



1-1-2014

Bone and cardiovascular health in the older person.

Cora McGreevy

Royal College of Surgeons in Ireland

Citation

McGreevy C. Bone and cardiovascular health in the older person. [MD Thesis]. Dublin: Royal College of Surgeons in Ireland; 2014.

This Thesis is brought to you for free and open access by the Theses and Dissertations at e-publications@RCSI. It has been accepted for inclusion in MD theses by an authorized administrator of e-publications@RCSI. For more information, please contact epubs@rcsi.ie.



— Use Licence —

Creative Commons Licence:



This work is licensed under a [Creative Commons Attribution-Noncommercial-Share Alike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/).

Bone and cardiovascular health in the older person.

Cora McGreevy MB BCh BAO(NUI), MRCPI

Royal College of Surgeons, Dublin, Ireland

A thesis submitted for the degree of Doctor of Medicine

Submitted June 2013

Supervisor: Professor David Williams

Department of Stroke and Geriatric Medicine, RCSI, Dublin, Ireland.



I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a Doctor of Medicine is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

Signed CMG

RCSI Student Number 10109668

Date 30 | 3 | 2014.

Acknowledgements

This thesis would not have been possible without the advice, support and patience of my supervisor, Professor David Williams whose knowledge, enthusiasm and engagement throughout this process have been invaluable. Sincere thanks also to Miriam Barry, our research nurse whose leadership helped bring these studies to fruition. Many thanks also to Professor Kathleen Bennett for her advice and assistance with statistical analysis. I would also like to acknowledge the financial and academic support of the Department of Geriatric and Stroke Medicine, Beaumont Hospital, Dublin. I would like to thank the patients and nursing staff of Beaumont Hospital and the nursing homes involved in my studies for their help and participation in my research.

On a personal note, my parents have always given me their unequivocal support throughout for which my mere expression of thanks does not suffice. Above all, I would like to thank my husband David for his support, encouragement and great patience at all times.

Dedicated to David, Evie, Harry & Elsa.

TABLE OF CONTENTS	Page
Table of Tables	i
Table of Figures	iii
Publications from this Thesis	v
List of Abbreviations	vi
Summary	vi-ix

CHAPTER 1

INTRODUCTION

Page

1.1 Osteoporosis

1

1.1.1 *Pathophysiology of Osteoporosis*

2

1.1.2 *Vitamin D and Osteoporosis*

3

1.2 Vitamin D

6

1.2.1 *Reference ranges for vitamin D*

7

1.2.2 *Prevalence of vitamin D deficiency*

8

1.2.3 *Risk factors for vitamin D deficiency*

12

1.3 Treatment of Osteoporosis

15

1.3.1 *Assessment of osteoporosis*

15

1.3.1.1 *Use of the FRAX tool*

15

1.3.2 *Non-pharmacological management*

16

1.3.3 *Pharmacological management*

17

1.3.3.1 *Vitamin D/ Calcium supplements*

17

1.3.3.2 *Bisphosphonates*

19

1.3.3.3 *Recombinant human PTH*

22

1.3.3.4 *Strontium Ranelate*

23

1.3.3.5 *Hormone Replacement Therapy*

24

1.3.3.6 *Selective Estrogen Receptor*

24

Modulators

1.3.3.7 *Calcitonin*

25

1.3.3.8 *Monoclonal Antibodies*

26

	Page
1.4	Management of Osteoporosis in the Nursing Home Setting 26
1.5	Vitamin D and Cardiovascular Disease 28
1.5.1	<i>Surrogate markers of vascular risk: endothelial dysfunction, arterial stiffness and wave reflection in humans</i> 30
1.5.2	<i>Vitamin D deficiency and arterial stiffness</i> 38
1.5.3	<i>Vitamin D and vascular calcification</i> 39
1.5.4	<i>Vitamin D and hypertension</i> 41
1.6	Potential role of confounders in the association between vitamin D deficiency and cardiovascular disease. 43
1.7	Aims and Objectives of Thesis 44

CHAPTER 2

REPEATABILITY OF THE MEASUREMENT OF AORTIC PULSE AVE VELOCITY(aPWV) IN THE CLINICAL ASSESSMENT OF ARTERIAL STIFFNESS OF ARTERIAL STIFFNESS IN COMMUNITY DWELLING OLDER PATIENTS USING THE VICORDER DEVICE.

2.1	<i>Introduction</i>	46
2.2	<i>Methods</i>	48
	<i>2.2.1 Ethical approval</i>	48
	<i>2.2.2 Subjects</i>	48
	<i>2.2.3 Arterial stiffness testing-studies 1 and 2</i>	48
	<i>2.2.4 Observers</i>	51
	<i>2.2.5 Statistical Approach</i>	51
2.3	<i>Results</i>	53
	<i>2.3.1 Results of Study 1: Repeatability of Vicorder PWV measurements using standard path length</i>	54
	<i>2.3.2 Results of Study 2-Repeatability of Vicorder PWV measurements using adapted path length</i>	57
2.4	<i>Discussion</i>	58
	<i>2.4.2 Strengths and weaknesses of the study</i>	60
2.5	<i>Conclusion</i>	62

CHAPTER 3

THE EFFECT OF VITAMIN D REPLACEMENT ON ARTERIAL STIFFNESS IN AN ELDERLY COMMUNITY BASED POPULATION.

3.1	<i>Introduction</i>	63
	3.1.1 <i>Aims of the study</i>	64
3.2	<i>Methods</i>	64
	3.2.1 <i>Ethical Approval</i>	64
	3.2.2 <i>Study Design</i>	65
	3.2.3 <i>Inclusion/Exclusion Criteria</i>	66
	3.2.4 <i>Screening and recruitment process</i>	67
	3.2.5 <i>Randomisation and Blinding</i>	68
	3.2.6 <i>Measures</i>	70
	3.2.7 <i>Vitamin D status</i>	72
	3.2.8 <i>Statistical Analysis</i>	72
	3.2.9 <i>Conduct of the study</i>	72
3.3	<i>Results</i>	73
	3.3.1 <i>Baseline clinical characteristics</i>	73
	3.3.2 <i>Relationship between vitamin D and PWV at baseline</i>	75
	3.3.3 <i>Response after 8 weeks in key variables</i>	76
	3.3.4 <i>Response after 8 weeks in other variables</i>	79

3.3.5	<i>Relationship between vitamin D and PWV at 8 weeks</i>	80
3.3.6	<i>Serum vitamin D levels after 8 weeks</i>	82
3.3.7	<i>Serum vitamin D levels and weight after 8 weeks</i>	82
3.4	<i>Discussion</i>	82
3.4.1	<i>Changes in vitamin D levels</i>	83
3.4.2	<i>Changes in PWV</i>	86
3.4.3	<i>Strengths and limitations of study</i>	88
3.5	<i>Conclusion</i>	89

CHAPTER 4

THE MEDICAL MANAGEMENT OF OSTEOPOROSIS IN A NURSING HOME POPULATION: A COMPARISON OF GERIATRIC LED AND GENERAL PRACTITIONER LED SERVICES.

4.1	Introduction	90
4.2	Methods	91
4.2.1	<i>Ethical Approval</i>	91
4.2.2	<i>Study Design</i>	91
4.2.3	<i>Inclusion/Exclusion Criteria</i>	91
4.2.4	<i>Recruitment Process</i>	92
4.2.5	<i>Statistical Analysis</i>	93
4.3.	Results	94
4.3.1	<i>Geriatrician led nursing home results</i>	94
4.3.2	<i>GP led nursing home results</i>	97
4.3.3	<i>Comparison of prescribing habits in geriatrician versus GP led facilities</i>	100
4.4	Discussion	101
4.5	Conclusion	106

CHAPTER 5

CONCLUSION

5.1	<i>Introduction</i>	108
5.2	<i>Conclusion of nursing home prescribing study</i>	108
5.3	<i>Conclusion of Vicorder repeatability study</i>	110
5.4	<i>Conclusion of Vitamin D/arterial stiffness study</i>	115

BIBLIOGRAPHY	116
---------------------	------------

<i>Appendices</i>	126
--------------------------	------------

Table of Tables		Page
Table 1.1	Risk Factors for Vitamin D Deficiency	10
Table 2.1	Subject demographics and haemodynamic measures	53
Table 2.2	Intra-class correlation coefficients (95% CIs) for PWV “within” nurse measures, “within” doctor and “between” nurse and doctor measures using the standard path length.	54
Table 2.3	Intra-class correlation coefficients (95% CIs) for PWV “between” nurse and doctor measures using the adapted path length.	57
Table 2.4	Comparison of previous studies validating the Vicorder device.	61
Table 3.1	Baseline clinical characteristics	74
Table 3.2	Mean/median changes in serum 25OHD and PWV over the 8 week trial period.	76

Table 3.3	Analysis of between group differences using the student t test (based on ranks of the data) including the baseline value (SD), week 8 (SD) and difference between them(SEM).	79
Table 3.4	Analysis of between group differences using the Mann Whitney U test (based on ranks of the data)	81
Table 4.1	A comparison of demographical data between geriatrician led versus GP led nursing home facilities	94
Table 4.2	Bone medications prescribed to high risk FRAX score Residents in geriatrician led nursing homes	95
Table 4.3	Bone medications prescribed to high risk FRAX score residents in GP led nursing homes	98
Table 4.4	Correlations between osteoporosis medications in geriatrician versus GP led facilities	100
Table 4.5	Multivariate predictors of odds of receiving an osteoporosis medication	101

Table of Figures	Page
Figure 1.1 Step by step metabolic process of vitamin D activation.	5
Figure 1.2 The arterial pressure wave generated with ventricular ejection propagates along the arterial tree. The speed at which the arterial pressure wave travels along the arterial tree is termed the pulse wave velocity.	31
Figure 1.3 An example of a typical healthy arterial pulse waveform.	33
Figure 1.4 Carotid-femoral pulse wave velocity (PWV) calculation.	34
Figure 1.5 Central aortic waveform and augmentation index (A)= Forward waveform, (B)=Reflected waveform (C)=Summation waveform as a result of early wave reflection in a patient with stiffened arteries.	35
Figure 1.6 Diagrammatic representation of the RANK/RANKL/OPG signalling pathway.	39
Figure 2.1 Measurement of carotid femoral pulse wave velocity using the Vicorder device.	49
Figure 2.2 Visual representation of pulse waves as captured by the Vicorder device.	50
Figure 2.3 "Within" Nurse PWV measures in the measurement of PWV using the standard path length.	55
Figure 2.4 "Between " Nurse PWV measures in the measurement of PWV using the standard path length.	55
Figure 2.5 Between doctor and nurse difference by mean for PWV	56

	using the standard path length.	
Figure 2.6	Between doctor and nurse difference by mean for PWV using the adapted path length.	58
Figure 3.1	Consort diagram of vitamin D/arterial stiffness clinical trial study design	69
Figure 3.2	Scatter plot of inverse correlation between vitamin D levels and PWV readings at baseline.	75
Figure 3.3	The dynamic changes in median PWV levels in groups receiving 100,000IU and 50,000IU vitamin D over the study period (Baseline to 8 weeks)	78
Figure 4.1	Medications prescribed to residents with known previous fractures in the geriatrician led facility.	96
Figure 4.2	Medications prescribed to residents with known previous fractures in GP led facilities.	99

Publications arising from this Thesis

McGreevy C, Williams D. New Insights about vitamin D and cardiovascular Disease. *Annals of Internal Medicine*. 2011 Dec 20; 155(12):820-6

McGreevy C, Williams D. Safety of drugs used in the treatment of osteoporosis. *Therapeutic Advances in Drug Safety*, August 2011 2: 159-172

McGreevy C, Barry M, Bennett K, Williams D. Repeatability of the measurement of aortic pulse wave(a PWV) activity in the clinical assessment of aortic stiffness in community-dwelling older patients using the Vicorder device. *Scand J Clin Lab Invest* 2013 Apr 2

List of Abbreviations

ALT-Alanine aminotransferase

BMD-Bone Mineral Density

FRAX- Fracture Risk Assessment Score

GP-General Practitioner

IM-intramuscularly

LFT – Liver Function Tests

OPG – Osteoprotegerin

PAD – Peripheral Arterial Disease

PWA-Pulse Wave Analysis

PWV – Pulse Wave Velocity

RCT-Randomised Controlled Trial

SC-Subcutaneous

Summary

It is increasingly recognised that vitamin D deficiency is highly prevalent in Ireland and worldwide particularly at higher latitudes. In addition to its well established role in bone health, initial animal studies have established a relationship between vitamin D deficiency and cardiovascular dysfunction including cardiac hypertrophy, fibrotic change and elevated blood pressure. Emerging evidence notes that age associated arterial stiffness is accelerated in the presence of cardiovascular disease and arterial ageing is a risk factor for adverse CV outcomes. PWV is currently accepted as the most simple, non- invasive, validated, robust and reproducible method to determine arterial stiffness.

Osteoporosis is highly prevalent both in Ireland and worldwide and represents a significant economic burden. Despite the high burden of osteoporosis in nursing homes, several studies have suggested that osteoporosis screening and therapies are underutilized in the nursing home population. Despite the accumulating evidence of efficacy, recent international studies of osteoporosis management in this setting indicated that intervention rates remain low, which raises concerns about underdiagnosis, and undertreatment of this disease.

In this thesis I sought to

-determine the prevalence of vitamin D deficiency among a screened population of community dwelling elderly patients.

-determine the repeatability of PWV measurement in a hospital setting in a cohort of older patients using the Vicorder apparatus.

-determine whether vitamin D replacement leads to changes in arterial stiffness in vitamin D deficient patients.

-compare the efficacy of two different doses of intramuscular vitamin D in providing supplementation and whether there is a difference in their effect on arterial stiffness.

-determine whether the medical management of osteoporosis in a nursing home population is different between a geriatric led and general practitioner led service and to assess the appropriateness of the medications prescribed.

I found that 61% of community dwelling elderly people in North Dublin were deficient in vitamin D. Physicians should have a low threshold when considering treatment of suspected vitamin D deficiency in the older population, given the high prevalence found in this and other studies. A vitamin D rich diet, exposure to sunlight along with oral supplementation should be recommended.

In the Vicorder repeatability study, I found high levels of both within- and between-observer repeatability, with values of intraclass correlation coefficients ranging from 0.8 – 0.93. Results showed that the highest repeatability was achieved using the traditional arterial path length (0.93) when compared with the adapted arterial path length (0.88). I conclude that this non-invasive method of assessing arterial stiffness has the potential to be included in the clinical assessment of older ambulant patients.

Previous studies have demonstrated that vitamin D deficiency may lead to impairment of vascular effects leading to abnormalities in central arterial stiffness. I

noted a significant improvement in Augmentation Index (Aix) from week 0 to week 8, with a mean difference of 3.803 ± 1.76 seen in the group who received the higher vitamin D dose ($p=0.033$). In the group that received 100,000IU vitamin D, median PWV decreased from $12.2(5.1-40.3)$ m/s to $11.5(4.3-14.9)$ m/s over the eight week study period ($p=0.22$). Further research is needed to investigate whether sufficient supplementation of vitamin D by a reliable method could result in positive functional changes in arterial stiffness. I was unable to find any correlation between PWV and hsCRP, MMP-9 or OPG as had been demonstrated in a number of previous studies. This may be due to our small sample size.

Low increases in vitamin D status followed the administration of 100,000IU and 50,000IU doses of cholecalciferol indicating that intramuscular use of these doses of vitamin D may not be adequate to achieve optimal vitamin D levels. Ultimately, the question of whether vitamin D supplementation improves vascular health can only be determined by performing large randomised controlled trials, specifically designed to answer this question. Until appropriate trial data are available, extending the prescribing indications for vitamin D beyond its current use in osteoporosis cannot be justified.

I calculated FRAX scores on patients recruited, a score which estimates the 10-year probability of hip fracture and major osteoporotic fractures. Results showed that when prescribing patterns for geriatric led NH residents were examined, only 33% of subjects with high FRAX scores were on calcium and vitamin D and only 11% were on bisphosphonate therapy. In a similar analysis in GP led NH residents, only 34% of those with high FRAX scores were on calcium and vitamin D with a further 14% on bisphosphonate therapy. In general, our findings demonstrated a high fracture risk

as determined by FRAX score in both types of facilities. This was coupled with a low level of use of anti-resorptive therapy and almost negligible DEXA scanning. There appeared to be no difference in prescribing patterns between the two types of nursing homes. Current guidelines for treating this subpopulation of older, often poorly mobile or bedbound elderly are unclear and further longitudinal research is needed to develop guidelines to aid the management of osteoporosis in the long-term care setting.

CHAPTER 1-INTRODUCTION

1.1 Osteoporosis

Osteoporosis is highly prevalent both in Ireland and worldwide and represents a significant economic burden. Over 75 million people worldwide are affected, with disease incidence increasing with advancing age. Over nine million osteoporotic fractures are sustained annually, ranking osteoporosis fourth in line to cardiovascular disorders, cancer, and diabetes in the non-infectious disease league table [1].

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, which leads to a consequent increase in bone fragility[2].

The diagnosis of osteoporosis is primarily based on bone mineral density(BMD).The World Health Organisation(WHO) has designated diagnostic criteria for osteoporosis based on axial skeletal measurements of bone density to allow screening of high risk individuals. BMD may be quoted as the number of standard deviations(SD) from the young adult mean from a normative population of the same ethnicity .This measure is known as the T score. Normal BMD is within 1 SD of the young adult mean(T score ≥ -1).Osteopenia is defined as between -1 SD and -2.5 SD within the young adult mean(T score between -1 and -2.5).The diagnosis of osteoporosis is made when the BMD value is at least -2.5 SD below the young adult mean(T score ≤-2.5). BMD increases in both men and women from early childhood and reaches a peak in the late teenage years or early adulthood. With increasing age, both men and women begin to lose bone gradually, although in women, there is also a more rapid phase of bone loss around the peri-menopausal period. The disease often does not become clinically apparent until a fracture occurs[3]. Consequently, many individuals experience pain, disability, and diminished quality of life as a result of having

osteoporosis. Fracture risk in later life is directly related to BMD and therefore the prevention of osteoporosis may be considered in terms of a maximisation of peak bone mass, avoidance or modification of lifestyle and environmental factors which cause bone loss, the use of specific anti-osteoporotic therapies and maintenance of postural stability and prevention of falls. The economic burden of the disease is substantial and is destined to grow as the prevalence of osteoporosis increases with age. In the United Kingdom, the over-50-year-old population is expected to grow by 25% by 2020. By 2020, annual osteoporotic fractures are predicted to increase by 21% to 230,000 per year, with costs growing by 20% to over £2.1 billion per year[4].

1.1.1 Pathophysiology of Osteoporosis

Bone architecture and its continual remodelling interact in the development of osteoporosis. The balance between bone resorption and bone deposition is determined by the activities of two principle cell types, osteoclasts and osteoblasts, which are both derived from the haematopoietic system. Osteoclasts have highly active ion channels in the cell membrane that pump protons into the extracellular space, thus lowering the pH in their own microenvironment[5]. This drop in pH dissolves the bone mineral. Osteoblasts, through an as yet poorly characterized mechanism, lay down new bone mineral. The balance between the activities of these two cell types governs whether bone is made, maintained, or lost.

A number of hormones including oestrogen, parathyroid hormone and testosterone are involved in modulating bone formation. Of these, oestrogen is now believed to have the most direct effect on bone cells, interacting with specific proteins, or receptors, on the surface of osteoblasts and osteoclasts[6]. It has been demonstrated that, following the loss of oestrogen, osteoclasts erode deeper than

normal cavities, leading to the removal of entire cancellous elements and loss of connection between the remaining ones[7]. This phenomenon is most likely due to the removal of the pro-apoptotic effect of oestrogens on osteoclasts[8].

The RANK (Receptor Activator of NF κ B) cell surface receptor has been found to be important in the stimulation of osteoclast activity by causing osteoclast precursor cells to develop into fully differentiated osteoclasts when RANK is activated by its cognate partner RANK ligand (RANKL)[9]. RANKL, in fact, is produced by osteoblasts and is one of perhaps many signalling molecules that facilitate cross-talk between osteoblasts and osteoclasts and help coordinate bone remodelling. Inhibitors of RANKL have recently shown promise as a potential treatment for osteoporosis in humans.

1.1.2 Vitamin D and Osteoporosis

Reaching peak bone mass is critical to the development and risk of age related bone loss and fracture risk in later life. A number of factors influence the attainment of peak bone mass and these include genetic, hormonal and environmental factors. Of the environmental factors, weight-bearing exercise and diet are the most crucial[10]. From a nutritional perspective, adequate intake of calcium and vitamin D (to aid calcium absorption) are vital for adequate bone mineralization and neuromuscular function. The amount of calcium absorbed through the intestine is highly dependent on both age and vitamin D status. In conjunction with parathyroid hormone, vitamin D is largely responsible for the regulation of calcium and phosphate homeostasis. As a result, vitamin D deficiency leads to calcium deficit, myopathy, osteomalacia in adults and rickets in children.

It is increasingly recognised that vitamin D deficiency is highly prevalent in Ireland and worldwide particularly at higher latitudes. There has been a resurgence of interest in vitamin D synthesis, metabolism and action due to the worsening worldwide trend towards nutritional insufficiency and the emerging knowledge regarding the nonhormonal actions of vitamin D[11]. Vitamin D obtained from sun exposure, food, and supplements is biologically inert and must undergo two hydroxylations in the body for activation[12][See Figure 1.1 below].

