................................................. 10
Figure 2.2 A Schematic Diagram of Action Spectrum of Previtamin D₃ Formation From 7-Dehydrocholesterol in Human Epidermis (----) & the Spectral Irradiance Curve for Sunlight (- - -). .................................................................................. 11
Figure 2.3 Metabolic Pathway of Vitamin D and Its Metabolites ............................................. 13
Figure 2.4 a&b. The Effect of Baseline Levels of 25-Hydroxyvitamin D (a) and Its Change (b) on Relative Risk of Falls Following Calciferol Intervention ................................................ 27
Figure 3.1 The Relationship Between Baseline Serum Parathyroid Hormone (PTH) and Serum 25-Hydroxyvitamin D (25(OH)D) Grouped in Intervals of 5 nmol/L ........................................... 80
Figure 3.2 The Relationship Between Changes in 25-Hydroxyvitamin D [Δ25(OH)D] and Parathyroid Hormone (ΔPTH) ........................................................................................................... 82
Figure 3.3 The Relationship Between Baseline 25-Hydroxyvitamin D [25(OH)D] and Change in Parathyroid Hormone (ΔPTH) ........................................................................................................... 82
Figure 3.4 Change in Parathyroid Hormone (PTH) as a Function of Both Change and Baseline 25-Hydroxyvitamin D [25(OH)D] ........................................................................................................... 83
Figure 4.1 a & b. Relationships Between Serum Parathyroid Hormone (PTH) and Serum 25-Hydroxyvitamin D [25(OH)D] ........................................................................................................... 91
Figure 4.2 Relationship Between Serum Parathyroid Hormone (PTH) and Serum 25-Hydroxyvitamin D [25(OH)D] According to Tertile of Calcium Intake in the Pooled Cohorts of Young Women ................................................................. 92
Figure 5.1 Relationships Between Baseline 25-Hydroxyvitamin D [25(OH)D] and (a) Parathyroid Hormone (PTH) and (b) Procollagen Type I Amino-Terminal Propeptide (P1NP). Data are Mean ± SEM ...................................................................................................................... 103
Figure 5.2 Effects of Three Regimens of Supplementation With Vitamin D₃ on Serum Levels of 25-Hydroxyvitamin D [25(OH)D] ........................................................................................................... 105
Figure 5.3 Effects of Three Regimens of Supplementation With Vitamin D₃ on Serum Levels of 25-Hydroxyvitamin D [25(OH)D] ........................................................................................................... 107
Figure 5.4 Data From All Subjects and All Visits Showing the PTH Change From Baseline (ΔPTH) in Relation to Baseline 25-Hydroxyvitamin D [25(OH)D] ................................................................. 108
Figure 5.5 Change From Baseline in Procollagen Type I Amino-Terminal Propeptide (ΔP1NP) in Subjects Who Received a Loading Dose of Calciferol at Baseline (i.e. Stat & Stat+Monthly groups) ...................................................................................................................... 110
Figure 5.6 Relationship Between Parathyroid Hormone Change From Baseline (ΔPTH) and Baseline 25-Hydroxyvitamin D [25(OH)D] According to Tertile of Total Calcium Intake Including Supplements.......................................................................................................................... 111

Figure 6.1 The Effect of Season-Adjusted Baseline 25-Hydroxyvitamin D [25(OH)D] Below & Above 50 nmol/L by Vitamin D Supplement Use on Change in Total Femoral Neck Bone Mineral Density (BMD)........................................................................................................................................... 134

Figure 6.2 The Effect of Season-Adjusted Baseline 25-Hydroxyvitamin D [25(OH)D] Below & Above 50 nmol/L by Vitamin D Supplement Use........................................................................................................................................... 135

Figure 7.1 Between-Gender Comparisons of Sine Curves of the Best Fit for 25-Hydroxyvitamin D [25(OH)D] Versus Day for Children (a), Adults (b) and Older Adults (c)........................................................................................................................................... 151

Figure 7.2 Sine Curves of the Best Fit For 25-Hydroxyvitamin D [25(OH)D] Versus Day for Different Age Groups........................................................................................................................................... 152

Figure 7.3 Mean Annual 25-Hydroxyvitamin D [25(OH)D] by Gender, Using Ethnicity Categories Recorded in the New Zealand National Health Index Database........................................................................................................................................... 152

Figure 7.4 Individuals Predicted to be Vitamin D Deficient, Insufficient or Either of These at Some Time During the Year, for Each Age Group as a Proportion of the Total Number for Each Gender........................................................................................................................................... 155

Figure 7.5 Individuals Predicted to be Vitamin D Deficient, Insufficient or Either of These at Some Time During the Year, for Each Ethnic Group as a Proportion of the Total Number for Each Gender........................................................................................................................................... 155