

**THE ROLE OF DIETARY VITAMIN D AND CALCIUM
IN DETERMINING BONE HEALTH AND STRENGTH**

**A THESIS SUBMITTED IN TOTAL FULFILMENT OF THE REQUIREMENT OF THE
DEGREE OF DOCTOR OF PHILOSOPHY**

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TABLE OF CONTENTS

ABSTRACT	VII
DECLARATION	IX
ACKNOWLEDGEMENTS	X
CHAPTER 1	1
1.1 INTRODUCTION	1
1.2 AIMS	3
1.3 BONE BIOLOGY	4
1.3.1 Functions of the skeleton	4
1.3.2 Composition of bone	4
1.3.3 Structure of bone	4
1.3.4 Cortical bone	4
1.3.5 Trabecular bone	5
1.4 CELLS OF BONES	5
1.4.1 The Osteoclast	6
1.4.1.1 Osteoclast origin	6
1.4.1.2 Osteoclast structure.....	7
1.4.1.3 Osteoclast function.....	7
1.4.2 The Osteoblasts	8
1.4.2.1 Osteoblast origin.....	8
1.4.2.2 Osteoblast structure.....	9
1.4.2.3 Osteoblast function	10
1.4.3 The Lining cells	11
1.4.3.1 Lining cell origin	11
1.4.3.2 Lining cell structure	11
1.4.3.3 Lining cell function.....	12
1.4.4 The Osteocyte	12
1.4.4.1 Osteocyte origin.....	12
1.4.4.2 Osteocyte structure.....	12
1.4.4.3 Osteocyte function	13
1.5 BONE REMODELLING	14
1.5.1 Activation Phase	14
1.5.2 Resorption Phase	15
1.5.3 Reversal Phase	15
1.5.4 Formation Phase	16
1.5.5 Osteoclastogenesis	17
1.6 HORMONES AND BIOCHEMICAL FACTORS KNOWN TO CONTROL BONE HOMEOSTASIS ...	18
1.6.1 Parathyroid Hormone	18
1.6.2 1,25 Dihydroxyvitamin D₃ (1,25D)	19
1.6.2.1 Metabolism of Vitamin D.....	19
1.6.2.2 Endocrine actions of 1,25D	19
1.6.2.3 Extra-renal Synthesis of 1,25D.....	21
1.6.3 Calcitonin	22
1.7 BONE BIOMECHANICS	23
1.7.1 Stress and strain	23

1.7.2	<i>Elasticity and Plasticity</i>	23
1.7.3	<i>Ultimate Failure</i>	24
1.7.4	<i>Mechanical forces on bone remodelling</i>	25
1.8	BONE MATERIAL PROPERTIES TESTING METHODS	26
1.8.1	<i>Three- and Four-point bone bending tests</i>	27
1.9	MEASUREMENTS OF BONE STRUCTURE, CELLULAR ACTIVITY AND BONE MATERIAL PROPERTIES	28
1.9.1	<i>Static measures of bone structure</i>	28
1.9.1.1	<i>2-Dimensional measurements</i>	28
1.9.1.2	<i>3-Dimensional measurements</i>	29
1.9.2	<i>Dynamic measures of bone cell activity</i>	30
1.9.2.1	<i>Bone formation</i>	30
1.9.2.2	<i>Bone resorption</i>	31
1.10	SUMMARY	31
1.11	REFERENCES	32
CHAPTER 2: MATERIALS AND METHODS	53	
2.1	INTRODUCTION	53
2.2	MATERIALS	53
2.3	ANIMALS	53
2.3.1	<i>Housing</i>	54
2.3.2	<i>Diet</i>	54
2.3.3	<i>Semi-synthetic diet</i>	54
2.3.4	<i>Fluorochrome Labelling Injections</i>	57
2.3.5	<i>Blood sample collection</i>	57
2.4	BLOOD BIOCHEMISTRY	57
2.4.1	<i>Serum calcium and phosphate</i>	57
2.4.2	<i>Serum 1,25-dihydroxyvitamin D₃</i>	58
2.4.3	<i>Serum 25-hydroxyvitamin D₃</i>	58
2.4.4	<i>Serum parathyroid hormone</i>	58
2.5	BONE HISTOLOGY	59
2.5.1	<i>Bone preparation</i>	59
2.5.2	<i>Von Kossa staining for calcium deposition with a haematoxylin and eosin counterstain</i>	60
2.5.3	<i>Tartrate resistant acid phosphatase (TRAcP) staining of osteoclasts</i>	60
2.6	BONE HISTOMORPHOMETRY	61
2.6.1	<i>Static and dynamic bone mineral measures</i>	61
2.6.2	<i>Three-dimensional analyses using micro-computed tomography</i>	61
2.7	MECHANICAL TESTING OF BONE PROPERTIES	63
2.8	TISSUE MESSENGER RNA ANALYSES	64
2.8.1	<i>Extraction of total RNA</i>	64
2.8.2	<i>Quantification of messenger RNA</i>	65
2.8.3	<i>Synthesis of cDNA</i>	65
2.8.4	<i>Quantitative Real Time Polymerase Chain Reaction (RT-PCR)</i>	66
2.9	STATISTICAL ANALYSES	68
2.9.1	<i>One-way analysis of variance</i>	68
2.9.2	<i>Two-way analysis of variance</i>	68