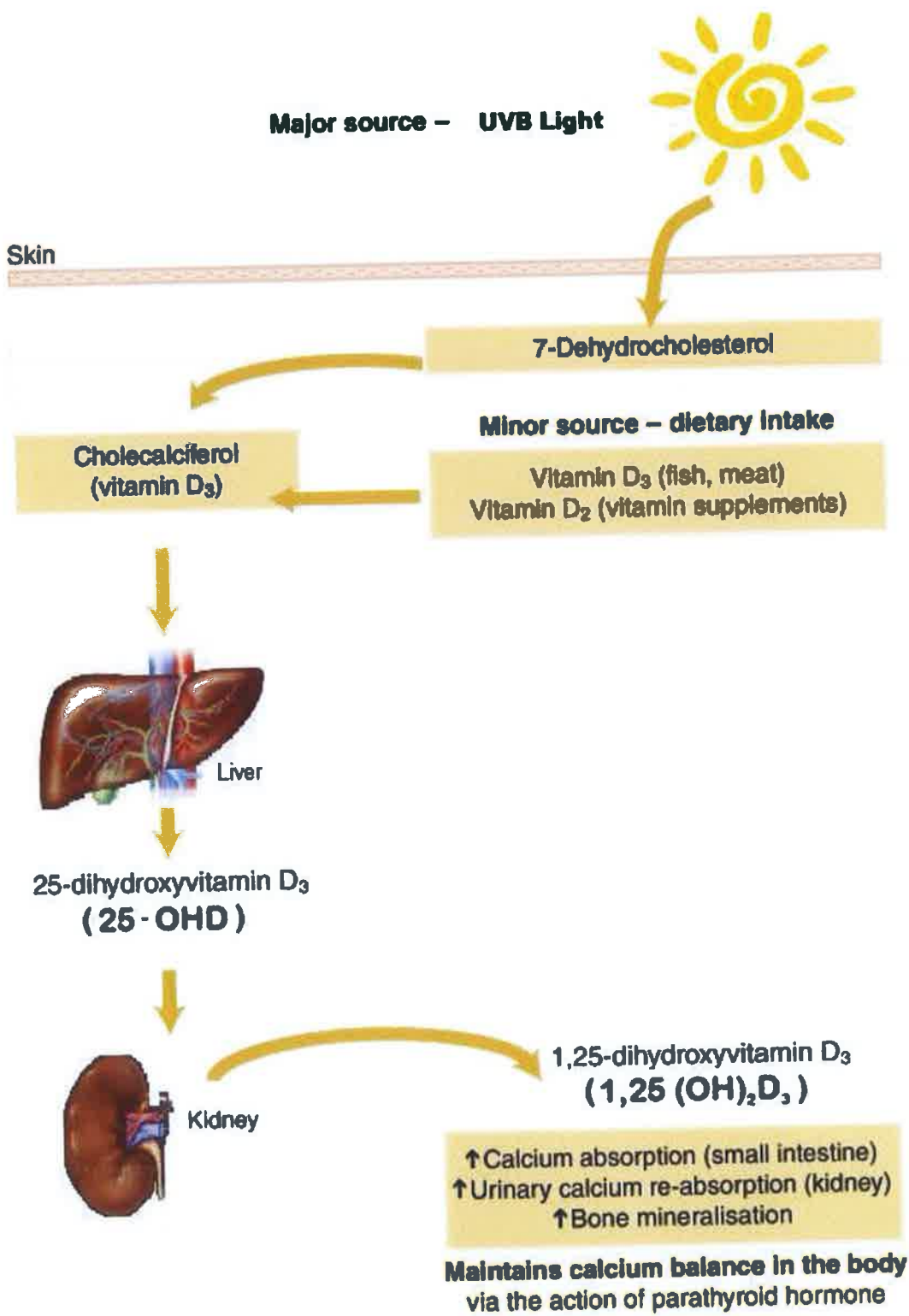


Figure 1.1: Step by step metabolic process of vitamin D activation.

Along with its role in the development of osteoporosis, vitamin D also plays a role in other regulatory mechanisms with experimental data suggesting that vitamin D affects cardiac muscle directly, controls parathyroid hormone secretion, regulates the renin-angiotensin-aldosterone system, and modulates the immune system. Because of these biologic effects, in addition to the development of osteoporosis, Vitamin D deficiency has been associated with hypertension, vascular disease and heart failure[13]. Clinical studies have shown cross sectional associations between lower vitamin D levels and plasma renin activity, coronary artery calcification, blood pressure (BP) and cardiovascular disease[14-16] which will be discussed in the section on vitamin D and cardiovascular disease later in this chapter.

1.2 Vitamin D

Vitamin D is a collection of fat-soluble steroids, the two dominant forms of which are vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol). The major circulating form of vitamin D in the blood is 25OHD (25-hydroxyvitamin D). Vitamin D₂ is manufactured by invertebrates and plants following exposure to Ultraviolet (UV) irradiation. Vitamin D₃ which is naturally present in a small range of foods is made endogenously in the skin when 7-dehydrocholesterol is exposed to UVB light between wavelengths of 270–300 nm, with maximal generation occurring between wavelengths of 295-297 nm. These wavelengths are only present in sunlight when the UV index is 3 or greater. The UV index is the internationally recognised standard measurement of the strength of UV solar radiation at a particular place on a particular day. Vitamin D₃ is produced in the skin when this level of sunlight is available and this is usually only possible during the warmer seasons in temperate regions. Apart from sunlight exposure, vitamin D₃ can also be obtained via dietary intake, and pharmaceutical supplementation. Dietary vitamin D typically comprises

only about 10-20% of circulating levels of vitamin D[17]. It has been suggested recently that the typical daily intake of vitamin D from food contributed less than UVB exposure to average year-round 25OHD levels in both Caucasian and Asian women[18].

Following the conversion of 7-dehydrocholesterol to cholecalciferol by UVB light, the first hydroxylation reaction takes place in the liver, where 25-hydroxycholecalciferol (25-OHD) is produced(Figure 1.1)[19]. This is the major ambient form of vitamin D in the body. The second hydroxylation reaction occurs in the kidneys, where 25-hydroxycholecalciferol is converted to 1,25-dihydroxycholecalciferol which is the major active form of vitamin D. Because of its long half-life, 25OHD measurements are clinically useful for assessing vitamin D status in patients[20].

1.2.1 Reference ranges for vitamin D

There is considerable controversy about what constitutes vitamin D deficiency. Current International Osteoporosis Foundation (IOF) guidelines define vitamin D insufficiency as 25OHD levels <50 nmol/L (<20 ng/ml) whereas vitamin D deficiency is defined as serum 25OHD levels below 25 nmol/L (10 ng/ml)[21].

There is no universal consensus on the level of serum 25OHD that reflects optimum vitamin D status. A recent position statement from the IOF recommended a target level of 75nmol/l, which is the level associated with maximal PTH suppression[6]. However, a recent report from the Institute of Medicine(IOM) which revised the dietary reference intakes for vitamin D and calcium for the United States and Canada

concluded that a serum 25OHD level of 50nmol/L (20ng/mL) was sufficient to ensure bone health[22]. The IOM do not support the recommendation that all adults should have vitamin D levels >75nmol/L and conclude that current evidence does not support the non-skeletal benefits for vitamin D or calcium. Furthermore, the IOM note that higher levels of both calcium and vitamin D may have adverse health outcomes including kidney stones and renal impairment.

1.2.2 Prevalence of vitamin D deficiency

Vitamin D deficiency is highly prevalent in Ireland and worldwide particularly at higher latitudes, because of the low levels of UVB light available in Winter. Ireland's northern latitude (51-55°N) makes its population particularly vulnerable to deficient vitamin D levels. One study of postmenopausal women aged 51-75 years, found that 48% of women had low levels of vitamin D during winter[23], and approximately 4% had low levels of vitamin D during summer[24]. Another Irish study found that vitamin D insufficiency, as defined by a 25OHD concentration of < 50nmol/l, was present in 75.4% of community dwelling postmenopausal women at baseline [25]. Supplementation with vitamin D has been shown to improve vitamin D status. A recent Irish study of seasonal variation of serum vitamin D and the effect of supplementation in community-dwelling older people found that vitamin D supplementation (typically cholecalciferol 800 IU/day) was associated with a mean serum 25OHD increase of 23.8 nmol/l[26] In this sample of over 500 subjects, 95.3% of the non-supplemented and 72.1% of the supplemented subjects were below the recommended serum 25OHD levels of 75 nmol/l.

Europe's largely northern latitude, coupled with the relatively short half-life of 25OHD (4-6 weeks)[27] result in levels falling significantly in winter and early spring. A 2007

study aimed at estimating the prevalence of hypovitaminosis D in the British population found that almost half of the 7437 subjects (all aged 45 years) had 25OHD concentrations <40 nmol/L during the winter and spring months[28].

In a review involving many European countries, Lips et al reported that a serum 25OHD lower than 25nmol/l was seen in 2-30% of adults, but found that the prevalence increased to 75% or more in institutionalized older persons[29].

Variation in vitamin D status within countries has been seen in a number of European studies including the SUPplementation en Vitamines et Minéraux Antioxydants (SUVIMAX)[30] and the Longitudinal Aging Study Amsterdam(LASA)[31] studies. The SUVIMAX study assessed vitamin D levels in French adults aged between 35 and 65 years and found a mean 25OHD level of 43nmol/l in the northern region and 94nmol/l in the south west of France [11]. In the Netherlands, the LASA study reported a serum 25OHD <25 nmol/l in 8% of men and 14% of women, and levels less than 50nmol/l in 45 % of men and in over 50% of the women [31].

Against expectations the SENECA (Survey in Europe On Nutrition in the Elderly, a Concerted Action) study which included 12 European countries, found a positive correlation between latitude and mean serum 25OHD, with lower concentrations in Greece and Spain than in Norway[32]. These stark variations in vitamin D status among European countries might be explained by reduced sunlight exposure, low dietary intake of vitamin D rich foods, low physical health status, limited fortification of food with vitamin D and differences in biochemical assays used[33]. Table 1.1 below lists common risk factors for vitamin D deficiency below.

Table 1.1: Risk Factors for Vitamin D Deficiency

Risk Factors for Vitamin D Deficiency

- Elderly
- Institutionalised or homebound persons
- Sunscreen with Sun Protection Factor(SPF) >15
- Darkly pigmented skin
- Air pollution
- Prolonged period of exclusive breastfeeding
- Northern latitudes
- Smoking
- Obesity
- Malabsorption syndromes
- Renal/liver disease
- Medications: Antiepileptics and human immunodeficiency virus medications

In the United States, a number of recent studies have described a high prevalence of 25-OHD deficiency (defined as 25OHD level <50nmol/L) and insufficiency (defined as levels between 50-75nmol/L) in the general population, with higher rates in older persons and racial and ethnic minorities. Ginde et al compared serum 25OHD levels from the Third National Health and Nutrition Examination Survey (NHANES III), collected during 1988 through 1994, with NHANES data collected from 2001 through 2004 (NHANES 2001-2004)[15]. During 2001 to 2004, only 23% of US adolescents and adults had serum 25OHD levels of 75nmol/L or greater. The more recent study

also describes a much higher prevalence (77% during NHANES 2001-2004) of vitamin D insufficiency (defined as levels between 50-75nmol/L) in the US population than previously reported[34].

A 2009 study of global vitamin D status found that although the exact interpretation of vitamin D insufficiency and deficiency varies, in addition to variability in assay techniques for 25OHD, serum 25OHD levels below 75nmol/L prevail in every region studied. Furthermore, very deficient levels of 25nmol/L or less are most prevalent in South Asian and the Middle Eastern regions[17]. This study included six regions (Asia, Europe, Latin America, Middle East/Africa, North America, and Oceania). More specifically, in Northern India, 96% of neonates, 91% of female schoolchildren, and over 80% of pregnant women were found to have serum 25OHD levels lower than 50 nmol/L[35]. Bangladeshi women were found to be vitamin D deficient across all age groups with levels of 37.5nmol/L or less found in 38% of high-income groups and 50% of low income group females[36]. A South American epidemiological study of postmenopausal osteoporotic women found that insufficient levels of 25OHD (<75 nmol/L) were found in 67%, 50%, and 42% of the Mexican, Chilean, and Brazilian populations respectively[37]. Overall, inhabitants of the Middle East and Africa register the highest prevalence of vitamin D deficiency worldwide, despite excellent UVB availability[17]. This may be explained by cultural dress which limits sun exposure and extended periods of breastfeeding without vitamin D supplementation. Up to 80% of adolescent girls in Saudi Arabia were found to have serum 25OHD levels <25nmol/L[38].

In a study of older Lebanese subjects, 37% of men and 56% of women had vitamin D levels < 25 nmol[39]. In all of these studies, recurrent predictors of hypovitaminosis

D throughout the Middle Eastern region are older age, female gender, multiple births, conservative dress, lower income groups and urban living [17].

Numerous studies have examined the frequency of vitamin D deficiency in the Oceanic region [40-42]. One such study which evaluated older nursing home residents in the Sydney area found that vitamin D deficiency [defined as levels <28 nmol/L] was present in almost 70% of men and 86% of women, with a mean 25(OH)D level of 17 nmol/L[40]. A separate study found that elderly Sydney based nursing home residents of Vietnamese descent were at four times the risk of deficiency (defined as <25 nmol/L) when matched with their European equivalents of Caucasian origin[41]. Younger residents of this region are also susceptible to low vitamin D levels. A large case series by Robinson et al demonstrated that significant levels of vitamin D deficiency remain despite adequate sunlight hours[42]. The median age of presentation was 15.1 months with 25OHD levels <20 mmol/L in 73% (90/ 123), and were significantly lower in the 6 month age group (88% v 68%, 20 nmol/L, p=0.033).

1.2.3 Risk factors for vitamin D deficiency

Mithal et al looked at the effects of various factors including age, gender, ethnicity, location, nutritional status, housing conditions, and physical fitness on vitamin D status [24]. Those living at lower latitudes with darker skin pigmentation and subject to cultural practices which decrease sunlight exposure are also extremely vulnerable to markedly deficient vitamin D levels [17]. In areas such as the Middle East, hypovitaminosis D is prevalent in all age groups, from very young children to the elderly[33].

Groups that appear particularly susceptible to severe deficiency worldwide include the elderly, female gender and those that are institutionalized. After comparable exposure to UVB sunlight, a 70-year-old individual produces 75% less vitamin D₃ than a 20-year-old individual [43]. A comparison of the amount of 7-dehydrocholesterol produced in the skin of young subjects compared with that produced in the skin from subjects aged between 77 and 82 found that aging significantly decreases the capacity of the skin to produce 7-dehydrocholesterol[22, 44]. The aforementioned epidemiological evidence has shown that the paediatric population of areas such as Asia, Middle East, Africa, and Oceania are susceptible to the development of rickets, in particular when their mothers are vitamin D deficient and when they are solely breastfed for long periods[17].

Low consumption of vitamin D rich foods such as oily fish and cod liver oil is also a risk factor for the development of vitamin D deficiency. Fortified foods such as low-fat milks, cereals and margarines are vitamin D rich but there are differing policies worldwide with regards to food supplementation. The United States and Canada fortify milk and a number of other dairy products and have a formal supplementation programme for infants which recommends 400IU (10µg) all year and 800IU (20µg) in winter for high-risk infants[22]. Both the United States and Canada mandate the fortification of infant formula with vitamin D: 40–100 IU/100 kcal in the United States and 40–80 IU/100 kcal in Canada[22]. In Britain, only margarine and infant formula milk are fortified with vitamin D (1-2.5 µg (40-100 IU) per 100 kcal and 8 µg (320 IU) per 100 g, respectively)[45].

Other risk factors for vitamin D deficiency including use of sunscreen with a sun protection factor (SPF) of 15 or more which has been shown to prevent approximately 99% of dermal vitamin D production[46]. Individuals with a high body

mass index (BMI) are also susceptible to vitamin D deficiency due to decreased bioavailability of vitamin D that is stored in excess adipose tissue[47].

Patients with stage 4 or 5 chronic kidney disease and an estimated glomerular filtration rate(eGFR) of <30 ml/minute per 1.73 m² of body-surface area, along with renal dialysis patients do not produce adequate levels of 1,25- dihydroxyvitamin D and need to supplement with vitamin D to suppress parathyroid hormone levels and reduce the risk of renal bone disease[48].

Patients on certain prescribed medications are also susceptible to low vitamin D levels. It is thought that long term treatment with some antiepileptic drugs such as phenytoin, carbamazepine, and phenobarbital can lead to osteomalacia as a result of induction of 1,25(OH)₂D₃ catabolism[49]. The nonnucleoside reverse transcriptase inhibitor (NNRTI) group of Highly Active anti-Retroviral Therapy (HAART) drugs used for the treatment of Human Immunodeficiency Virus (HIV) have also been implicated as a risk factor for vitamin D deficiency as they appear to increase the catabolism of 25OHD through induction of the CYP450 metabolic system[50].

1.3 Treatment of osteoporosis

1.3.1 Assessment of osteoporosis

The National Osteoporosis Foundation (NOF) recommends that pharmacologic therapy should be reserved for postmenopausal women and men aged 50 years or older who present with the following [National Osteoporosis Foundation guidelines, 2010][51]

- A hip or vertebral fracture (Vertebral fracture may be clinical or morphometric [ie, identified on a radiograph alone]).
- T-score less than -2.5 at the femoral neck, total hip, or spine after appropriate evaluation to exclude secondary causes
- Low bone mass (T-score between -1.0 and -2.5 at the femoral neck, total hip, or spine) and (1) 10-year probability of hip fracture of 3% or more or (2) a 10-year probability of any major osteoporosis-related fracture of 20% or more based on the UK-adapted WHO algorithm of the Fracture Risk Assessment Score (FRAX) score[52].

1.3.1.1 Use of the FRAX tool

FRAX is a computer-based diagnostic tool that provides models for the assessment of fracture probability in men and women. FRAX integrates clinical risk factors and bone mineral density at the femoral neck to calculate the 10-year probability of hip fracture and the 10-year probability of a major osteoporotic fracture (clinical spine, forearm, hip or shoulder fracture)[53]. The models used to develop the FRAX

diagnostic tool were derived from studying patient populations in North America, Europe, Asia and Australia. Clinical risk factors assessed include a prior fragility fracture, parental history of hip fracture, current tobacco smoking, long-term use of glucocorticoids, rheumatoid arthritis, other causes of secondary osteoporosis and daily alcohol consumption[53]. FRAX assessments are intended to provide guidance for determining access to treatment for osteoporosis in differing healthcare systems.

Using the FRAX calculator, ten-year probabilities of major osteoporotic fractures (e.g., hip, clinical vertebral, proximal humerus, distal forearm) can be estimated. This is especially useful when BMD testing is not feasible, as FRAX can compute fracture risk using body mass index (BMI) as a proxy for BMD. It has been constructed using primary data from population-based cohorts around the world.

These NOF guidelines recognize that people who have already suffered a fragility fracture are at highest risk of subsequent fractures, and advocate evaluation and treatment. Fragility fracture is a type of pathologic fracture that occurs as a result of normal activities, such as a fall from standing height or less. There are three fracture sites said to be typical of fragility fractures: vertebral fractures, fractures of the neck of the femur, and Colles fracture of the wrist. Prior fragility fracture is associated with at least a doubling of risk for subsequent fracture, regardless of the site of the prior fracture [54].

1.3.2 Non-pharmacological management of osteoporosis

A number of non-pharmacological factors have been suggested for the prevention of fractures in patients with osteoporosis. Growing evidence suggests that physical exercise reduces the risk of falling in older people. Gait training, appropriate use of assistive devices, and exercise programmes with balance training have emerged as

key components of exercise programmes for community dwelling older people[55]. Previous meta-analyses[56, 57] have suggested that an exercise programme combining low impact weight bearing exercise and high-intensity strength training maintains bone density in men and postmenopausal women. A recent Cochrane review suggests a small statistically significant, but possibly important effect of exercise on bone density compared with control groups[58].

Calcium and vitamin D also play major roles in the development of osteoporosis. Patients with low calcium intake and a low vitamin D status are at risk of developing secondary hyperparathyroidism, increased bone resorption, osteopenia and fractures. and further guidance on dietary needs is contained in the next section. For older persons, current recommendations advise the consumption of adequate dietary calcium (>1100 mg/day) together with maintaining adequate vitamin D status (>50 nmol 25OHD) to reduce risk of fracture. The increasing range of calcium fortified foods can assist in increasing the dietary calcium intake of older people. In addition to the usual dairy based food sources, vitamin D supplements are likely to be required for older people with reduced mobility and access to sunlight[59].

1.3.3 Pharmacological management of osteoporosis

1.3.3.1 Vitamin D and calcium supplements

As detailed earlier, Vitamin D is increasingly being recognized as a key element in overall bone health, muscle function and balance [60-62]. A meta-analysis of 12 double-blind, randomized, controlled trials of non-vertebral fractures (n = 42,279) and 8 randomized controlled trials of hip fractures (n = 40,886) which compared oral

vitamin D (with or without calcium) with either calcium alone or placebo performed to evaluate the efficacy of oral supplemental vitamin D in preventing non-vertebral and hip fractures among older individuals (>65 y) demonstrated that non-vertebral fracture prevention with vitamin D is dose-dependent, and a higher dose reduced fractures by at least 20% in individuals aged 65 years or older[61].

It has therefore been recommended that calcium and vitamin D be given together for the treatment of osteoporosis[63], and there is now a broad consensus that older adults should receive at least 800 to 1,000 IU per day of vitamin D in addition to calcium to reduce risk of falls and fractures[21]. Premenopausal women and men younger than 50 years without risk factors for osteoporosis should receive a total of 1000 mg of calcium daily[64]. Postmenopausal women, men older than 50 years, and other persons at risk for osteoporosis should receive a total of 1200-1500 mg of calcium daily. However, a recently published and widely reported meta-analysis of 15 randomized blinded placebo-controlled trials evaluated calcium alone supplement use (at least 500 mg daily) in more than 12,000 patients older than 40 years of age and found an increased risk of myocardial infarction (MI) in those receiving calcium supplementation alone (at least 500mg daily)[65]. Of note, the authors excluded studies that involved co-administered calcium and vitamin D supplements.

Furthermore, a recently published re-analysis of the Women's Health Initiative data demonstrated that women allocated to calcium and vitamin D who were not taking personal calcium supplements were at increased risk of cardiovascular events[66]. A meta-analysis of trials involving 29,000 people found that calcium supplements used with or without vitamin D modestly increase cardiovascular risk, suggesting their use in osteoporosis management should be reassessed.

1.3.3.2 **Bisphosphonates**

Bisphosphonates are stable analogues of inorganic pyrophosphate.

Bisphosphonates have a high affinity for hydroxyapatite crystals, and by binding at sites of active bone resorption, these agents can inhibit osteoclastic resorption. All oral bisphosphonates have a poor absorption and a bioavailability of less than 5%. Bone uptake is 20-80%, with the remainder being rapidly excreted through the kidney. They have a short plasma half-life but have a half-life of several years in bone. Bisphosphonates are the first line treatment for postmenopausal osteoporosis and are also used in steroid-induced osteoporosis and osteoporosis in males.

- Alendronate

Alendronate is given at doses of either 10mg/daily or 70mg/weekly and is available in combination with 5600IU of vitamin D. Like all oral bisphosphonates, it necessitates the patient to sit upright for 30 minutes post ingestion because of the recognised side effect of oesophagitis. It is therefore contraindicated in patients with oesophageal abnormalities or other factors that delay gastric emptying. Caution is advised with patients with renal insufficiency (GFR < 35ml/min).

The Fracture Intervention Trial (FIT) was a large RCT of alendronate that included postmenopausal women with low femoral neck bone mass (BMD \leq 0.68 g/cm²) and with (FIT I)[67] and without (FIT II)[68] prevalent vertebral fractures. The results from both arms of FIT found that alendronate significantly increased lumbar and hip BMDs and decreased the risk of new vertebral fractures. However, alendronate reduced the

incidence of hip fractures only in the group with prevalent vertebral fractures and did not reduce the incidence of non-vertebral fractures in either study arm.

The FOSamax Interventional Trial (FOSIT) was a large multinational RCT of alendronate designed primarily to examine the efficacy of alendronate in reducing the incidence of non-vertebral fractures[69]. Mean increases in BMD were significantly greater in the alendronate than in the placebo group at both the lumbar spine and the femoral neck at 12 months. The incidence of non-vertebral fractures was significantly lower in the alendronate group compared with the placebo group.

- Risedronate

Risedronate is given in doses of either 5mg/daily or 35mg/weekly. It has similar indications and contraindications to Alendronate.

A head-to-head comparison between alendronate and risedronate, the Fosamax Actonel Comparison Trial (FACT) examined BMD and suppression of bone turnover markers over 1 year [70]. This RCT included 1,053 women with low bone mass (T score at any site <-2.0) randomized to Alendronate 70 mg or risedronate 35 mg oral once weekly. Alendronate was associated with greater gains in BMD at all sites (lumbar spine, femoral neck and total hip) and greater reductions in markers of bone turnover than risedronate. The tolerability profiles were similar. However, the correlation of these results to a difference in a fracture risk reduction is unclear and was not examined in this trial.

- Ibandronate

Ibandronate is given as either 150mg monthly or 3mg intravenously every 3 months. The oral iBandronate Osteoporosis vertebral fracture trial in North America and Europe (BONE) study examined the efficacy of ibandronate to prevent new vertebral fractures. Patients were randomised to receive either placebo or oral ibandronate in daily doses of 2.5mg or intermittent doses (20mg every other day for 12 doses) every 3 months. After 3 years, the mean increases in BMD were significantly greater in the daily and intermittently ibandronate treated group compared to the placebo group[71].

- Zoledronic acid

This yearly intravenous infusion is licensed for osteoporosis in post-menopausal women. A phase II RCT examined the efficacy of different dosing regimens of zoledronate in the treatment of osteoporosis and found that BMD significantly increased at the lumbar spine and femoral neck in all zoledronate treatment groups as compared with placebo[72]. In a more recent study, treatment with zoledronic acid reduced the risk of morphometric vertebral fracture by 70% during a 3-year period, compared to placebo (3.3% incidence of morphometric vertebral fracture in the zoledronic acid group compared to 10.9% in the placebo group)[68]. Additionally, there was a reduction in the risk of hip fracture by 41%. Nonvertebral fractures, clinical fractures, and clinical vertebral fractures were reduced by 25%, 33% and 77%, respectively. Patients receiving zoledronic acid also had a significant improvement in BMD and bone metabolism markers. The results of this trial indicate

that a once-yearly infusion of zoledronic acid during a 3-year period significantly reduces the risk of vertebral, hip, and other fractures in patients with postmenopausal osteoporosis. Zoledronic acid is well tolerated and has an acceptable safety profile.

1.3.3.3 *Recombinant human PTH (parathyroid hormone)*

Recombinant human PTH is available as a treatment for post-menopausal osteoporosis. It is responsible for stimulating bone turnover and can induce renewed modelling and increase cortical thickness of bone.

- Teriparatide

Teriparatide is a biological product that contains a portion of human parathyroid hormone. Teriparatide is approved for the treatment of osteoporosis patients with increased risk of fracture. Among 2532 postmenopausal women with osteoporosis randomly assigned to receive PTH or placebo, PTH decreased new vertebral fractures [73]. The licensed dose of teriparatide is 20µg/day. It is often reserved for patients with high risk of fracture or those that have failed or are intolerant of other anti-osteoporosis therapies.

1.3.3.4 Strontium Ranelate

Strontium Ranelate is a dual action bone agent (DABA). Its mechanism of action is unclear but it potentiates osteoblast proliferation and differentiation along with inhibiting osteoclast activity at resorption sites.

It is licensed for the treatment of postmenopausal osteoporosis but is not recommended for use in patients with a GFR < 30mls/min. A recent study looked at whether strontium ranelate reduced the risk of vertebral and nonvertebral fractures over a 5 year period [74]. The authors analyzed a subgroup of 1489 female patients over 80 years of age (mean 83.5+/-3.0 years) with osteoporosis from the SOTI (spinal osteoporosis therapeutic intervention) and TROPOS (treatment of peripheral osteoporosis) studies randomized to strontium ranelate 2 g/d or placebo. All received a supplement of calcium plus vitamin D. By intention to treat, vertebral fracture risk was reduced by 31%, nonvertebral fracture risk by 27% and major nonvertebral fracture risk by 33%[74].

In a recent development, the Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicines Agency(EMA) found an increased risk for adverse cardiovascular events, including MI, in women receiving strontium ranelate compared with those who received placebo, following a routine benefit/risk assessment of trials involving about 7500 patients[75]. No increased risk of death was found. As a result of this finding, the EMA is recommending the following restrictions:

- Strontium ranelate should be used only for the treatment of severe osteoporosis in postmenopausal women at high risk for fracture and severe osteoporosis in men at increased risk for fracture.

- Strontium ranelate should not be used in patients with current or past history of ischemic heart disease (such as angina or MI), peripheral arterial disease, or cerebrovascular disease.
- Strontium ranelate should not be used in patients with hypertension that is not controlled by treatment.

1.3.3.5 *Hormone Replacement Therapy (HRT)*

Oestrogen provides a protective effect on the skeleton, which is illustrated by the rapid bone loss which follows the onset of menopause in females. HRT was the first choice treatment for post-menopausal osteoporosis prior to the introduction of bisphosphonates in the early 1990's. However, as a result of adverse findings of increased stroke [76] and increased risk of breast cancer[77], the use of HRT in the treatment of postmenopausal osteoporosis has a limited role and necessitates careful weighing up of relative risks and benefits when evaluating patients for possible treatment.

1.3.3.6 *Selective Estrogen Receptor Modulators (SERMs)*

SERMs act as weak oestrogens in some organ systems, while acting as oestrogen antagonists in others and compounds such as tamoxifen and raloxifene were initially lauded as a safer replacement for HRT in the treatment of postmenopausal osteoporosis. Raloxifene's positive effects on bone are well established [78, 79]. However, results from a number of randomized controlled trials and observational data suggests that raloxifene and tamoxifen are less potent on the skeleton than

oestrogen[[80, 81], Furthermore, the benefits of these agents in fracture risk reduction must be weighed against their increased risk of venous thromboembolism and stroke. As a result, the role of SERMs in the clinical treatment of postmenopausal osteoporosis is currently viewed as limited.

- Raloxifene

Raloxifene is the only SERM approved for the prevention and treatment of postmenopausal osteoporosis and the recommended daily dose is 60mg daily.

1.3.3.7 *Calcitonin*

Calcitonin is a polypeptide hormone that is produced in humans primarily by the parafollicular cells of the thyroid. Calcitonin acts directly on osteoclasts, resulting in inhibition of bone resorption resulting in attenuation of subchondral bone turnover. It also acts directly on chondrocytes, attenuating cartilage degradation and stimulating cartilage formation. It is usually administered in intra-nasal form. However, due to the recent publication of studies that demonstrated a higher cancer risk in patients receiving calcitonin-containing medicines compared to those patients receiving placebo, long term use of calcitonin for the treatment of post-menopausal osteoporosis is no longer recommended by the European Medicines Agency (EMA)[82].

1.3.3.8 Monoclonal Antibodies

- Denosumab

Denosumab was approved by the US Food and Drug Administration (FDA) in June 2010. It is a human monoclonal antibody to receptor activator of nuclear factor-kappaB ligand (RANKL), inhibits osteoclast activity, decreases bone resorption, and increases bone density. Because of this, its use has been investigated in populations prone to osteoporosis (eg, postmenopausal women, men receiving androgen-deprivation therapy for prostate cancer). In a randomized placebo-controlled trial, Cummings et al studied 7868 women with osteoporosis (age range, 60-90 y) who received either denosumab 960 mg subcutaneously every 6 months for 36 months or placebo. Compared with placebo, denosumab decreased the risk of vertebral, and nonvertebral fractures in women with osteoporosis[83]. Denosumab is indicated for prevention of fracture in postmenopausal women with osteoporosis and high fracture risk (ie, history of osteoporotic fracture, failed other treatments) and is given as a 60mg subcutaneously every 6 months. Recent reports indicate that patients with renal impairment who receive denosumab have an increased risk for the development of hypocalcemia[84].

1.4 Management of Osteoporosis in the Nursing home setting

Previous United States (US) epidemiologic studies have demonstrated that 70% to 85% of nursing home residents have osteoporosis. Despite the high burden of osteoporosis in nursing homes, several studies have suggested that osteoporosis screening and therapies are underutilized in the nursing home population[85]. Colón-Emeric et al examined osteoporosis treatments in 67 US nursing homes (895

residents) and found that residents with osteoporosis or recent fracture had moderate use of calcium (69%) and vitamin D (63%) but prescribing frequency was also low for other pharmacologic therapies including bisphosphonates(19%) and calcitonin(14%) with other osteoporosis medications being prescribed to less than 5% of the cohort[86].

A US study of >180,000 nursing home residents found that only 9.1% received anti-resorptive medications and/or supplements indicated for osteoporosis treatment despite the expected high prevalence of osteoporosis in this setting[87]. In a study of over 29,000 nursing home residents, only 25% of those diagnosed with osteoporosis received antiosteoporosis drugs. Females were more likely than males to receive antiosteoporosis drugs (adjusted odds ratio [OR], 1.41; 95% confidence interval [CI], 1.26 to 1.57) with both increasing age and level of cognitive impairment inversely related to receipt of antiosteoporosis drugs [88]. Furthermore, a study which compared the use of anti-resorptive therapy between nursing home residents and community dwelling older adults found that there was under-usage of anti-osteoporosis treatments in both cohorts. Nursing home residents were less likely to be prescribed anti-resorptive treatment compared with their community dwelling counterparts [89].

Despite the accumulating evidence of efficacy, a recent review of osteoporosis management indicated that intervention rates remain low, which raises concerns about underinvestigation, underdiagnosis, and undertreatment[90]. Perhaps most concerning is that consistently low intervention rates persist even among those patients who have suffered a previous fragility fracture.

It appears that the low level of fracture protection overall, combined with the high variability amongst different healthcare settings, suggests that there is significant room for improvement in the management of osteoporosis care in nursing homes. There has been no previous research on osteoporosis medication use in nursing homes in Ireland.

1.5 Vitamin D deficiency and cardiovascular disease

The association between vitamin D deficiency and cardiovascular disease corresponds with the influence of latitude, with higher rates of ischaemic heart disease (IHD) noted in countries with lower levels of UVB exposure[91]. Vitamin D levels have been shown to be seasonal with higher levels in Summer[92] and the rate of IHD has been shown to display similar seasonal patterns[93, 94].

Initial animal studies established a relationship between vitamin D deficiency and cardiovascular dysfunction including cardiac hypertrophy, fibrotic change, elevated blood pressure, as well as alterations of serum calcium, parathyroid hormone, and renin levels[14]. The studies supported a role for vitamin D in maintaining cardiovascular health through both a direct action of the vitamin on cardiomyocytes and indirect actions on circulating hormones and calcium[16]. The vitamin D receptor (VDR) is found throughout the body in several tissue types such as lymphocytes, colonic, liver and cardiac cells[13, 15]. Previous studies have demonstrated associations between low vitamin D levels and plasma renin activity, calcification of cardiac vessels, hypertension and cardiovascular disease [95-98]. Additionally, epidemiological studies have reported a trend towards a higher prevalence of

ischaemic heart disease and hypertension with increasing distance from the equator, and attribute these rates to the higher rates of vitamin D deficiency in regions with less exposure to sunlight [99, 100].

Wang et al studied over 1700 Framingham Offspring Study participants (mean age 59 years) without prior cardiovascular disease and measured their 25OHD levels[101]. During a follow up period of 5 years, 120 individuals had an initial cardiovascular event. Participants with lower vitamin D levels <37nmol/L were found to have a hazard ratio of 1.62 (95% confidence interval 1.11 to 2.36, $P=0.01$) for developing incident cardiovascular events in comparison to those with 25-OHD levels greater than 37nmol/L[101]. This effect was seen in subjects with hypertension only. The authors concluded that low vitamin D levels are correlated with incident cardiovascular disease and proposed several potential mechanisms that might explain their findings. These included the role of 1,25 OHD in the renin-angiotensin axis by direct inhibition of renin gene expression and the potential role of vitamin D in vascular function including inflammation, smooth muscle growth and thrombosis[101]. As the positive findings were only found in subjects with hypertension, the authors proposed that hypertension could enhance the adverse effects of hypovitaminosis D on the cardiovascular system given their joint roles in vascular remodelling.

A prospective case-control study of over 18,000 men found a significant correlation between low 25OHD levels and an increased risk of myocardial infarction, which remained after adjustment for traditional cardiovascular risk factors[102]. This study confirmed the findings of other smaller studies that looked at the association between vitamin D status and cardiovascular risk. These cross-sectional studies have linked lower vitamin D levels with acute stroke[103], myocardial infarction[104],

congestive cardiac failure[105], and cardiovascular disease[106]. Low 25OHD levels were found in patients presenting to hospitals with myocardial infarction[104] and stroke[103].As vitamin D has a long half-life, it is likely that these low levels predated the cardiovascular incidents.

1.5.1 Surrogate markers of vascular risk: endothelial dysfunction, arterial stiffness and wave reflection in humans.

To fully understand arterial stiffness, one must understand the main functions of the arterial system and mechanisms affecting blood flow through arterial tree. Along with acting as a conduit for delivering blood from the left ventricle into the circulation, the arterial system buffers the pulsatile blood flow from the left ventricle and convert it to steady and almost continuous flow to the peripheral vasculature. This is called the 'windkessel function'[107]. This function is possible because arteries are compliant, with the ability to expand due to pressure and the ability to recoil. Although the windkessel model of the circulation helps to explain the importance of elastic and conduit characteristics of arteries, this theory assumes that the arterial tree has separate elastic and conduit compartments and also does not take the existence of wave reflection into account. In fact most of the arterial tree has both of these functions combined, although one or the other tends to predominate in any given arterial segment. That combination of function leads to wave reflection which is discussed in the following section. The arterial pressure wave is generated with ventricular ejection which propagates through the arterial tree. The speed at which the arterial pressure wave travels along the arterial tree is termed the pulse wave velocity(Figure 1.2).

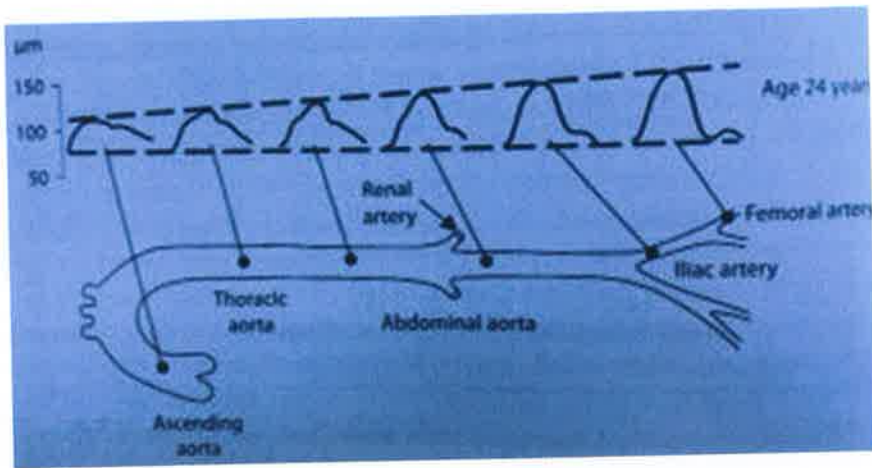


Figure 1.2- The arterial pressure wave generated with ventricular ejection propagates along the arterial tree. The speed at which the arterial pressure wave travels along the arterial tree is termed the pulse wave velocity[adapted from 108].

Age associated changes in arterial structure and function have long been recognised as part of normal ageing. However, emerging evidence notes that age associated arterial stiffness is accelerated in the presence of cardiovascular disease and arterial ageing is a risk factor for adverse CV outcomes[109]. A number of surrogate markers of vascular ageing have been explored extensively in both the research and clinical settings and these include endothelial dysfunction and more recently arterial stiffness and wave reflection.

Endothelial dysfunction: Normal healthy endothelium acts as a medium for exchange of materials between blood and tissues, and plays a key role in the mechanisms of blood flow, inflammation cascades and blood clotting. It secretes nitrous oxide which acts as a defence against atherosclerosis by regulating vessel tone, cellular adhesion, resistance to clotting, smooth muscle cell production and vessel wall inflammation[110]. Endothelial dysfunction is defined by a change of the actions of the endothelium toward decreased vasodilation, creation of a proinflammatory state,

and prothrombotic state. It is associated with most forms of cardiovascular disease, such as hypertension, coronary heart disease, chronic heart failure, peripheral vascular disease along with diabetes mellitus, and chronic renal failure[111]. Endothelial dysfunction plays an important role in the pathogenesis of atherosclerosis, with in vivo evidence of its contribution to plaque initiation and progression[112]. The extent of endothelial dysfunction has been shown to have prognostic value for predicting future cardiovascular events. Endothelial dysfunction is also associated with increasing arterial stiffness [112]. The current method for assessing endothelial dysfunction is flow mediated dilatation(FMD) which measures the diameter of an artery by non-invasive ultrasound before and after increasing stress(provided by reactive hyperaemia).The degree of dilatation appears to reflect arterial NO release[113, 114].FMD has been shown to be reproducible and reliable[115], but can be technically difficult and requires specialised training.

Arterial stiffness and wave reflection:

Arterial stiffness is a generic term that describes the rigidity of the arterial wall. Many terms exist in this field, often with slightly different interpretations. Arterial stiffness has been chosen as a generic term in this thesis to avoid confusion and describes the rigidity of arterial walls[116].The arterial wall is structurally made up of three layers, with varying degrees of elasticity which is present to counter the pulsatile ejection of blood from the heart. The circulation distributes cardiac output via a series of branching networks, and this model serves to explain the concept of wave

reflection. At every branching of an artery, a small proportion of the forward travelling pulse is reflected backwards.

Thus the arterial waveform is a summation of both the forward travelling wave pulse and the reflected wave travelling back towards the heart[Figure 1.3].

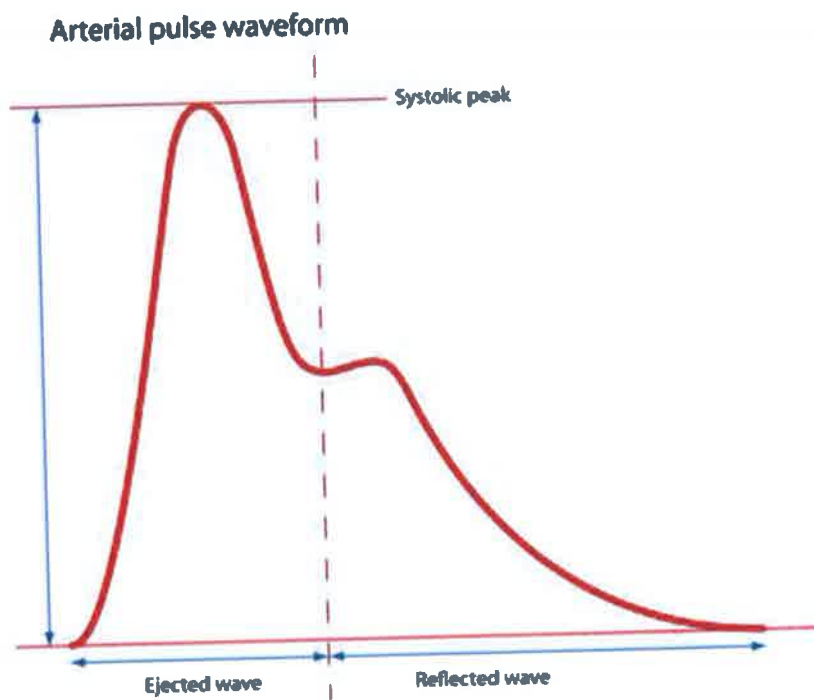


Figure 1.3: An example of a typical healthy arterial pulse waveform.

This arterial wave travels down the large aorta from the heart and gets reflected at the bifurcation of the aorta into the iliac arteries. In a healthy subject, the reflected wave usually returns in the diastolic phase, after the closure of the aortic valves. The returned wave helps in the perfusion of the heart through the coronary vessels as it pushes the blood through the coronary arteries.

It is possible to calculate the velocity of this wave (ie; Pulse wave velocity) from the delay between two BP curves located at two different sites in the arterial tree and

this is feasible once the distance between measuring sites is known (Figure 1.4). Given that pulse waves travel faster in stiffer arteries, the measurement of PWV is considered an effective surrogate measure of arterial stiffness [117]. PWV is currently accepted as the most simple, non-invasive, validated, robust and reproducible method to determine arterial stiffness [118].

PULSE WAVE VELOCITY

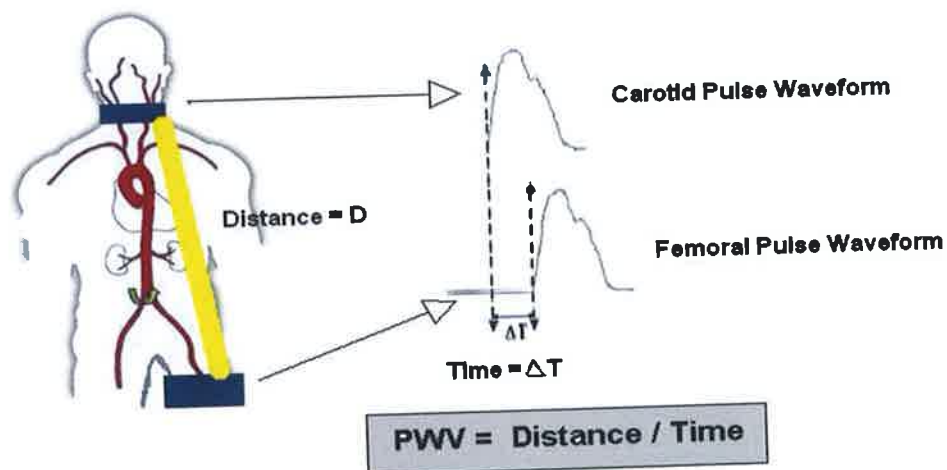


Figure 1.4 Carotid-femoral pulse wave velocity (PWV) calculation.