2.9.3	<i>Tukey's post-hoc test</i>	68
2.9.4	<i>Linear and multiple-linear regression analysis</i>	68
CHAPTER 3: EVALUATION OF THE METHODOLOGIES FOR MECHANICAL STRENGTH TESTING OF RODENT TIBIA BONE.		70
3.1	INTRODUCTION	70
3.2	MATERIALS AND METHODS	71
3.2.1	<i>Animals</i>	71
3.2.2	<i>Micro-computed topographical analyses</i>	72
3.2.3	<i>Three-point mechanical strength testing of bone</i>	72
3.2.4	<i>Data expression and statistical analysis</i>	73
3.3	RESULTS	73
3.3.1	<i>Reproducibility of measures of mechanical testing</i>	73
3.3.2	<i>Validation of mechanical strength measures with bone histomorphometric analyses</i>	78
3.3.2.1	Correlation Between Cortical bone volume and Three-Point Testing	78
3.3.2.2	Evaluation of the Predictors of the Mechanical Parameters.....	78
3.4	DISCUSSION	84
3.5	REFERENCES	88
STATEMENT OF AUTHORSHIP		90
CHAPTER 4: VITAMIN D DEFICIENCY CAUSES IN TRABECULAR BONE LOSS WHILE PRESERVING CORTICAL BONE ARCHITECTURE AND STRENGTH IN GROWING RODENTS		93
4.1	ABSTRACT	94
4.2	INTRODUCTION	94
4.3	MATERIALS AND METHODS	96
4.3.1	<i>Animals</i>	96
4.3.2	<i>Biochemical analyses</i>	96
4.3.3	<i>Micro Computed Topographical analyses</i>	97
4.3.4	<i>Three Point Mechanical Strength Testing</i>	97
4.3.5	<i>Statistical analyses</i>	98
4.4	RESULTS	98
4.4.1	<i>Biochemistry</i>	98
4.4.2	<i>Bone structure and strength</i>	98
4.5	DISCUSSION	104
4.6	REFERENCES	106
STATEMENT OF AUTHORSHIP		108
CHAPTER 5: THE EFFECT OF DIETARY CALCIUM ON 1,25(OH)₂D₃ SYNTHESIS AND SPARING OF SERUM 25(OH)₂D₃ LEVELS		110
5.1	ABSTRACT	111