Augmentation Index (AIx)

The stiffer the arteries are, the faster the wave returns, adding to the forward wave and augmenting the systolic pressure. Therefore, augmentation of the aortic pressure wave is an index of wave reflection [119]. This can be expressed in absolute terms as the augmentation pressure or as a percentage of pulse pressure as the

Augmentation index (AIx)(Figure 1.5) defined as the difference between the 2 systolic peaks expressed as a percentage of the pulse pressure[120]. Large values of AIx indicate increased wave reflection from the periphery and/or earlier return of the reflected wave as a result of increased PWV (owing to increased arterial stiffness) and vice versa. PWV and wave reflection indexes often change in parallel because the PWV affects the timing of the merging of incident and reflected waves[121].

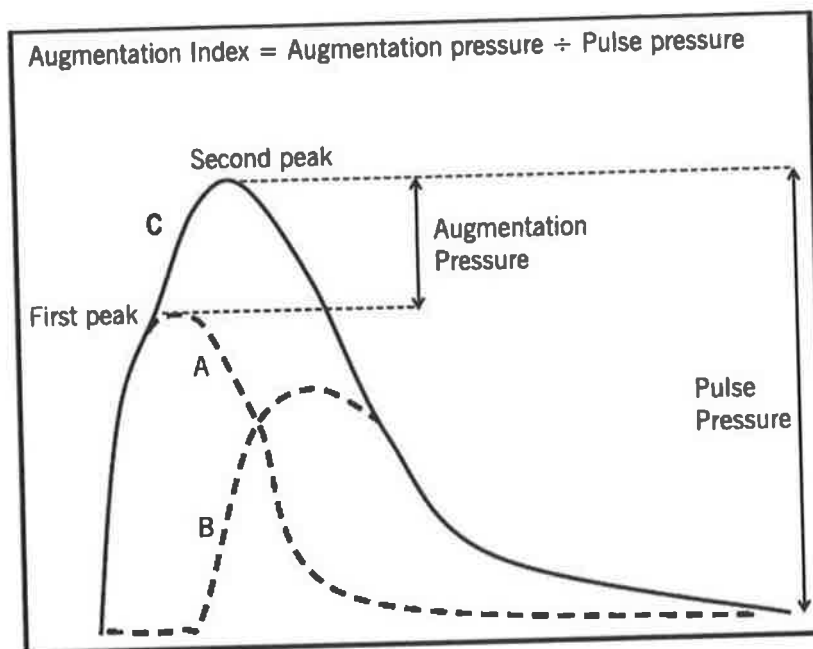


Figure 1.5: Central aortic waveform and augmentation index. (A)= Forward waveform,(B)=Reflected waveform ,(C)=Summation waveform as a result of early wave reflection in a patient with stiffened arteries(adapted from [120]).

When arteries become stiffer(with age or disease), this relationship is changed as the forward wave speeds up, hits the branch points early resulting in an earlier reflectance wave that arrives at the heart while it is still in systole and not yet relaxed

to allow blood flow to its arteries. As a result of this, the vascular supply to heart is compromised and puts the contracting heart under additional strain.

A number of commercial devices exist for arterial stiffness measurement although at present no consensus exists as to which is the most accurate or reproducible. The SphygmoCor® system (ArtCor, Sydney, Australia) uses a single high fidelity applanation tonometer to obtain a proximal (i.e. carotid artery) and distal pulse (i.e. radial or femoral) recorded sequentially a short time apart and calculates PMV from the transit time between the two arterial sites [122]. The arterial pulse waveform is a contour wave generated by the heart when it contracts, and it moves along the walls of the arterial vessels. A novel relatively operator-independent device (Vicorder® system (Skidmore Medical, Bristol, UK) is now available which has potential advantages for screening programmes and use in intervention studies [123]. It has compared favourably with the more established SphygmoCor device, considered by some to be the gold standard for measuring arterial stiffness in normal individuals [124]. The Vicorder measures simultaneous pressure waveforms by a volume displacement technique, using blood pressure cuffs placed around the sites of interest. It measures arterial stiffness by measuring carotid to femoral pulse wave velocity (PWV) [124]. Arterial pulse waveforms are recorded using PVR (pulse volume recording) measurements from a standard vascular cuff. The waveforms can be acquired in up to two sites simultaneously to provide pulse transit time (TT). The Vicorder simultaneously records the oscillometric blood pressure wave from the proximal and distal ends of the arterial segment under consideration. These waves are simultaneously sampled at 1.8 sec intervals. Once a screen of data has been captured, the region of the foot for each proximal and distal wave is identified and time marked. The time delay is then computed by cross correlating the proximal time

markers with the distal time markers and an average time delay is computed from the time to the peak in the cross correlation function. Given, its non-invasive properties, we felt it an appropriate device for use in the elderly population and to our knowledge, it has not been validated in this cohort previously. In both the repeatability study and the clinical trial, we used two methods to measure arterial path length- standard path length and an adapted path length measure. Standard path length is the distance from the suprasternal notch to the top of the thigh cuff as indicated by the manufacturer. The rationale for the use of an adapted path length was that a previous validation study had found that there was a significant difference in transit times between the Vicorder when compared to the highly validated SphygmoCor[[124] . Findings showed that the Vicorder tended to report lower PWV values at higher values of aortic stiffness when compared with the SphygmoCor device and the authors recommended the use of a formula for calculation of an adapted path length[124].

Formula

Adapted path length = Suprasternal notch to top of thigh cuff-notch to carotid pulse +65mm

The Vicorder is further described in Chapter 2.

1.5.2 Vitamin D deficiency and arterial stiffness.

Previous evidence has suggested that arterial stiffness may precede and contribute to the development of cardiovascular disease and is a predictor of long term morbidity and mortality[125]. A recently published study by Dong et al aimed to determine the effect of daily vitamin D supplementation on arterial stiffness as measured by pulse wave velocity (PWV)[126]. The experimental group received 2000IU cholecalciferol orally daily and the control group received 400IU cholecalciferol daily. Results showed that vitamin D supplementation with 2000IU daily led to a reduction in PWV (and therefore reduced arterial stiffness) from baseline to post-test [5.41 +/- 0.73 m/sec to 5.33 +/-0.79 m/sec) ($P=0.031$)]. A 2011 study by Al Mheid et al investigated the mechanisms underlying the link between 25OHD and arterial stiffness and found that vitamin D insufficiency was associated with increased arterial stiffness as measured by PWV[127].

Furthermore, vitamin D deficiency correlates with higher circulating concentrations of matrix metalloproteinase-9 (MMP-9), which controls vascular wall remodelling. Plasma MMP9 levels increase in the circulation in unstable angina [128]and acute coronary syndrome[129]. Vitamin D supplementation has been shown to be associated with decreased serum matrix metalloproteinase-9 concentrations [130]. In this interventional study, patients were randomised to receive 3 monthly intramuscular doses of cholecalciferol ['high' (50 000 IU) or 'low' (500 IU)] over one year. Both 'high' and 'low' groups demonstrated comparable and significant increases in vitamin D status and plasma MMP9 levels related inversely to vitamin D status.

1.5.2 Vitamin D and vascular calcification.

Previous studies have revealed an inverse association between 25-hydroxyvitamin D concentrations and subclinical atherosclerosis as measured by computed tomography-derived calcified atherosclerotic plaque (CP) or carotid intima-media thickness[131, 132]

A number of bone proteins have also been found to be components of the arterial wall. These include osteopontin(OPN), osteocalcin(OCN), matrix-gla proteins(MGP) and osteoprotegerin(OPG). More recently, OPG has attracted the most interest for its role in vascular calcification. OPG inhibits the binding of RANK L (receptor activator of nuclear factor-K ligand) to the RANK receptor, thereby blocking intercommunication between osteoblast cells and osteoclast precursors(see Figure 1.6 below). This action inhibits the differentiation of the osteoclast precursor into a mature osteoclast.

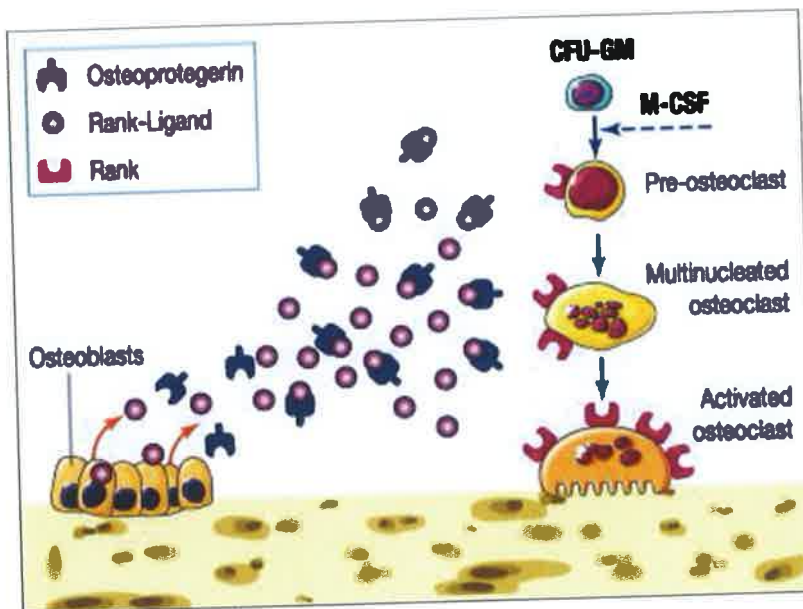


Figure 1.6: Diagrammatic representation of the RANK/RANKL/OPG signalling pathway [138].

In- vitro studies and those in animal models, both suggest that OPG inhibits vascular calcification[133]. In an OPG deficient mouse model, deficiency of OPG resulted in marked vascular calcification along with osteoporosis [134]. Subsequent to this study, OPG was seen as a putative link between bone and vascular disease. Despite the inverse relationship between OPG and vascular calcification in animal studies, clinical studies on humans suggest that serum OPG levels may be elevated in association with calcification of vessels, ischaemic heart disease and stroke[135]. This has led to increased interest in the potential of OPG as a possible biomarker of vascular disease. One theory proposed is that OPG may support the development of endothelial dysfunction by inhibiting RANKL signalling which results in the impairment of nitrous oxide (NO) release [136]. Inhibition of NO release is an initial step in the development of endothelial dysfunction, a forerunner for the future development of atherosclerosis. Furthermore, in vitro studies have implicated OPG with the promotion of leukocyte/endothelial cell adhesion which plays a key role in the onset of endothelial dysfunction [137]. These studies contribute to evidence that increased OPG serum levels are correlated with symptomatic and unstable cardiovascular disease. The exact role of elevated OPG as a potential marker of vascular damage has not, as yet, been fully elucidated.

Data collected in the National Health and Nutrition Examination (NHANES) study also examined the link between vitamin D and atherosclerosis. Low serum 25OHD levels were found to be correlated with higher levels of peripheral arterial disease (PAD) providing support for the theory that vitamin D may have potent anti-atherosclerotic properties[139].

Hypovitaminosis D is also associated with decreased levels of HDL cholesterol-associated apolipoprotein A-I[140] and vitamin D supplementation has been shown to have a beneficial effect on the elastic properties of the arterial wall (compliance coefficient (CC), distensibility coefficient (DC), intima-media thickness (IMT) and the Young's Modulus (E)) in a randomized, placebo-controlled interventional study in postmenopausal women[141]

1.5.3 Vitamin D and hypertension

Previous observational studies have suggested possible links between low 25OHD levels and a subsequent higher risk of hypertension. A Swedish study found that men with 25OHD concentrations $<37.5\text{nmol/L}$ had a 3-fold higher prevalence of confirmed hypertension compared to those with higher 25OHD levels [Odds Ratio = 3.3, 95%CI: 1.0–11]. This study suggests that low plasma 25OHD concentration may have an association with a higher prevalence of confirmed hypertension [142].

However, findings from randomized-controlled trials of vitamin D supplementation and blood pressure have so far showed inconsistent results, possibly as a result of differences in sample sizes, vitamin D preparations used and duration of studies [143-145].

The proposed mechanism for the link between vitamin D and high blood pressure revolves around the role of vitamin D in the inhibition of the Renin-Angiotensin System (RAS). This data is mainly derived from in vitro and animal studies [95, 146, 147]. Increased activation of the RAAS was seen in the vitamin D receptor (VDR) and 1α -hydroxylase knockout mice [95] with resolution of the ensuing hypertension and myocardial abnormalities noted after administration of vitamin D [147]. High renin

levels have been associated with low 1,25(OH)₂D levels in a clinical study of patients with known hypertension[148]. Further randomized clinical trials are needed to fully elucidate the clinical relevance of vitamin-D mediated suppression of the RAAS.

There is increasing evidence that secondary hyperparathyroidism and hypocalcaemia, which are commonly seen in patients with hypovitaminosis D may be an alternative explanation for the association between vitamin D deficiency and hypertension. Previous observational studies have shown a correlation between parathyroid hormone and hypertension[149, 150]. The pathogenesis for this correlation is as yet unclear, but Fitzpatrick et al suggest that parathyroid hormone may increase arterial stiffness and induce atherosclerotic changes by acting on smooth muscle cells in the endothelium[151]. One double blind, randomized, controlled trial examined the effects of calcium and vitamin D on blood pressure and PTH levels compared with calcium alone over an 8 week period. Compared with calcium, supplementation with a combination of vitamin D and calcium resulted in a significant increase in serum 25OHD of 72% ($p < 0.01$), a decrease in serum PTH levels of 17% ($P = 0.04$), along with significant decreases in systolic blood pressure(SBP) and heart rate[152].

Furthermore, a possible association of 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃] and blood pressure levels has been demonstrated in normotensive men[153]. This study examined levels of serum calcitriol, parathyroid hormone, serum calcium, blood pressure, diet and demographic variables in normotensive men. After adjustment for possible confounders, multivariate analyses revealed a significant association between calcitriol levels and systolic blood pressure suggesting a possible link between the active metabolite of vitamin D and blood pressure levels[153].

1.6 Potential role of confounders in the association between vitamin D deficiency and cardiovascular disease.

As previously mentioned, many observational studies have reported inverse correlations between levels of serum 25-hydroxyvitamin D (25OHD) and the risk of a wide range of conditions including vascular disease[154], autoimmune disease[43], type 2 diabetes mellitus [106], obesity[45] and more recently cognitive impairment[155].

A recent editorial by Grey et al[156] commented that "it seems intuitively unlikely that a single hormone could play a substantial role in preventing or ameliorating the diverse range of diseases that have been linked to low levels of vitamin D". There are a number of possible explanations for these links which include the influence of confounders. It has been suggested that vitamin D may only act as a surrogate marker for poor health status reflecting an inability to get outdoors for UVB exposure due to possible increased body mass index (BMI), multiple comorbidities and poor exercise tolerance. This view is supported by the study by Llewelyn et al[155] who examined whether low levels of serum 25 OHD were correlated with an increased risk of cognitive decline in an older Italian cohort. Multivariate analysis showed that the MMSE (Mini Mental State Exam) scores of patients with a lower baseline level of serum 25 OHD (<25nmol/L) disimproved by an additional 0.3 MMSE points per year more than those with sufficient levels of 25OHD (>75nmol/l) at baseline. Interestingly, other markers of poor health status such as impaired mobility, significant depressive symptoms and lower total energy intake were more commonly seen in the vitamin D deficient group suggesting that deficiency may be in fact be a marker of poorer health status in general.

1.7 Aims and Objectives of this thesis

The aims of this thesis are

1. To determine the prevalence of vitamin D deficiency among a screened population of community dwelling elderly patients.
2. To determine the repeatability of PWV measurement in a hospital setting in a cohort of older patients using the Vicorder apparatus.
3. To determine whether vitamin D replacement leads to changes in arterial stiffness in vitamin D deficient patients.
4. To compare the efficacy of two different doses of intramuscular vitamin D in providing supplementation and whether there is a difference in their effect on arterial stiffness.
5. To determine whether the medical management of osteoporosis in a nursing home population is different between a geriatric led and general practitioner led service and to assess the appropriateness of the medications prescribed.

The objectives will then be

1. To determine the repeatability of PWV measurement in an older population using the Vicorder device.
2. To examine the prevalence of vitamin D deficiency in a community dwelling population using a cohort of screened elderly patients.

3. To perform a randomised clinical trial to determine whether vitamin D replacement leads to changes in arterial stiffness in vitamin D deficient patients.
4. To examine the difference in prescribing patterns of geriatricians and general practitioners of osteoporosis related medications in the nursing home setting.

CHAPTER 2

REPEATABILITY OF THE MEASUREMENT OF AORTIC PULSE WAVE VELOCITY(aPWV) IN THE CLINICAL ASSESSMENT OF ARTERIAL STIFFNESS OF ARTERIAL STIFFNESS IN COMMUNITY DWELLING OLDER PATIENTS USING THE VICORDER DEVICE.

2.1 Introduction

Pulse wave velocity (PWV) is an indicator of arterial stiffness and appears to be predictive of future cardiovascular risk [[157-160]. Recently developed non-invasive approaches in the measurement of arterial stiffness have made this method applicable for the examination of larger populations. Carotid to femoral PWV is considered the “gold standard” measurement for arterial stiffness because it is the most simple, noninvasive, robust, and reproducible method[118].

The recent Vicorder® system measures simultaneous pressure waveforms by a volume displacement technique, using blood pressure cuffs placed around the carotid and femoral sites[124]. This technique has shown a good intraobserver and interobserver variability with little operator training, and PWV values are in good agreement with those from SphygmoCor applanation tonometry [124, 161]. Given its non-invasive properties, we felt it an appropriate device for use in the elderly population and to our knowledge, it has not been validated in this cohort previously.

We used two methods to measure arterial path length- standard path length and an adapted path length measure. Standard path length is the distance from the suprasternal notch to the top of the thigh cuff as indicated by the manufacturer. The

rationale for the use of an adapted path length was that a previous validation study had found that there was a significant difference in transit times between the Vicorder when compared to the highly validated SphygmoCor[[124] . Findings showed that the Vicorder tended to report lower PWV values at higher values of aortic stiffness when compared with the SphygmoCor device and the authors recommended the use of a formula for calculation of an adapted path length[124].

Formula

Adapted path length = Suprasternal notch to top of thigh cuff-notch to carotid pulse +65mm

The aim of this study was to assess the 'within' and 'between' observer repeatability of PWV measurement performed in a hospital setting by clinical staff with limited previous experience of arterial stiffness and pulse wave velocity measurement in a cohort of community dwelling older patients using both standard and adapted path length measures.

A further aim was to prove the reliability in advance of using the Vicorder to measure arterial stiffness as part of a clinical trial evaluating arterial stiffness in a vitamin D deficient older population.

2.2 Methods

2.2.1 Ethical approval

Approval was obtained from the local Research Ethics Committee as part of a larger clinical trial (see Chapter 3) and written informed consent was obtained from all participants.

2.2.2 Subjects

PWV was measured in 25 consecutive patients (15 males, 10 females) all aged 65 and over attending Beaumont day hospital who were undergoing screening for the aforementioned clinical trial. Patients did not speak/sleep during assessment. They had been asked to refrain from eating/drinking/smoking for 4 hours prior to assessment as described in the expert consensus document on arterial stiffness measurement[118].

2.2.3 Arterial stiffness testing

Study 1: Repeatability of Vicorder PWV measurements using standard path length

After an initial rest period of 15 minutes with subjects in a supine position in a quiet, temperature controlled room, brachial blood pressure was measured using an automatic device, a Welch Allyn Vital Signs Monitor 300 blood pressure monitor. Blood pressure was measured twice by the nurse and twice by the doctor and documented as an average value of the nurse/doctor measurements. PWV were measured using the Vicorder® system (Skidmore Medical, Bristol, UK). The Vicorder device estimates aortic stiffness by measuring carotid to femoral (aortic)

pulse wave velocity (aPWV) using an oscillometric technique and was chosen for use in this trial because it represented a non-invasive method of assessing arterial stiffness[124]. Arterial pulse waveforms were recorded using PVR (pulse volume recording) measurements from a standard vascular cuff. The waveforms can be acquired in up to two sites simultaneously to provide pulse transit time (TT) and pulse wave velocity (PWV) measurements for the assessment of arterial stiffness.

PWV measurements were obtained by placing a 100mm blood pressure cuff around the upper thigh to capture the femoral pulse reading and a 30mm plethysmographic partial inflatable sensor cuff around the neck at the level of the carotid artery(see Figure 2.1 below). Standard path length was defined as the distance from the suprasternal notch to the top of the thigh cuff as indicated by the manufacturer.



Figure 2.1: Measurement of carotid femoral pulse wave velocity using the Vicorder device(Smart Medical TM).

The cuffs were inflated to 60mmHg and high quality waveforms were recorded for 10 seconds with the patient lying supine, using a volume displacement method. The nurse/doctor assessed the quality of the pulse waves captured visually on screen (see Figure 2.2 below).



Figure 2.2: Visual representation of pulse waves using the Vicorder device.

Study 2: Repeatability of Vicorder PWV measurements using adapted path length

At the same session as Study 1, the doctor and nurse took one further measurement each of PWV using an adapted path length calculated using the aforementioned formula.

As with study 1, the nurse took measurements first, followed by the doctor.

2.2.4 Observers

The nurse was a registered general nurse (RGN qualified for 18 years) with previous experience of general nursing care. The doctor involved was qualified for 12 years with experience in general and geriatric medicine. Prior to the start of the study, each observer had performed less than a total of 20 PWV measurements on a small number of work colleagues.

2.2.5 Statistical Approach

Two main approaches in assessing observer variation in the recording of PWV were used: correlation coefficients and Bland Altman plots.