5.2	INTRODUCTION	111
5.3	MATERIAL AND METHODS	112
5.3.1	<i>Animals</i>	112
5.3.2	<i>Biochemical analyses</i>	113
5.3.3	<i>Messenger RNA analyses</i>	113
5.4	RESULTS	115
5.5	DISCUSSION	122
5.6	REFERENCES	125
 CHAPTER 6: DIETARY CALCIUM AND VITAMIN D ARE BOTH REQUIRED TO OPTIMISE BONE REMODELLING AND STRENGTH IN ADULT RATS		128
6.1	ABSTRACT	129
6.2	INTRODUCTION	130
6.3	MATERIALS AND METHODS	132
6.3.1	<i>Animals</i>	132
6.3.2	<i>Biochemical analyses</i>	132
6.3.3	<i>Micro-Computed Topographical analyses</i>	133
6.3.3.1	Trabecular bone	133
6.3.3.2	Cortical bone	133
6.3.4	<i>Three Point Mechanical Strength Testing</i>	134
6.3.5	<i>Data expression and statistical analyses</i>	134
6.4	RESULTS	134
6.4.1	<i>Serum Biochemistry</i>	134
6.4.2	<i>Body weights and bone length</i>	135
6.4.3	<i>Bone Structure, Content and Strength</i>	135
6.4.3.1	Bone Structure and Content.....	135
6.4.3.2	Bone strength.....	136
6.4.3.3	Relationships between structure and strength	136
6.5	DISCUSSION	146
6.6	REFERENCES	152
 CHAPTER 7: SUMMARY AND CONCLUSIONS		157
7.1	SUMMARY	157
7.2	THE ROLE OF MECHANICAL TESTING OF LONG BONES TO ASSESS BIOLOGICAL FUNCTION	157
7.3	THE EFFECT OF VITAMIN D AND DIETARY CALCIUM ON BONE STRUCTURE AND STRENGTH	158
7.4	LIMITATIONS	161
7.5	FUTURE DIRECTIONS	163
7.6	REFERENCES	165

ABSTRACT

Adequate dietary vitamin D and calcium intake have shown to be important in regulating skeletal development and bone mineralization. Vitamin D insufficiency is associated with increased fracture risk suggesting that a minimum 25-hydroxyvitamin D (25D) production in bone may be essential for maintaining a healthy skeleton. However, the required level of vitamin D to maintain/improve bone quality is still undetermined. This thesis investigates the regulation of dietary vitamin D on bone in young adult rats as well as the interaction between vitamin D requirement and dietary calcium intake on bone structure and the mechanical measures of bone quality in aged rats. Bone mineral content in trabecular and cortical bones and a number of biochemical factors known to regulate renal metabolism of 1,25D hydroxyvitamin D₃ (1,25D) such as PTH, calcium, 25D and 1,25D were examined. Enzymes responsible for the production of 1,25D, 25-hydroxyvitamin D₃-1 α -hydroxylase (CYP27B1) and the catabolism of 1,25D (25-hydroxyvitamin D-24-hydroxylase (CYP24)) mRNA expression in the kidney was also studied. Furthermore, bone mechanical quality was determined using 3-point bending on rat tibia with the aim to validate mechanical testing as well as determining the effects of varying levels of dietary vitamin D and calcium on bone strength.

We have previously shown that serum 25D levels, which represents the level of vitamin D status is a strong determinant of bone volume. Despite that vitamin D deficiency results in trabecular bone loss in the femur and vertebrae, further cortical bone analysis demonstrated that cortical bone volume, bone mineral distribution and strength was preserved in short term vitamin D deficiency suggesting that the effect of vitamin D deficiency in young adult rats varies between trabecular and cortical regions.

To further understand the reported effects on low dietary calcium induced bone loss, mRNA expressions of the renal enzymes were examined. High renal CYP27B1 mRNA expressions and

serum 1,25D levels in long term low dietary calcium animals suggested that the effects of bone loss may be due to 25D metabolism leading to the reduction in vitamin D status. CYP24 and other liver enzymes were not regulated by the low calcium diet.

We have reported that circulating levels of serum 25D are greater in animals fed a diet containing high levels of calcium. Thus, the effects of a high calcium diet to protect against bone loss may be due to the subsequent effects on the maintenance of circulating 25D levels. μ -CT analysis demonstrated that both femoral and tibial cortical bone volume as well as trabecular bone volume is higher in animals that are fed high calcium and vitamin D diet. Furthermore, 3-point bending demonstrated the greatest maximum load to failure was achieved in the same dietary group. Cortical bone volume and the sagittal loading are both strong determinants of ultimate load suggesting that mechanical forces and bone mineral content are crucial in maintaining the quality and function of bone strength. In addition, these results have validated our mechanical testing suggesting that bone strength is affected despite the subtle changes in cortical bone volume which may be the result of ovariectomy or dietary changes.

The studies of this thesis reveal a complex interaction between dietary calcium and vitamin D, and show that physiological changes in biochemical factors can affect structure and strength in different regions of bone. More importantly, it also demonstrate the optimal levels of dietary calcium and vitamin D that are required to prevent the development of osteoporosis

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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