Intraclass correlation coefficients (ICCs) are used when quantitative measurements are made on units that are designated into groups. The ICC describes how strongly units in the same group resemble each other. A "0" means that there is no correlation between the variables, while -1 or 1 means there is a perfect negative or positive correlation between variables. It is commonly used in the assessment of consistency or reproducibility when quantitative measurements are made by different observers measuring the same quantity. ICC's measured for different populations may not be comparable.

Pearson's correlation coefficient (r) is the linear relationship between 2 variables. With ICCs, the data is centred and scaled using a pooled mean. However with Pearson's

correlation coefficients, each variable is centred and scaled by its own mean and standard deviation. The closer the value of r is to 1, the higher the correlation between variables. While correlation shows the relationship between two different measurements, it is best used when the reference method is low in error. However, all PWV measurement techniques have an inherent error. Thus, correlation may not necessarily demonstrate accurate performance of a PWV measurement technique. Coefficient of variation is the ratio of the standard deviation to the mean. It is a useful measure for comparing the degree of variation from one data series to another. Bland Altman plots are a statistical method used to compare two different measurement techniques. The Bland-Altman graph plots the difference between two techniques against their averages. The resulting scatter diagram allows the clinician to determine bias in any of the measurements and LOA (Limits of Agreement). Bland-Altman plots allow a visual inspection of the association between the differences in PWV measurement between observers [162]. We used the Bland-Altman 95% limits of agreement approach (LOA) (mean difference $\pm 2SD$) to assess within-observer and between-observer differences in the paired measures made on the same patient [163]. Limits of agreement are the reference intervals between the differences in means between two raters observations. For non-normal data a Spearman correlation was used. A Fisher z transformation on the correlation coefficients was applied to compare the values between nurses and doctors. All data entry was double-checked and analysis was undertaken using SPSS (SPSS Inc., Chicago, Illinois, USA (v. 13), without the exclusion of any patients or 'outlying' values. Only 'anonymized' patient data were available for analysis (which included unique patient study number, age and gender).

2.3 Results

Complete data were available for all of the 25 (15 males, 10 females) study participants who presented for PWV assessment. All were in normal sinus rhythm and independently mobile. A detailed analysis of subject demographics is presented in Table 2.1 below.

Table 2.1: Subject demographics and haemodynamic measures.

	Median(IQR)	Mean +/- SD	Range
Age(years)	80(6)	79.7+/-5.6	67-92
Weight(kg)	65.7(31.7)	67.7+/-18.4	30.2-105.2
Height(cm)	165(14)	165+/-9.5	148-185
Body Mass Index(BMI)	24.7(7.2)	24.7+/-5.4	10.4-34.1
Systolic Blood Pressure(SBP)(mm Hg)	138(41)	133.6+/-23	83-172
Diastolic Blood Pressure(DBP)(mm Hg)	68(7)	69.4+/-10.9	60-95
Heart Rate(HR)(bpm)	72(8)	70.9+/-11.1	50-89

Values are expressed as means +/- SD or median (IQR). All results not marked were based upon independent t tests.

2.3.1

Results of Study 1: Repeatability of Vicorder PWV measurements using standard path length

Intra class correlations

Table 2.2 shows intra-class correlation coefficients (95% CIs), Pearson correlations and LOA for PWV “within” nurse, “within” doctor measures and between nurse-doctor measures.

Table 2.2: Intra-class correlation coefficients (95% CIs) for PWV “within” nurse measures, “within” doctor and “between” nurse and doctor measures using the standard path length.

	Pearson's correlation coefficient	Coefficient of variation(%) ^S	Limits of agreement(m/s ⁻¹)	Intra-class correlation coefficient
“Within” nurse	0.85**	23	(-2.97,3.68)	0.91
“Within” doctor	0.70**	25	(-4.92,5.55)	0.80
Between “Nurse-doctor”	0.87**	23	(-2.88,2.94)	0.93

** p<0.001 \$ based on mean of two measures (calculated as SD/Mean)

Average intra-class correlations were higher “within nurse” measurements (0.91) when compared to “within” doctor measurements (0.80). “Between” nurse and doctor ICC's was 0.83. The difference in intra-class correlations between doctors (0.80) and nurses (0.91) was non-significant(p = 0.16). Limits of agreement were widest in the “within” doctor readings with lower limits of agreement seen in the “within” nurses

measurements. Overall LOA's appear high for all measures and may be related to taking the average of 2 measurements (as opposed to a single/initial measurement).

Bland Altman plots for PWV measurements using standard path length are shown in Figures 2.3, 2.4 and 2.5.

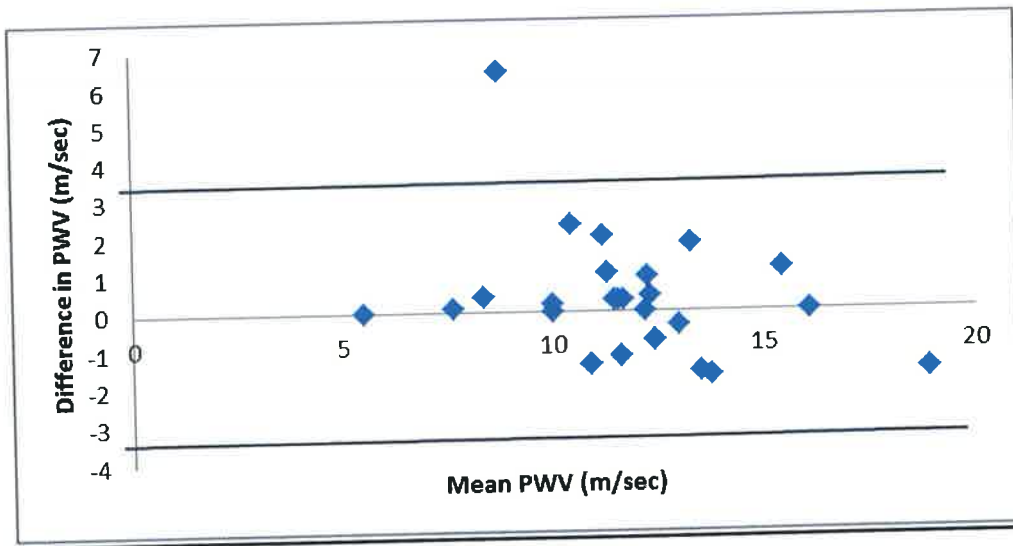


Figure 2.3: "Within" Nurse PWV measures in the measurement of PWV using the standard path length.

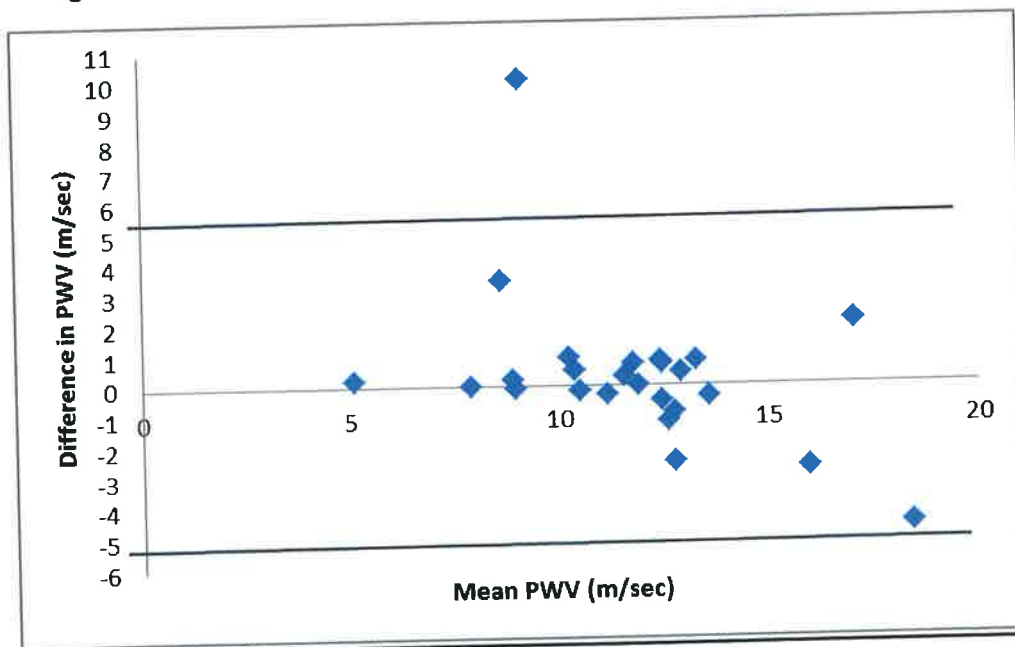


Figure 2.4: "Within" doctor PWV measures in the measurement of PWV using the standard path length.

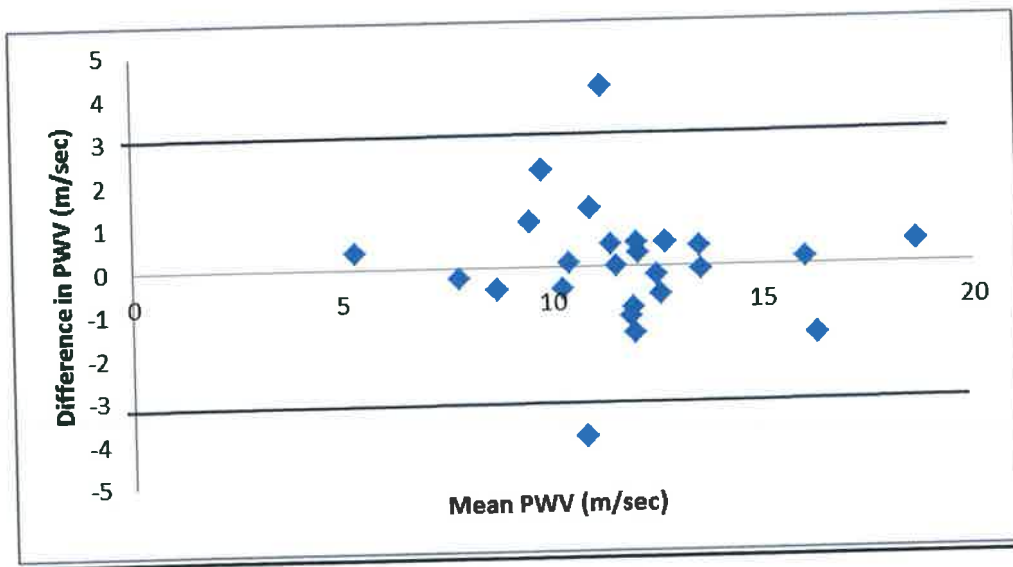


Figure 2.5: Between doctor and nurse difference by mean for PWV using the standard path length.

2.3.2

Results of Study 2-Repeatability of Vicorder PWV measurements using adapted path length

Intra class correlations

“Between” nurse and doctor measures were calculated using the adapted path length and the ICC was calculated as 0.88 as depicted in Table 2.3.

Table 2.3: Intra-class correlation coefficients (95% CIs) for PWV “between” nurse and doctor measures using the adapted path length.

	Pearson correlation	Coefficient of variation(%) ^s	Limits of agreement(m/s ⁻¹)	Intra-class correlation coefficient
“Between” nurse and doctor	0.793**	24.9	(-3.31,3.17)	0.88

** $p < 0.001$

2.3.5 Bland and Altman plot for PWV measures using adapted path length

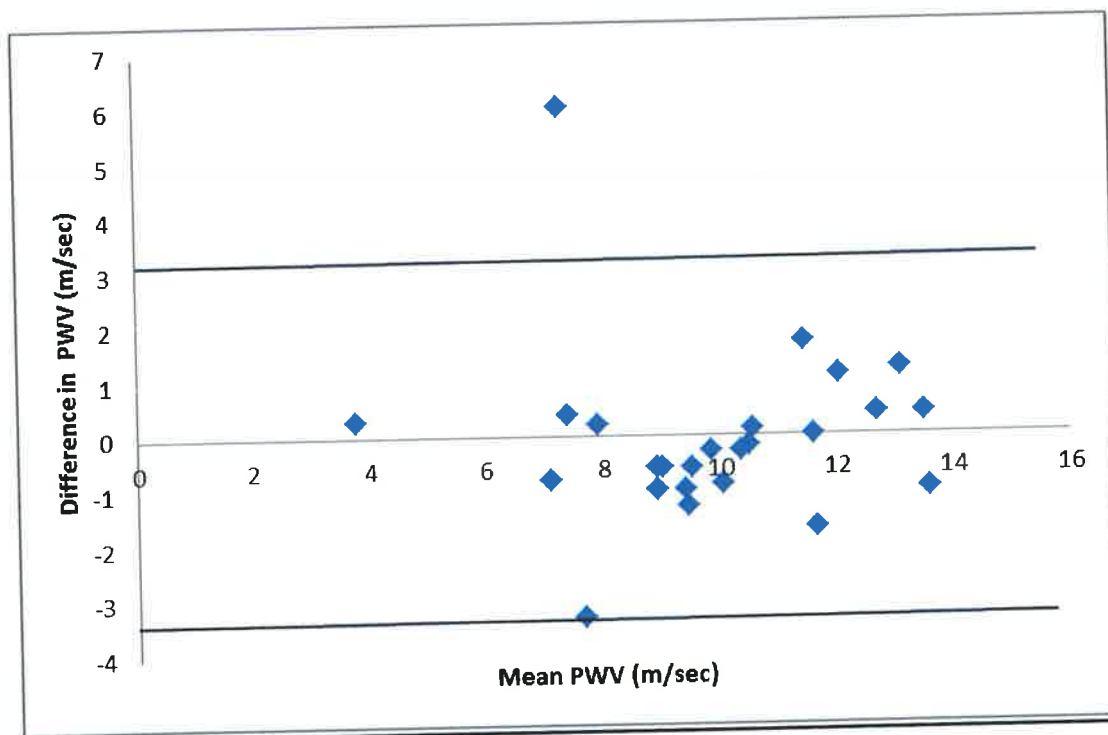


Figure 2.6: Between doctor and nurse difference by mean for PWV using the adapted path length.

There does not appear to be any pattern in the scatter plots which may have been suggestive of bias. There is a wide scatter in all of the Bland Altman plots which reflects the wide LOA. The inclusion of outlying values seen in the plots may have contributed to the wide LOA seen in all plots.

2.4 Discussion

This study aimed to evaluate the repeatability of PWV measurements obtained with the Vicorder, a novel non-invasive operator-independent device for the evaluation of arterial properties, performed by clinical staff with limited experience in this

setting. We found acceptable levels of both within- and between-observer repeatability, with values of intraclass correlation coefficients ranging from 0.8 – 0.93. Our results must be viewed with caution, given the wide LOA noted in all measures. The wide LOA found in our study may be due to the relative inexperience of our observers or the use of averages of 2 measures. However, in weighing up the appropriateness of different methods of assessing repeatability, we suggest that ICC's are the most appropriate test as LOA's are harder to apply to the error of a single measurement and are more difficult to interpret.

Our results showed that the highest repeatability was achieved using the standard arterial path length (0.93)[Study1] when compared with the adapted arterial path length (0.88)[Study 2]. There is some controversy in the literature at present as to which arterial path measurement to use. Hickson et al. recommended the use of an adjusted formula for calculation of arterial path length as detailed earlier[124]. A Dutch study compared measurements taken with both the Vicorder with the SphygmoCor and found that the Limits of Agreement (LoA) of both instruments exceeded a value of 1.5m/s and that the LoA of the Vicorder PWV were too wide to use this technique reliably in adults [164]. Two studies have found a significant difference in transit times between the Vicorder when compared to the highly validated SphygmoCor[124] Findings showed that the Vicorder tended to report lower PWV values at higher values of aortic stiffness when compared with the SphygmoCor device and the authors recommended the use of an adjusted formula for calculation of an adapted arterial path length[124] .Kracht et al validated the Vicorder device for aortic pulse wave velocity measurements in children and adolescents and found that intra- and inter-observer repeatabilities were excellent with coefficients of variation of 5.6% and 5.8% and interclass correlation coefficients

of 0.8 and 1.0. The authors concluded that Vicorder PWV values were similar to those obtained by the previously validated SphygmoCor and that the best agreement was with the use of the path length that most accurately replicated the aortic tree (Suprasternal notch to femoral recording point via the umbilicus)[161]. A recent study by Sugawara et al indicated that the choice of arterial path length measurements elicits markedly different PWV values[165]. The authors conclude that a standardization of arterial path length measurement may be required. It is currently not clear which path length[123] is the most appropriate since validation of pulse wave velocity with invasive studies has proven difficult, a problem acknowledged by the published guidelines on validation of haemodynamic non-invasive measurement devices[166]. The difficulty arises as the invasive measure chosen typically equates to the PWV within the aorta and does not take into account the additive effect that may arise from the iliac and carotid vessels [125, 167]. This lack of a comparable invasive measure for PWV is a limitation of this study. The validity of the PWV measures obtained depends mainly on the correct measurement of this path length[168].

The Vicorder system was user friendly even when operated by inexperienced technicians. This is the first study which has validated the Vicorder device in the older ambulant population.

2.4.1 Strengths and weaknesses of the study

We rigorously followed current consensus guidelines on the assessment of PWV [118]. The nurse and doctor each made three independent measurements on each patient at a single session. The six measurements of PWV were performed in quick succession using the same equipment, with the nurse blinded to her colleague's measurements and no knowledge of the patient's previous medical history. Our main interest lay in the repeatability of PWV in a consecutive series of patients and to avoid bias, we deliberately only collected limited data on patient demographics. Although we did not estimate sample size in advance and our sample size is small, our study is comparable with other published studies (Table 2.4) [124, 161, 164, 166, 169, 170].

Table 2.4-Comparison of previous studies validating the Vicorder device.

	Instruments compared	N(number of participants)	LOA(m/s ⁻¹)	Coefficient of variation	ICCs
McGreevy	Vicorder	25	-2.8-2.9	23	0.93
Von Leeuwen-Segarceanu ¹⁶⁴	Vicorder Sphygmocor	38	-4.24-4.72 -1.53-1.71	-	
Kracht ¹⁶¹	Vicorder	14	-1-1.7	5.6/5.8	0.8/1
Hickson ¹²⁴	Vicorder Sphygmocor	122		2.8	
Shahin ¹⁷⁰	Vicorder Sphygmocor	30	-1.07-1.09 -1.79-1.85		0.94 0.92

There is no objective evidence of quality of pulse wave traces with the Vicorder as compared to the quality control functions of the SphygmoCor and this is a weakness of this study. A further weakness is that only two measures of PWV were carried using the adapted path so a full analysis of repeatability using this measure was unable to be carried out.

Overall, the Vicorder system was user friendly even when operated by inexperienced technicians. This is the first study which has looked at validation of the Vicorder device in the older ambulant population.

2.5 Conclusion

This study found good 'within' and 'between' observer repeatability of PWV measurement performed by medical professionals with no previous experience of vascular investigations to be adequately reproducible in a hospital setting.

Furthermore, we found that this technique can be quickly acquired for use in research or clinical settings. We conclude that, with further longitudinal studies, this non-invasive method of assessing arterial stiffness may have the potential to be included in the clinical assessment of older ambulant patients.

CHAPTER 3

THE EFFECT OF VITAMIN D REPLACEMENT ON ARTERIAL STIFFNESS IN AN ELDERLY COMMUNITY BASED POPULATION.

3.1 Introduction

Vitamin D deficiency is associated with hypertension, vascular disease and heart failure. Furthermore, previous studies have demonstrated that vitamin D deficiency may lead to impairment of vascular effects, eventually leading to abnormalities in central arterial stiffness [139, 171]. Low serum 25OHD levels have been found to be associated with a higher level of peripheral arterial disease (PAD) suggesting support for the theory that vitamin D may have potent anti-atherosclerotic properties[139, 141].

Pulse wave velocity (PWV) is accepted as a simple, non-invasive, validated, robust and reproducible method to determine arterial stiffness[118]. The arterial pressure waveform is a composite of the forward pressure wave created by ventricular contraction and a reflected wave. In the case of stiff arteries, PWV rises and the reflected wave arrives back at the central arteries earlier, adding to the forward wave and augmenting the systolic pressure. The Vicorder® system (Skidmore Medical, Bristol, UK) measures simultaneous pressure waveforms by a volume displacement technique, using blood pressure cuffs placed around the sites of interest [124] and has been detailed in an earlier chapter.

A number of molecules such as osteoprotegerin(OPG) and MMP-9 may be elevated in association with calcification of vessels, ischaemic heart disease and stroke[135] resulting in increased interest in their potential as possible biomarkers of vascular disease.

Yearly intramuscular cholecalciferol (300,000U) has traditionally been used to treat vitamin D deficient patients[165]. Patients who received this treatment demonstrated significant improvement in 25OHD levels, from 25.5 to 81 nmol/L with 11% remaining deficient. However, concern has been expressed regarding the use of megadoses of vitamin D given recent suggestions of an association between increased fracture risk and the use of annual high dose vitamin D[172]. There has been no previously published study using doses of 100,000IU cholecalciferol and a follow up period of 8 weeks was chosen due to the above findings.

3.1.1 Aims of the study:

Primary aim:

(i) To determine whether vitamin D deficiency is associated with increased arterial stiffness as determined by PWV and to compare the effects of 50,000IU of cholecalciferol with 100,000IU of cholecalciferol on arterial stiffness

Secondary aim:

(ii) To determine the effects of supplementation with two different doses of IM cholecalciferol on serum 25OHD levels.

3.2 Methods

3.2.1 Ethical Approval

Ethical approval was obtained from the Beaumont Hospital Ethics Committee (Appendix A). Further approval for the setting up of a randomised clinical trial was

obtained from the Irish Medicines Board (Appendix B). Many studies have shown cholecalciferol to be superior to ergocalciferol in terms of efficacy in raising 25OHD levels which led us to choose cholecalciferol as the treatment for this trial [173-176]. I hypothesised that if 300,000IU sufficiently repletes vitamin D levels over 1 year, 100,000IU should replace levels over a 4 month period. A detailed protocol was written and strictly adhered to [Appendix C]. Vicotrat D3 (Heyl Pharmaceuticals, Berlin, Germany) was chosen as an appropriate vitamin D product due to EU licensing regulations [Appendix D].

3.2.2 Study Design

The study was designed as a double-blind randomized controlled clinical trial to compare the effects of 50,000IU of cholecalciferol with 100,000IU of cholecalciferol on arterial stiffness and to determine whether vitamin D deficiency is associated with arterial stiffness. The primary outcome measure was whether the difference in PWV(m/s) between group A (prior to and following 100,000 IU cholecalciferol administration) (Δ PWV1) and group B (prior to and following administration of 50,000IU cholecalciferol) (Δ PWV2) was statistically significant assuming equal or similar baseline values of PWV.

Power calculation

Our power calculation was based on a study by Dong et al [126] which detected a difference in PWV after vitamin D supplementation after 16 weeks of 0.38 m/sec.

Assuming an equal sample size and similar baseline PWV values to Dong et al, a total of 136 patients (2 equal groups of 68 patients) were required to detect a difference of 0.38 with a pooled SD of 0.77 (effect size of $0.38/0.77 = 0.49$ SD) to have at least 81% power for a two-tailed t-test.

Only 119 of the required 136 patients were recruited due to logistical difficulties with repeat attendances at our outpatient clinics. We randomised a total of 119 subjects to each treatment arm over a period of 1 year to receive either

- I. Cholecalciferol 50,000IU single dose intramuscularly [n=60]
- II. Cholecalciferol 100,000IU single dose intramuscularly [n=59]

The CONSORT diagram (Figure 3.1) gives details of participant numbers[177].

3.2.3 Inclusion criteria

- Community dwelling patients aged 65 and over
- Not receiving vitamin D preparations
- No change in medications in last 3 months
- Vitamin D deficiency- serum 25OHD levels <50nmol/l.

Exclusion criteria

- Patients already on vitamin D supplementation
- Liver function tests (bilirubin, aminotransferases and alkaline phosphatase) > 3 times the upper limit of normal

- Patients with a history of or current malignancy, known hypoparathyroidism, history of renal stones,
- Renal dysfunction, defined as a creatinine greater than 200µmol/l
- Unable to read or give informed consent
- Hypercalcaemia (Total calcium > 2.6mmols)
- Recent (over the previous 3 months) change in medication
- Hypersensitivity to cholecalciferol or any of its excipients as listed in the SPC
- Hypervitaminosis D or evidence of vitamin D toxicity

3.2.4 Screening and recruitment process

Older (aged 65 and over) patients from a geriatric clinic (either day hospital or outpatient clinic) at Beaumont Hospital, Dublin had vitamin D levels assessed along with Full Blood count(FBC),Urea and Electrolytes(U/E),Liver function tests(LFT's), Calcium(Ca²⁺) and Phosphate(PO⁴) levels as part of standard care. Patients found to be vitamin D deficient (levels < 50nmol/L) were approached with regards to recruitment onto the study by an initial telephone call by a member of the research team). The study was explained to them and they were sent a copy of the patient information leaflet (Appendix E). If he/she was willing to be a subject in the study, a timeframe was agreed upon for the investigations proposed to be performed at visits

0 weeks and 8 weeks. Informed consent was then given by the participant on their week 0 visit. Each participant's GP was sent an information letter regarding the trial (Appendix F).

3.2.5 Randomisation and blinding

Subjects were randomised using a restricted (stratified) random assignment of patients on a 1:1 basis into two parallel treatment arms (either 50,000IU IM cholecalciferol or 100,000IU IM cholecalciferol). Computer generated random lists were used where every patient was randomly assigned to either the high dose arm or the low dose arm. The injections were prepared by a nurse independent of the study and placed in identical sealed brown envelopes. They were administered on a blind basis by a member of the research team. Neither the participants nor the investigators knew which treatment the patient received, until the code was broken at the end of the study, when data collection was complete.

Once recruited, all participants had baseline weight, height, waist circumference PWV and Pulse Wave Analysis (PWA) carried out at week zero and had blood samples taken for OPG, MMP-9, PTH and 25OHD levels. Subsequently they were randomised into 2 groups- vitamin D replacement in the form of intramuscular (IM) cholecalciferol 100,000IU or 50,000IU. At week 8, parameters including BP, PWV and PWA were rechecked to assess for changes in these cardiovascular parameters and vitamin D levels and clinical biochemical parameters were rechecked. This was a double blinded, single centre randomized clinical trial (EUDRA number-2010-024417-31).

After the 119 participants were randomised to either group, a further 17 were lost to the study prior to the 8 week follow up. This is further detailed in the CONSORT diagram (Figure 3.1) below[177].

Study Design

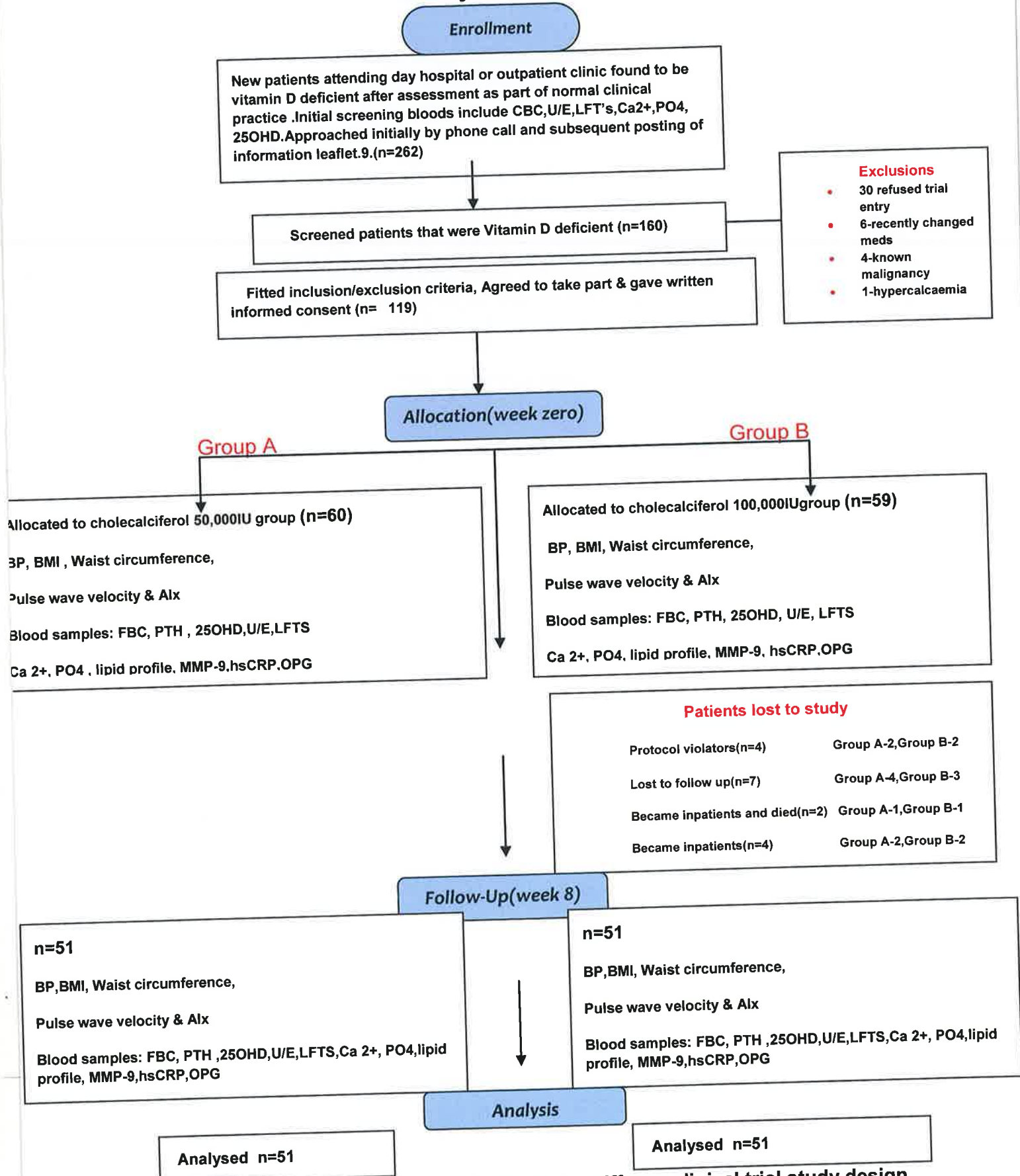


Figure 3.1: CONSORT diagram of vitamin D/arterial stiffness clinical trial study design

3.2.6 Measures

Once recruited, patients had baseline weight, height, waist circumference and Pulse Wave Velocity (PWV) carried out using the Vicorder device. As detailed in chapter 2, after an initial rest period of 15 minutes with subjects in a supine position in a quiet, temperature controlled room, brachial blood pressure was measured using an automatic device, a Welch Allyn Vital Signs Monitor 300 blood pressure monitor. Two BP readings were taken at 0 and 8 weeks and an average reading for each visit was documented.

PWV was measured using the Vicorder® system (Skidmore Medical, Bristol, UK). The Vicorder device estimates aortic stiffness by measuring carotid to femoral (aortic) pulse wave velocity (aPWV) using an oscillometric technique and was chosen for use in this trial because it represented a non-invasive method of assessing arterial stiffness[124]. Arterial pulse waveforms were recorded using PVR (pulse volume recording) measurements from a standard vascular cuff. The waveforms can be acquired in up to two sites simultaneously to provide pulse transit time (TT) and pulse wave velocity (PWV) measurements for the assessment of arterial stiffness.

PWV measurements were obtained by placing a 100mm blood pressure cuff around the upper thigh to capture the femoral pulse reading and a 30mm plethysmographic partial inflatable sensor cuff around the neck at the level of the carotid artery. Standard path length was defined as the distance from the suprasternal notch to the top of the thigh cuff as indicated by the manufacturer. The cuffs were inflated to 60mmHg and high quality waveforms were recorded for 10 seconds with the patient

lying supine, using a volume displacement method. Two PWV readings using the standard path length were taken and an average value documented. A further 2 readings using an adapted path length (as calculated by the formula [**Adapted path length = Suprasternal notch to top of thigh cuff-notch to carotid pulse +65mm**]) were taken and an average value was documented.

Patients' samples were centrifuged on arrival in the laboratory with serum removed and frozen immediately (-18°C) and batched for a number of weeks until sufficient numbers for analysis were achieved. 25OHD was analysed using the LC/MS method. Solvent delivery and sample introduction were performed using a Waters ACQUITY Ultra Performance LC (UPLC) system (Waters, MA, USA) equipped with a thermostat for both the sample and column compartments maintained at 4 and 35°C respectively. Full blood counts were measured using a Bayer ADVIA 120 automated blood counter analyser. U/E and LFTs were carried out on a Beckman Coulter AU5400. PTH analysis was performed on a Roche Elecsys 2010. Calcium/PO4 and PTH were all measured in the hospital laboratory and were processed within 4 hours of being drawn. Serum 25OHD were centrifuged and serum stored at -20°C for subsequent batch analysis by LC/MS method in the hospital laboratory (repeatability co-efficient of variation of 5.6% with laboratory imprecision value of 7.9%). Blood was centrifuged with plasma separated and stored at -80°C for subsequent analysis. Total OPG and MMP-9 were measured in an external independent laboratory using commercial enzyme-linked immunosorbent assay (ELISA) kits. The OPG Duoset (R&D Systems, Minneapolis, USA) had intra and inter-assay variations of <4%, with a minimal detection limit of 31.25pg/ml. The MMP-9 Quantikine (R&D Systems, Minneapolis, USA) had an intra- and inter-assay variations of <5%, with a minimal detection limit of 0.156ng/ml. HsCRP, triglycerides, HDL, LDL and total cholesterol

were measured on the Randox Daytona analyser (Randox, Antrim, Northern Ireland).

3.2.7 Vitamin D status

Vitamin D deficiency was classed as 25OHD less than 50nmol/L.

3.2.8 Statistical analysis

Descriptive statistics are presented as mean +/- SD for continuous measures and proportions for categorical data unless stated otherwise. We tested the assumption of normality for all variables by applying the Kolmogorov-Smirnov test for normality in SPSS. Differences between randomised groups were compared at baseline and the difference between baseline and 8 week follow-up using independent t test for data that were continuous and distributed normally and Mann Whitney U tests for non-normal data. Comparisons between categorical variables between the 2 randomised groups were compared using Chi square tests or Fisher exact test for small sample size. A repeated measure ANOVA was used to examine whether there was a change in variables from 0 to 8 weeks between the two groups. P value <0.05 was considered statistically significant. All analyses were conducted in SPSS (v18.0)(PASW statistics, Chicago,IL).

3.2.9 Conduct of the study

In accordance with IMB guidance, an independent safety monitoring committee was appointed to oversee the safety of the study. All serious adverse events were reported in accordance with guidance provided by the European Commission.³ data monitoring committee meetings were held over the trial period.⁶ SUSAR reports

were sent to the IMB during the study as 6 trial participants became inpatients and 2 of these subsequently died of unrelated causes.

3.3 Results

3.3.1 Baseline clinical characteristics

A total of 102 subjects completed the study and were included in the statistical analysis (51 in each group). The average baseline plasma level of 25OHD was 27.5 nmol/L which indicates a significant vitamin D deficiency status (<50nmol/L) in this elderly cohort. Clinical characteristics of the 2 randomised groups of subjects are compared in Table 3.1 below. No significant differences were noted for baseline characteristics with the exception of potassium. Mean calcium and median PTH levels were of normal levels in both groups. At baseline, median PWV was 11.4m/s in the group who received 50,000IU vitamin D and 12.2m/s in the group receiving the higher dose of vitamin D. Mean Augmentation index was 29% in both groups at baseline.

Table 3.1: Baseline clinical characteristics.

Clinical Characteristics	Group allocated to 50,000IU vitamin D	Group allocated to 100,000IU vitamin D	P value
N	51	51	
Males/Females ^a	26/25	28/23	0.16
Age(year)	80.5+/-6.6	79.3+/-7	0.37
Height(cm) ^b	164(140-185)	165(73-186)	0.7
Weight(kg)	74+/-6.5	73.1+/-22	0.77
BMI (kg/m ²)	26.9+/-9.5	26.6+/-6.4	0.85
SBP(mm Hg)	135.1+/-18.9	129.4+/-17.8	0.13
DBP(mm Hg)	70.9+/-11.5	69.5+/-10.9	0.53
25OHD(nmol/L)	27.3+/-13.5	27.7+/-11.7	0.87
PTH(pg/ml) ^b	46(19-115)	49(23-138)	0.9
WBC count(10 ⁹ /L)	6.74+/-1.98	6.72+/-2.42	0.96
Haemoglobin(g/dl)	12.8+/-1.57	12.4+/-1.42	0.18
Platelet(10 ⁹ /L)	225.3+/-80.2	216.2+/-62.2	0.52
Urea (mmol/l) ^b	6.9(2.4-15.6)	6.9(1.4-15.4)	0.79
K+(mmol/l)	4.3+/-0.42	4.1+/-0.35	0.01
Na(mmol/l)	138+/-2.5	138.1+/-3.4	1
Chloride(mmol/l)	102.2+/-2.9	102.3+/-3.8	0.66
Creatinine(umol/L)	85.8+/-25.2	85.4+/-26.2	0.93
Bilirubin ^b (umol/L)	9(4-25)	9(5-33)	0.85
Alt (I.U./L) ^b	18(4-173)	15(3-85)	0.11
Alk Phos(I.U./L)	98.1+/-30.3	103.06+/-39.5	0.47
Ca(mmol/L)	2.4+/-0.9	2.38+/-0.11	0.87
PO ⁴ (mmol/L)	1.1+/-0.17	1.07+/-0.17	0.37
PWV(m/s) ^b	11.4(6.3-17.3)	12.2(5.1-40.3)	0.27
Adapted PWV(m/s) ^b	10.2(4.2-17.3)	10.8(3.9-29.9)	0.11
Alx(%)	28.5+/-7.2	29.4+/-6.9	0.52
Triglyceride(mmol/l)	1.3+/-0.59	1.3+/-0.54	1
Total Cholesterol(mmol/l)	4.24+/-1.12	4+/-0.93	0.24
HDL(mmol/l)	1.36+/-0.43	1.3+/-0.36	0.44
LDL(mmol/l)	2.23+/-0.89	2+/-0.76	0.16
HsCRP(mg/dl)	3.5+/-4.31	4.4+/-6.25	0.39
OPG(pg/ml)	2527.3+/-938.8	2337.6+/-990.8	0.32
MMP-9(ng/ml)	5.07+/-3.41	5.04+/-3.53	0.96

Values are expressed as means +/- SD or median (range). All results not marked were based upon independent t tests.

^a Tests of significance between groups were based on Fisher's exact test.

^b Tests of significance between groups were based on Mann-Whitney U tests.

3.3.2 Relationship between vitamin D and PWV at baseline.

There was an inverse trend between vitamin D levels and PWV readings at baseline (Figure 3.2). A non-significant correlation of -0.120 ($p=0.245$) was seen between PWV and vitamin D at baseline and a similar non-significant inverse trend between vitamin D and adapted PWV was also noted [Pearsons correlation -0.05 ($p=0.63$)].

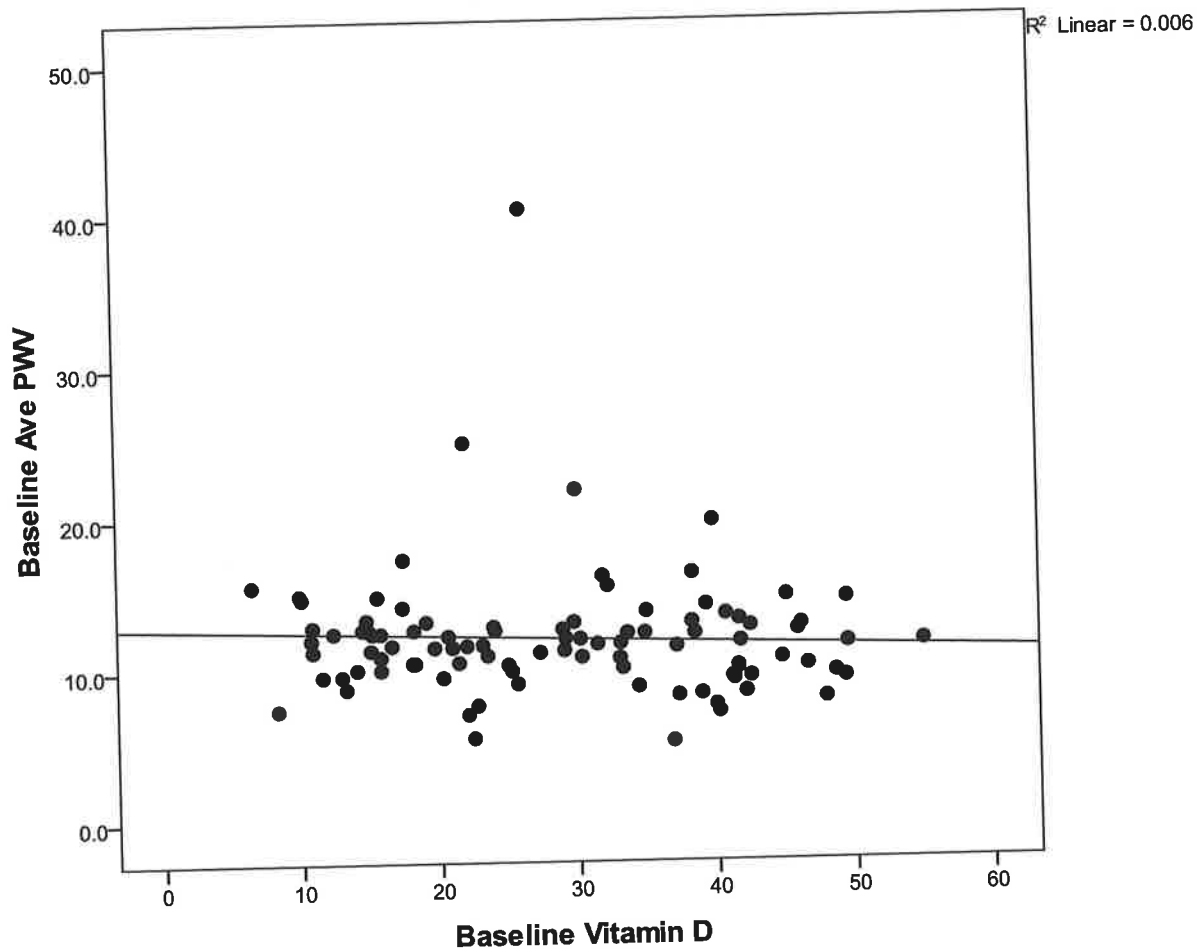


Figure 3.2: Scatter plot of inverse trend between vitamin D levels and PWV readings at baseline.

3.3.3 Responses after 8 weeks in key variables(Serum 25OHD, PWV, adapted PWV, Aix)

Differences between data at baseline and week 8 were computed for each variable (changes from baseline).

Table 3.2 below shows the mean/median changes that occurred in the key variables over the 8 week trial period and a between group comparison between the group receiving 50,000IU and 100,000IU cholecalciferol.

Table 3.2: Mean/median changes in serum 25OHD and PWV over the 8 week trial period.

	Dose of vitamin D given	Week 0	Week 8	P value	p-value for between group comparison
Serum 25OHD levels(nmol/L)	50,000IU	27.3(+/-13.5)	41.2(+/-13.6)	p<0.001	0.022
	100,000IU	27.7(+/-11.7)	48.5(+/-16.8)	P<0.001	
PWV(m/s) ^a	50,000IU	11.4(6.3-17.3)	11.1(6-15.6)	p=0.76	0.097
	100,000IU	12.2(5.1-40.3)	11.5(4.3-14.9)	p=0.22	
Adapted PWV(m/s) ^a	50,000IU	10.2(4.2-17.3)	10.6(3.7-17)	p=0.77	0.071
	100,000IU	10.8(3.9-29.9)	10.7(4-13.4)	p=0.49	
Aix(%)	50,000IU	28.5+/-7.2	28.4+/-0.9	p=1	0.033
	100,000IU	29.4+/-6.9	25.6+/-1.2	p=0.032	

Values are expressed as means +/- SD or median (range). All results not marked were based upon independent t tests.^a Tests of significance between groups were based on Mann-Whitney *U* tests. Between group comparisons were carried out using 2 way ANOVA.

Significant changes over the 8 week study period were seen in serum 25OHD levels between the 2 randomised groups with a greater increase in levels at week 8 in the group that had received 100,000IU. A mean increase of 20.7nmol/L was seen in this group compared with a mean increase of 13nmol/L noted in the group that received the lower 50,000IU vitamin D. The responses in serum 25OHD levels seen in both lower and high dose vitamin D groups were statistically significant ($p < 0.001$). When a between group analysis was performed using ANOVA, a significant difference was found ($p = 0.022$).

In the group that received 100,000IU vitamin D, median PWV decreased from 12.2 m/sec to 11.5 m/sec over the 8 week study period but this trend did not meet statistical significance ($p = 0.22$) [Figure 3.3]. In the group receiving the lower dose (50,000IU), median PWV decreased from 11.4m/sec to 11.1m/sec ($p = 0.76$). Analysis of adapted PWV levels showed a decrease in PWV readings from a median of 10.8 to 10.7m/sec in participants who received 100,000IU vitamin D but this was not statistically significant. There was a significant decrease in Alx from week 0 to week 8, with a mean decrease of 3.803 ± 1.76 seen in the group who received the higher vitamin D dose ($p = 0.032$). Between group analysis between the groups confirmed a statistically significant difference ($p = 0.033$).

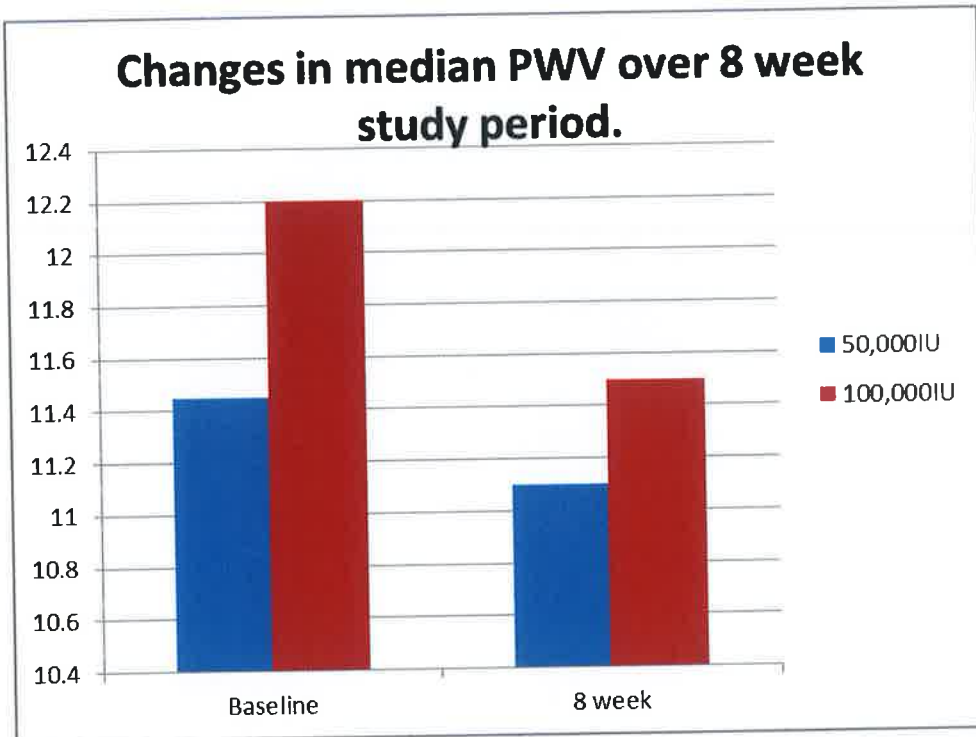


Figure 3.3: The dynamic changes in median PWV levels in groups receiving 100,000IU and 50,000IU vitamin D over the study period (Baseline to 8 weeks). In the group receiving 100,000IU, median PWV decreased from 12.2 m/sec to 11.5 m/sec over the 8 week study period but this trend did not meet statistical significance ($p=0.22$). In the group receiving the lower dose (50,000IU), median PWV decreased from 11.4m/sec to 11.1m/sec ($p=0.76$).

3.3.4 Responses after 8 weeks in other variables

The following variables were found to have a significant difference in values over the 8 week study period : Calcium, PO⁴ and systolic BP. As the data was normally distributed, differences between randomised groups were assessed using parametric statistics (2 sample t-test) (Table 3.3)

Table 3.3: Analysis of between group differences using the student t test (based on ranks of the data) including the baseline value (SD), week 8 (SD) and difference between them (SEM) where A= 100,000IU cholecalciferol, B=50,000IU cholecalciferol

	Baseline (mean, SD)	Week 8 (mean, SD)	Difference (mean, SEM)	t-test, p-value
Calcium				
50,000IU	2.40 (0.10)	2.40 (0.10)	0.006 (0.010)	T=4.51, p<0.001
100,000IU	2.38 (0.12)	2.44 (0.11)	0.058 (0.012)	
PO4				
50,000IU	1.08 (0.18)	1.11 (0.17)	0.025 (0.021)	T=1.13, p=0.26
100,000IU	1.07 (0.17)	1.07 (0.13)	-0.008(0.021)	
SBP				
50,000IU	135.1 (18.9)	132.4 (18.6)	-2.73 (2.22)	T=2.03, p=0.045
100,000IU	129.4 (17.8)	133.5 (18.7)	4.07 (2.51)	

Table 3.4 shows between group differences using the Mann Whitney U test. Variables that were found to have a significant difference in values over the study period were ALT, Total cholesterol and LDL. Significant increases in values were noted in the groups that received the 100,000IU dose of vitamin D (see Table 3.4 below).

Table 3.4: Analysis of between group differences using the Mann Whitney U test(based on ranks of the data) including the baseline value (median), week 8 (median) and difference between them(SEM).

	Dose of vitamin D given	Baseline (median)	Week 8 (median)	Difference (median, week 8-baseline)	Mann Whitney test, p-value (difference)
BMI	50,000IU	26.7	26.6	0	0.48
	100,000IU	25.9	25.3	0	
PWV	50,000IU	11.45	11.1	-0.3	0.66
	100,000IU	12.2	11.55	-0.3	
Adapted path PWV	50,000IU	10.2	10.6	-0.3	0.51
	100,000IU	10.8	10.7	-0.2	
Bilirubin	50,000IU	9.0	9.0	0.0	0.76
	100,000IU	9.0	9.0	0.0	
ALT	50,000IU	18	17	0	0.008
	100,000IU	15	20	1.0	
Phosphate	50,000IU	94.0	94.0	0.0	0.10
	100,000IU	99.0	108.0	6.0	
Cholesterol	50,000IU	4.13	4.11	-0.02	0.003
	100,000IU	3.97	4.15	0.19	
HDL	50,000IU	1.3	1.31	0.01	0.11
	100,000IU	1.2	1.34	0.08	
LDL	50,000IU	2.14	2.03	-0.04	0.002
	100,000IU	2.08	2.10	0.13	
CRP	50,000IU	2.08	2.34	0.03	0.42
	100,000IU	1.64	1.84	0.0	
OPG	50,000IU	2545	2500	-100	0.55
	100,000IU	2200	2340	-40	
MMP9	50,000IU	4.09	4.91	0.74	0.95
	100,000IU	4.65	4.76	0.022	

3.3.5 Relationship between vitamin D and PWV at 8 weeks.

A non-significant inverse trend [Pearsons correlation -0.159 (p=0.12)] between vitamin D levels and PWV was seen at 8 weeks. A similar non-significant inverse trend was seen between vitamin D levels and adapted PWV measures at 8 weeks.[Spearman's Correlation -0.113(p=.272).

3.3.6 Serum vitamin D levels after 8 weeks

Only 3/51(5.8%) who received 100,000IU vitamin D reached levels of sufficiency(>75nmols/L), with none of the participants who received 50,000IU achieving sufficient vitamin D levels 8 weeks post therapy.

3.3.7 Serum vitamin D levels and weight after 8 weeks.

A negative correlation between weight and vitamin D levels was noted at 8 weeks(Pearson correlation -.360, p<0.001) indicating that individuals with higher weight tended to have lower vitamin D levels at the 8 weeks assessment.

3.4 Discussion

We conducted an 8 week randomized clinical trial in a community dwelling cohort of elderly Irish population to determine whether vitamin D deficiency is associated with increased arterial stiffness and whether supplementation with vitamin D will lead to improved vascular stiffness in an elderly cohort of patients.

The main findings of this clinical trial include:

1. High prevalence of vitamin D deficiency (61%) in the screened elderly population was detected.
2. Although statistically significant increases in vitamin D status were seen with both 100,000IU and 50,000IU doses of cholecalciferol, serum levels were still insufficient post-trial indicating that intramuscular use of these doses of vitamin D may not be adequate to achieve optimal vitamin D levels.
3. A significant decrease in Augmentation index was seen in the group that received 100,000IU vitamin D.
4. Decreases in PWV readings were seen over the study period in both groups but these changes were non- significant.

3.4.1 Changes in vitamin D levels

We found that 61% of patients screened had a serum 25OHD level of <50nmol. These results are similar to other Irish studies which found prevalences of this level of deficiency in 75% and 55% of patients screened [25, 178]. Our samples were taken over a period of a year (from October to October) and seasonality was not adjusted for.

Our randomized clinical trial demonstrates that even high doses of intramuscular cholecalciferol failed to achieve adequate vitamin D levels in this older cohort. We found that the group who received 100,000IU of cholecalciferol had a mean increase

of 20.7nmol/L with a mean increase of 13nmol/L noted in the group that received the lower 50,000IU vitamin D. Both of these increases were statistically significant($p < 0.001$). However, only 3/51 (5.8%) who received 100,000IU vitamin D reached levels of sufficiency (>75 nmols/L), with none of the participants who received 50,000IU achieving sufficient vitamin D levels 8 weeks post therapy.

Current evidence suggests that levels of at least 50 nmol/L are required for optimal bone and muscle function[22] with estimates of optimal vitamin D status focusing on 75 nmol/L as the threshold for peak bone health[179]. This finding is in agreement with a recently published RCT which compared the efficacy of oral and intramuscular routes of vitamin D therapy[180]. The authors compared a single dose of 300,000IU IM cholecalciferol with 300,000IU of oral vitamin D given in 6 divided doses. Results showed that although both treatment arms significantly increased the serum 25OHD level, there was a marginally significant trend in rising 25OHD level in favour of the oral route during the entire follow-up time, with the increase in serum 25OHD level from baseline to three months significantly higher in patients who received oral therapy compared to parenteral treatment.

Similarly an interventional study using a younger cohort of participants found that a single megadose of 600,000IU IM cholecalciferol resulted in only 35% of participants achieving optimal (>75 nmol) vitamin D status after 8 weeks with a further 25% remaining deficient after treatment[181]. Another study compared the tolerability and efficacy of high dose (300,000IU) oral vitamin D₃ with a one off intramuscular dose of 300,000IU ergocalciferol and found that although they were both well tolerated, the oral vitamin D₃ had greater potency than the IM vitamin D₂, with a higher, sustained serum 25OHD response and more efficacious PTH suppression[182]. There are limited studies which have examined the bioavailability of intramuscular preparations of vitamin D. In their initial 2008 study, Romagnoli et al compared PO and IM cholecalciferol preparations and demonstrated a sharp increase in 25OHD levels when cholecalciferols was given orally, in contrast to a sustained and gradual

increase after IM administration with this study having a cut off follow up point at 2months[183].The same study group recently published a study evaluating the longterm bioavailability and metabolism of a high IM dose(600,000IU) of cholecalciferol and found that serum 25(OH)D continues to increase after IM administration with the highest levels seen after 120 days[184].

Our results are in contrast to a number of recent studies which found that high dose IM cholecalciferol was effective in increasing vitamin D levels to sufficient levels in an older population but these studies used 300,000IU and 600,000IU of cholecalciferol respectively[178, 185].

Our biochemical outcomes may be as a result of our use of dosages of 100,000IU and 50,000IU which we hypothesised should replace vitamin D levels over a 4 month period. However, we were reluctant to use higher doses of IM cholecalciferol given recent suggestions of a possible increase in falls and fractures post annual high dose vitamin D[171].We followed patients up after an 8 week period which may have been too early for optimal vitamin D status to have been reached as a peak in serum 25OHD values after 12 weeks was seen in Cipriani et al's study[183].Furthermore, differences in uptake in patients may be as a result of differences in bioavailability. The delayed increase in 25(OH)D after IM vitamin D administration has been linked to the oily depot preparation, which in turn leads to its deposition in the injection site, thereby producing a slow and gradual release[185].

Mean BMI for our cohort in both randomised groups was 26, which is classed as "overweight". We found a weak negative correlation between weight and vitamin D levels at 8 weeks (-0.36, $p < 0.001$) suggesting that heavier individuals tended to have lower vitamin D levels at 8 weeks. Wortsman et al found that larger doses of vitamin D may be required in treating vitamin D deficient obese persons due to decreased

vitamin D bioavailability[47]. Giusti et al found that there was a wide variability in serum 25OHD in response to IM 300,000iu cholecalciferol, with logistic regression analysis showing that BMI had a significant association with normalization of vitamin D levels[187]. The authors concluded that variable response to IM supplementation may be as a result of poor intestinal absorption or high rates of catabolism of vitamin D[188]. It may be the case that the intramuscular use of cholecalciferol may not be efficacious in the older population and that despite compliance issues, regular oral treatment may be safer and more effective.

3.4.2 Changes in PWV

To our knowledge, no previous clinical trial has evaluated the effects of different doses of cholecalciferol on arterial stiffness using the Vicorder apparatus. Our study found that there was a significant improvement in Alx readings from week 0 to week 8, with a mean difference of 3.803 ± 1.76 seen in the group who received the higher vitamin D dose ($p=0.032$). Between group differences confirmed a statistically significant difference in Alx between the 100,000IU and 50,000IU group ($p= 0.03$).

A recently published study by Dong et al aimed to determine the effect of daily vitamin D supplementation on arterial stiffness as measured by pulse wave velocity (PWV)[126]. Results showed that vitamin D supplementation with 2000IU daily led to a reduction in PWV (and therefore reduced arterial stiffness) from baseline to post-test [5.41 ± 0.73 m/sec to 5.33 ± 0.79 m/sec] ($P=0.031$).

Our study demonstrated that the group that received 100,000IU vitamin D, median PWV decreased from $12.2(5.1-40.3)$ m/s to $11.5(4.3-14.9)$ m/s over the 8 week study period ($p=0.22$). In the group receiving the lower dose (50,000IU), median PWV

decreased from 11.4(6.3-17.3) m/s to 11.1(6- 15.6) m/s. Analysis of adapted PWV levels showed a decrease in PWV readings from a median of 10.8(3.9-29.9) m/s to 10.7(4-13.4)m/s in participants who received 100,000IU vitamin D. Although a definite trend was noted, these changes did not reach statistical significance.

Our study noted an inverse trend (non-significant) between serum vitamin D levels and PWV at baseline. In a post-hoc analysis, we also found that there was a non significant trend towards falling PWV levels as serum vitamin D levels rose over the 8 week period [Pearson correlation -0.159,p=0.123]. Given this definite trend, further research is required to investigate whether sufficient supplementation of vitamin D by a reliable method could result in positive functional changes in arterial stiffness.

ALT, total cholesterol and LDL all increased over the 8 week period. A recent meta-analysis confirms an increase in LDL and total cholesterol post vitamin D supplementation[189]. The effect of vitamin D therapy on serum LDL levels was particularly significant in obese subjects (BMI>30) and in studies of shorter duration. Like our study, this meta-analysis found that reductions in HDL and triglycerides were non-significant. Conflicting results were seen with regards to SBP, with an increase in values noted in the group that received the higher dose of vitamin D and a decrease in value noted in participants who received the lower vitamin D dose.

Furthermore, a number of recent studies failed to see any benefits to markers of cardiovascular health such as blood pressure and hsCRP post vitamin D supplementation[190, 191].

We were unable to find any correlation between PWV and hsCRP, MMP-9 or OPG as had been demonstrated in a number of previous studies[192-194]. This may be due to our small sample size.

3.4.3 Strengths and limitations of study

Our relatively small sample size as a result of recruitment difficulties limits the power of the study and suggests that clinical trials with a larger sample size are warranted. Details regarding sun exposure and dietary vitamin D intake were not collected at the time of recruitment, which would have contributed to our study. Just under 10% of patients were lost to follow up and may reflect the frailty and co-morbidities of the population we chose to study. A decision was made not to include a placebo group as one of our secondary aims was to compare the effects of two different doses of vitamin D on arterial stiffness.

Strengths of this double blind randomized controlled trial were that we measured carotid-femoral PWV, which is the “gold standard” for the non-invasive assessment of arterial stiffness. No previous clinical trial has evaluated the effects of different doses of cholecalciferol on arterial stiffness using the Vicorder apparatus in an older population and further larger trials are encouraged. Our laboratory analysis of serum levels of 25OHD used the LC/MS method which is the gold standard for vitamin D assays[195]. Furthermore, we established that the Vicorder is a simple, non-invasive method of assessing arterial stiffness in an ambulant elderly population which needs further evaluation using larger studies of the older population.

3.5 Conclusion

Vitamin D deficiency remains an issue in the older Irish population and poor responses to intramuscular therapy suggest that higher IM doses or oral therapy may be more beneficial. High dose vitamin D resulted in an improvement in augmentation index with non-significant improvements noted in PWV readings. Further research is needed to expand our knowledge of the pathogenesis of arterial stiffness and its association with vitamin D deficiency in the older population.

CHAPTER 4

THE MEDICAL MANAGEMENT OF OSTEOPOROSIS IN A NURSING HOME POPULATION: A COMPARISON OF GERIATRIC LED AND GENERAL PRACTITIONER (GP) LED SERVICES.

4.1 Introduction

Nursing home residents are at very high risk of osteoporotic fractures since fracture rate in this population is between 3 and 11 times higher than in age and gender matched community dwellers[196,197]. Despite the high burden of osteoporosis in nursing homes, several studies have suggested that osteoporosis screening and therapies are underutilized in the nursing home population. An American study of >180,000 nursing home residents found that only 9.1% received anti-resorptive medications indicated for osteoporosis treatment despite the expected high prevalence of osteoporosis in this setting[87]. Despite a known previous history of fracture in 44% of residents, a very small proportion of these residents were on bisphosphonate treatment (6%) with 21% on calcium/vitamin D preparations in a recent study[198]. However, implementation of effective fracture prevention efforts should be a priority at the time of admission to nursing homes since fracture incidence is the highest during the first months after admission[199]. In a systematic review of the pharmacological management of osteoporosis in the nursing home setting, Parikh et al found that the infrequent use of pharmaceutical agents in nursing

home residents for primary and secondary prevention of osteoporosis was the most consistent finding across all studies[200].

A study of 67 nursing home facilities in North America found substantial variation in the quality of osteoporosis treatment across nursing homes and recommended interventions that improve osteoporosis quality of care[86]. Of patients with a known previous history of fracture, only 36% received any bone protection, including calcium and vitamin D[86]. There has been no previous research on osteoporosis medication use in nursing homes in Ireland.

The aim of this study was to determine whether the medical management of osteoporosis in a nursing home population is different between a geriatric led and general practitioner led service and to assess the appropriateness of the medications prescribed.

4.2 Methods:

4.2.1 Ethical Approval

Ethical approval was obtained from the Beaumont Hospital Ethics Committee (Appendix F).

4.2.2 Study Design

This study was designed to compare prescribing habits of osteoporosis related drugs between one geriatrician led nursing home (St Joseph's Community Unit, Raheny, Dublin 5) and 3 general practitioner(GP) led facilities[Swords Nursing Home, Swords, Co. Dublin, Skerries Nursing Home, Skerries, Co. Dublin, Tara Winthrop Nursing Home, Swords, Co. Dublin]. Management of each facility gave written permission for the study prior to commencement[See Appendix G for protocol].

4.2.3 Inclusion criteria:

- All nursing home residents aged 65 and over.

Exclusion criteria:

- Nil

4.2.4 Recruitment Process

Members of the research team visited the facilities and chose every second name from the list of residents provided by the nursing home manager. Details of the study were discussed with residents and they were given an information leaflet (see Appendix H). Subsequent to this, informed consent was obtained. In the case of patients with known cognitive impairment ($MMSE < 22/30$), informed assent was obtained from their Next-of-Kin (NOK) and an information leaflet given to the NOK (see Appendix I). Following consent or assent, medication data over a 7 day period was collected from medication charts. Demographic information including the resident's age, gender and past medical history was noted [Appendix J]. We also assessed patients 10 year probability of fracture using the FRAX tool. FRAX is a computer-based algorithm that provides models for the assessment of fracture probability in men and women. The approach uses easily obtained clinical risk factors to estimate 10-year fracture probability, with or without femoral neck bone mineral density (BMD), to enhance fracture risk prediction [53]. It has been constructed using primary data from population-based cohorts around the world. We also calculated a Barthel score for each resident. This is an ordinal scale used to measure performance in activities of daily living (ADL'S) such as dressing, washing

and toilet use. Each performance item is ranked on a scale with specific points being attributed to each level or ranking. For example, for feeding, the patient is given a score of 0 if unable, scores 1 if he/she needs help, or 2 if they are independent with feeding. The Barthel score has been validated and shown to have high inter-rater reliability(0.95) and test-retest reliability(0.89) as a measure of physical disability[201].

4.2.5 Statistical Analysis & Power Calculation.

As no similar study had previously been published, an initial pilot study of 30 residents of a geriatrician led home and 30 residents of a GP led home was carried out. From this initial pilot study, we found that 21/30 residents in the geriatrician led home had a 10 year major osteoporotic fracture risk >20% compared with 11/30 in a GP led home. From these pilot study figures, the larger study was powered to detect a difference of 29% on treatment outcome between the GP-led and geriatrician-led groups. A total sample of 168 patients (84 in Geriatrician led homes:84 in GP led homes) was calculated to be necessary to achieve a two-sided level of significance of 0.05 and power of 81%. Categorical variables were compared using the Chi square test. Logistic regression models were used to examine the odds of receiving an osteoporosis medication using all the variables collected. A backwards stepwise method was used which removed any variables that were not significant after adjustment until the most significant variables were left at the end (using a criteria of $p < 0.10$ for entering or removing variables). 95% confidence intervals are presented. Analysis was performed using SPSS statistical package (v18). Significance at $p < 0.05$ was assumed.

4.3 Results

168 residents were studied in total (84 in the geriatrician led facility and 84 in GP led facilities). Table 4.1 compares demographical data between geriatrician led and GP led facilities.

Table 4.1: A comparison of demographical data between geriatrician led and GP led facilities. Data is presented as means +/- SD or median (range).

Type of Nursing Home	Geriatrician led NH(n=84)	GP led NH(n=84)
Age(years)	82.8+/- 7.6	83.7+/-6.9
LOS(days)	385(85-1984)	973(51-2515)
Barthel score(0-20)	7(0-20)	10(1-20)
Mean weight(kg)	67.9+/-17.9	67.4+/-13.6
Mean BMI(kg/m ²)	26+/-6.7	25.9+/-4.4
Gender(%females)	73% female	63% female

4.3.1 Geriatrician led nursing home results

36/84(43%) residents were prescribed at least one osteoporosis drug at the time of the study. When FRAX scores were computed, 36(43%) of residents sampled had a 10 year probability of a major fracture of $\geq 20\%$ on their FRAX scores. The National

Osteoporosis Foundation (NOF) has recommended prescription treatment for anyone in the U.S. with a 10-year risk of major fracture (hip, clinical vertebral, wrist, or humerus) that exceeds 20% [64].

Table 4.2 shows the osteoporotic medications prescribed to the aforementioned high risk residents in the geriatrician led facility. The most commonly prescribed medications were calcium/vitamin D combinations, followed by oral bisphosphonates. There was low prescription rates of vitamin D alone, intravenous bisphosphonates, strontium and teriparatide.

Of the residents with high risk major osteoporotic FRAX scores, only 3/36(0.02%) had previously had DEXA scans.

Table 4.2: Bone medications prescribed to high risk FRAX score residents in geriatrician led nursing homes.

Medications prescribed	High risk major osteoporotic fracture residents (n=36)
<u>Vitamin D alone</u>	3(0.08%)
<u>Calcium/Vitamin D</u>	12(33%)
<u>PO Bisphosphonate</u>	4(11%)
<u>IV Bisphosphonate</u>	2(5%)
<u>Strontium</u>	1(0.02%)
<u>Teriparatide</u>	1(0.02%)
<u>Denusomab</u>	0
<u>No treatment</u>	13(36%)

Figure 4.1 below depicts the osteoporotic medications prescribed to residents with known previous fractures in the geriatrician led nursing home. Of a total of 20 residents with known previous fragility fracture, 7 were on calcium/vitamin D combinations, with further low numbers on oral bisphosphonates, strontium etc. 3 residents had been on appropriate treatment but this had been discontinued for various reasons including dysphagia and low GFR. A further 5(25%) residents with previous fractures were on no medication, with no documented reason.

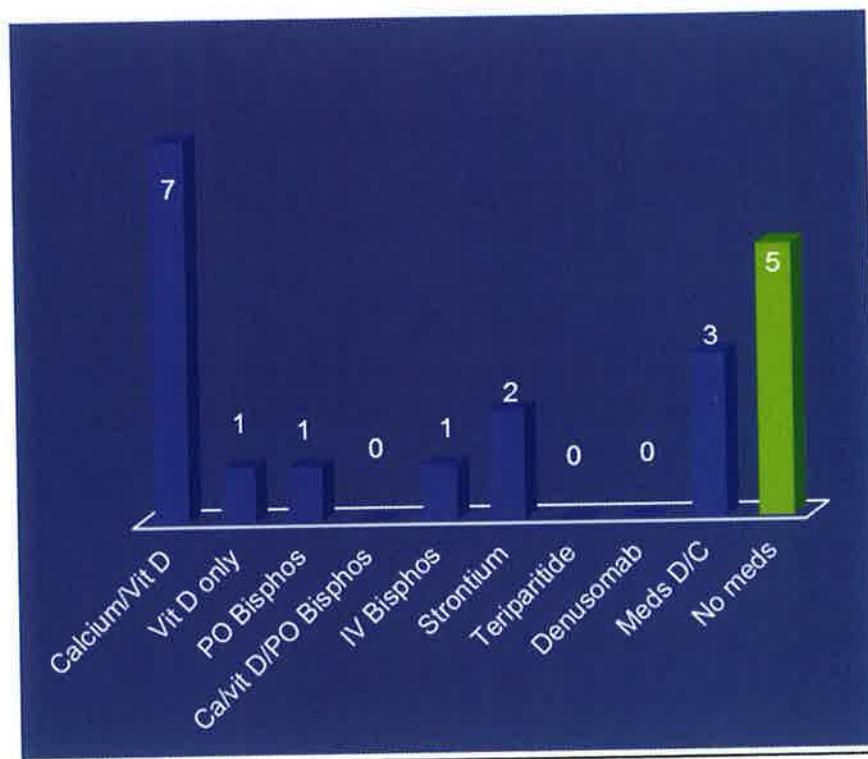


Figure 4.1: Medications prescribed to residents with known previous fractures in the geriatrician led facility.

4.3.2 GP led nursing home results

In the GP led facilities, 30/84(36%) of all residents were prescribed at least one osteoporotic medication at the time of the study. When FRAX scores were computed, we found 33(42%) had a 10 year probability of a major fracture of $\geq 20\%$ on their FRAX scores. Table 4.3 shows the osteoporotic medications prescribed to the aforementioned high risk residents in the geriatrician led facility. Similar to the geriatrician led cohort, the most commonly prescribed medications were calcium/vitamin D combinations, followed by oral bisphosphonates. There was low prescription rates of vitamin D alone, intravenous bisphosphonates, strontium and teriparitide and zero use of denosumab.

Of the residents with high risk major osteoporotic FRAX scores, only 1/35(0.02%) had previously had DEXA scans.

Table 4.3: Bone medications prescribed to high risk FRAX score pts in GP led nursing homes.

	High risk major osteoporotic fracture residents (n=33)
Vitamin D alone	1(0.02%)
Calcium/Vitamin D	12(34%)
PO Bisphosphonate	5(14%)
IV Bisphosphonate	1(0.02%)
Strontium	0
Teriparitide	0
Denusomab	0
No treatment	14(42%)

Figure 4.2 below depicts the osteoporotic medications prescribed to residents with known previous fractures in GP led facilities. Of a total of 13 residents with known previous fragility fracture, 5 were on calcium/vitamin D combinations, with low numbers on oral bisphosphonates, vitamin D only etc. 1 resident had been on appropriate treatment but this had been discontinued because of swallowing difficulties. A further 2(15%) residents with previous fractures were on no medication, with no documented reason.

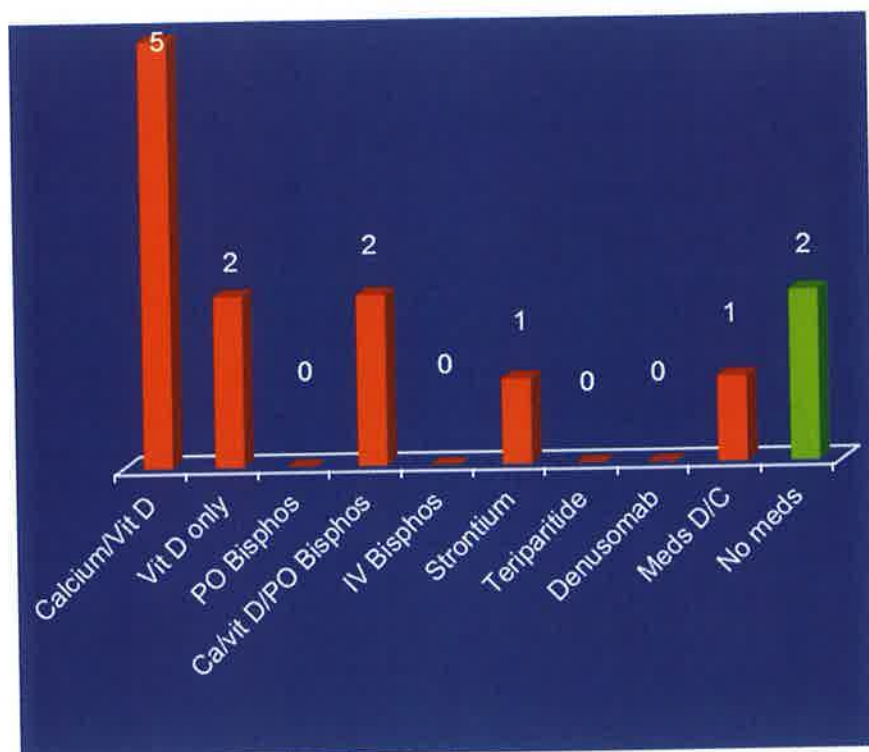


Figure 4.2: Medications prescribed to residents with known previous fractures in the GP led facilities.

4.3.3 Comparison of prescribing habits in geriatrician versus GP led facilities

We found no significant difference using the Chi-square test between osteoporosis prescribing habits in geriatrician versus GP led facilities as shown in Table 4.4.

Table 4.4 Percentage of the GP and geriatrician-led facilities receiving osteoporosis medications and associations (Chi-square test)

	Geriatrician-led nursing home	GP led nursing homes	Chi-square test, p
Vitamin D alone	4.8%	4.8%	p=1.0
Calcium/Vitamin D	31%	28.6%	p=0.74
PO bisphosphonates	9.5%	9.5%	p=1.0
IV bisphosphonates	2.4%	1.2%	p=1.0 [§]
Strontium	4.8%	2.4%	p=0.68 [§]
Teriparitide	1.2%	0.0%	p=1.0 [§]

[§] Fishers exact test was used due to small sample size.

In a logistic regression analysis, we used a backwards stepwise method removing any variables that were not significant after adjustment until the most significant variables were left at the end. We found that residents of male gender were statistically less likely to receive osteoporotic medications compared with their female counterparts(see Table 4.5 below). Similarly, residents of older age were less likely to be prescribed osteoporosis treatment than their younger counterparts. Residents with a previous history of fracture, secondary osteoporosis and smoking were significantly more likely to be prescribed osteoporotic medications.

Table 4.5: Multivariate predictors of odds of receiving an osteoporosis medication.

	Odds Ratio	P value	95% Confidence Interval(CI)
Previous fracture	5.57	0.003	1.79-17.3
Smoker	8.22	0.039	1.11-60.85
Male Gender	0.16	0.013	0.04-0.68
Secondary osteoporosis	37.8	0.001	4.68-305
Age	0.86	<0.001	0.80-0.94

4.4 Discussion

This is one of the first studies to look at prescribing habits of osteoporosis medications in the Irish long term care setting. This prospective study compared prescribing habits of osteoporotic medications prescribed to 168 residents in both

geriatrician led and GP led nursing homes. From our analysis, we found little difference in disease burden or osteoporosis prescribing patterns between GP led and geriatrician led facilities. We found that at the time of the study 43% of residents sampled in the geriatrician led facility were on at least one osteoporotic drug compared with 36% of GP led facilities. These figures are higher than those reported by Wright[87] and Jachna et al[85] previously. However, when prescribing patterns for geriatric led NH residents were examined, our results showed that only 33% of subjects with high FRAX scores were on calcium and vitamin D and only 11% were on bisphosphonate therapy. In a similar analysis in GP led NH residents, only 34% of those with high hip fracture risk scores were on calcium and vitamin D with a further 14% on bisphosphonate therapy. As bisphosphonate therapy is generally considered first line therapy, the use of these agents seems remarkably low in both types of facilities. Oral bisphosphonates can be difficult to tolerate due to their GI side effect and in these cases, an intravenous bisphosphonate is licensed for use. However, the use of IV bisphosphonates were similarly very low in both geriatrician and GP led facilities. Other osteoporotic drugs including strontium and teriparatide were used in a very small number of cases in treating high risk FRAX score residents in both types of facilities with zero use of denosumab seen at the time of the study.

In various surveys, the prevalence of effective osteoporosis treatment amounted from 9% to 38% [202,203,204]. This confirms the marked undertreatment of osteoporosis in this high-risk population[88, 205].

The prevalence of vertebral fracture in nursing home residents is not known. Whether the detection of such fracture and their inclusion into the Fracture Risk Assessment (FRAX) algorithm could improve fracture probability evaluation in this

specific population, and thereby further identify patients at increased risk of fracture deserving therapy, is not established.

On examining the association between several factors and the use of medications indicated for treatment of osteoporosis, we found that osteoporosis medication use was disproportionate across gender. Male residents were statistically less likely to receive anti-osteoporotic medications compared with their female counterparts in both types of facilities. This is obviously a concern as fracture risk rises exponentially in males from the age of 70[1]. Furthermore, fracture risk may be underestimated by FRAX in males[198] which may indicate further under-treatment of high risk male residents in this study.

In general, our findings demonstrate a high fracture risk as determined by FRAX score in both types of facilities. This was coupled with a low level of fracture protection and almost negligible DEXA scanning.

One potential explanation for this under-treatment of osteoporosis in the nursing home setting is that the treating physician may have an expectation of a lack of a favourable risk to benefit ratio for osteoporosis therapies, given the advanced age, limited life expectancy and multiple comorbidities suffered by many nursing home residents. This is borne out by our findings that older residents had lower odds of receiving osteoporotic medications than their younger counterparts. Many nursing home residents may have a concurrent diagnosis of cognitive impairment or dementia which may also discourage osteoporosis treatment due to perceived risk of complications with medications, lack of adherence with treatment and shorter life expectancy[206].

Similarly, given the often silent nature of osteoporosis, the treating physician may have considered other medical conditions that were more symptomatic and favoured treating them instead of osteoporosis[207]. As seen in a number of our cases, treatment may have been initially started appropriately, but subsequently discontinued due to swallowing difficulties, low GFR etc.

Guidelines for treating this subpopulation of older, often poorly mobile or bedbound elderly are unclear and this may result in uncertainty regarding appropriate management. However, guidance is clearer for older individuals who have already experienced one or more clinical fragility fractures.

Study Limitations

This study is limited by the fact that sample size is relatively small. Notable absences from the medical notes in both types of facilities included information on Dexa scans which may have been carried out prior to the resident's admission to each facility were not available in the medical notes. Availability of hip BMD measurements from Dexa scans would have enabled us to calculate a more accurate fracture risk score. However, Rodondi et al found that a systematic assessment of bone mineral density and/ or vertebral fracture does not appear to modify the 10-year fracture probability obtained by the FRAX tool based on age and medical history in this specific elderly population[198]. Information regarding intolerances or reasons for anti-osteoporotic medications being discontinued were frequently unavailable in the medical notes. We did not collect information on the use of hip protectors or look at whether residents had had recent falls in either type of facility.

Our findings suggest that there is significant room for improvement in the management of osteoporosis care in the Irish nursing home setting.

Recommendations for the management of osteoporosis in the long term care setting would include baseline blood tests for all new residents including calcium and renal profiles(to calculate GFR). Supplemental blood tests such as serum protein electrophoresis should be carried out if myeloma is suspected. Empirical treatment with vitamin D should be offered to all residents if calcium levels are normal given the high likelihood that residents will be vitamin D deficient. FRAX scores should be calculated for all new residents with treatment considered for residents with a 10-year risk of major fracture (hip, clinical vertebral, wrist, or humerus) that exceeds 20% [64]. Assuming no contraindications(suitable GFR and no history of GI motility problems), these high risk residents should be considered for PO or IV bisphosphonate therapy.

Once therapy is started adherence to and tolerance of the medication should be assessed regularly and adjusted if appropriate. Teriparatide should be reserved for patients with recurrent fractures on bisphosphonates and those intolerant of bisphosphonates. Denosumab may also be considered for patients with difficulty tolerating bisphosphonates or difficulty adhering or complying with this therapy. Each individual case must be judged on its own merit given the complex medical morbidities that many residents have. All patients with known previous fractures should be on vitamin D, calcium and bisphosphonate therapy if appropriate due to the increased risk of a second fracture[208]. If patients are mobile and do not have difficulty with leaving the nursing home surroundings, then a baseline DEXA if not carried out in the previous 3 years may be appropriate.

Non pharmacological therapy: As 90% of hip fractures are due to falls, severe modifiable risk factors should be assessed including visual, hearing deficits, medication review(particularly psychoactive medications) and orthostatic BP assessment. Regular weight bearing exercise should also be encouraged if feasible[89].

4.5 Conclusion

This study raises important questions about the prescription of osteoporosis medications in long term care facilities in Ireland. Although, we found little difference in prescribing patterns between geriatrician led versus GP led facilities, we did note high fracture risk as determined by FRAX score coupled with a low level of fracture protection and almost negligible DEXA scanning.

High risk fracture residents infrequently received an indicated osteoporosis treatment and when residents were treated, the choice of agents was often suboptimal with very low percentages on bisphosphonates, teriparitide or novel therapies such as denosumab. Many residents appear not to have been treated appropriately with anti-resorptive therapy. Future longitudinal research is needed to create guidelines to aid management of osteoporosis in subpopulations such as elderly nursing home residents.

CHAPTER 5

CONCLUSIONS

5.1 Introduction

The aims of this thesis were

1. To determine whether the medical management of osteoporosis in a nursing home population is different between a geriatric led and general practitioner led service and to assess the appropriateness of the medications prescribed.
2. To determine the repeatability of PWV measurement in a hospital setting in a cohort of older patients using the Vicorder apparatus.
3. To determine the prevalence of vitamin D deficiency among a screened population of community dwelling elderly patients.
4. To determine whether vitamin D replacement leads to changes in arterial stiffness in vitamin D deficient patients.
5. To compare the efficacy of two different doses of intramuscular vitamin D in providing supplementation and whether there is a difference in their effect on arterial stiffness.

5.2 Conclusion of nursing home prescribing study

The economic burden of osteoporosis is substantial and is destined to grow as the prevalence of osteoporosis increases in line with the ageing population. By 2020, annual osteoporotic fractures in the UK are predicted to increase by 21% to 230,000 per year, with costs growing by 20% to over £2.1 billion per year[4]. In Ireland,

osteoporosis related fractures are estimated to cost €404 million/year which amounts to approximately 4.2% of all public health expenditure in Ireland [209].

Previous research indicates that osteoporosis screening and therapies are underutilized in the nursing home population. In order to investigate the medical management of osteoporosis in the long term care setting, we carried out an observational study looking at the influence of geriatricians and general practitioners on prescribing of osteoporosis related medications in nursing homes (NH). We calculated FRAX scores on patients recruited, a score which estimates the 10-year probabilities of hip fracture and major osteoporotic fractures. Results showed when prescribing patterns for geriatric led NH residents were examined, only 33% of subjects with high FRAX scores were on calcium and vitamin D and only 11% were on bisphosphonate therapy. In a similar analysis in GP led NH residents, only 34% of those with high hip fracture risk scores were on calcium and vitamin D with a further 14% on bisphosphonate therapy. In general, our findings demonstrated a high fracture risk as determined by FRAX score in both types of facilities. This was coupled with a low level of use of anti-resorptive therapy and almost negligible DEXA scanning. There was no significant difference in prescribing habits for osteoporosis treatments noted between the two types of facilities.

Recommendations from this study

Current guidelines for treating this subpopulation of older, often poorly mobile or bedbound elderly are unclear. Recommendations have been as to assessment of long-term care residents for osteoporosis and further longitudinal research is needed to develop firm guidelines to aid the management of osteoporosis in this setting.

5.3 Conclusion of Vicorder repeatability study

A nurse and doctor carried out PWV measurements on 25 consecutive patients using the Vicorder device and were blinded to each other's results. We found acceptable levels of both within- and between-observer repeatability, with values of intraclass correlation coefficients ranging from 0.8 – 0.93. Our results showed that the highest repeatability was achieved using the traditional arterial path length (0.93) when compared with the adapted arterial path length (0.88). LOA were wide and may be due to the relative inexperience of our observers. However, in weighing up the appropriateness of different methods of assessing repeatability, we suggest that ICC's are the most appropriate test as LOA's are harder to apply to the error of a single measurement and are more difficult to interpret.

We found that this technique can be quickly acquired for use in research or clinical settings. We conclude that this non-invasive method of assessing arterial stiffness has the potential to be included in the clinical assessment of older ambulant patients

Recommendations of this study

The Vicorder is a simple, non-invasive method of assessing pulse wave velocity and has been shown to have repeatability. We recommend its use in the clinical setting particularly in the evaluation of older patients.

5.4 Conclusion of vitamin D deficiency prevalence study

It is increasingly recognised that there is a worldwide prevalence of vitamin D deficiency and Ireland appears to be no exception. Previous Irish studies have noted

prevalences of vitamin D deficiency (< 50 nmol/l) varying between 55-75% of community dwelling cohorts[25, 178]. We found that 61% of community dwelling elderly people in North Dublin were deficient in vitamin D.

Recommendations of this study

Physicians should have a low threshold when considering treatment of suspected vitamin D deficiency in the older population, given the high prevalence found in this and other studies. A vitamin D rich diet, exposure to sunlight along with oral supplementation should be recommended. Given Ireland's latitude, these figures highlight the need to explore strategies for patient education regarding the importance of vitamin D to enable individuals to attain dietary targets.

5.5 Conclusion of vitamin D/arterial stiffness study

Vitamin D has a myriad of biological effects in addition to its traditionally ascribed roles in calcium metabolism and bone health. Vitamin D deficiency has been associated with hypertension, vascular disease and heart failure[13]. Clinical studies have shown cross sectional associations between lower vitamin D levels and plasma renin activity, coronary artery calcification, blood pressure (BP) and cardiovascular disease[14-16]. Previous observational and cross-sectional data support a link between low vitamin D metabolite levels and cardiovascular health. However, such associations are prone to confounders, and to date interventional trials have been less promising. We performed a randomised clinical trial to determine whether

vitamin D replacement leads to changes in arterial stiffness in vitamin D deficient patients. We sought to determine whether vitamin D deficiency is associated with increased arterial stiffness as determined by PWV and to compare the effects of 50,000IU of cholecalciferol with 100,000IU of cholecalciferol on arterial stiffness.

Amongst our findings was that low increases in vitamin D status followed the administration of 100,000IU and 50,000IU doses of cholecalciferol indicating that intramuscular use of these doses of vitamin D may not be adequate to achieve optimal vitamin D levels.

In clinical practice, IM cholecalciferol is commonly used to treat vitamin D deficiency particularly in elderly patients where compliance with supplements is often poor. Vitamin D levels post therapy are not checked routinely and there is often a presumption that levels are corrected adequately post IM injection. In this study, only 3/51(5.8%) who received 100,000IU vitamin D reached levels of sufficiency(>75nmols/L), with none of the participants who received 50,000IU achieving sufficient vitamin D levels 8 weeks post therapy. Intramuscular use of vitamin D may have decreased bioavailability when compared with oral and intravenous routes[185]. It may be that despite compliance issues, regular oral treatment may be safer and more effective than intra-muscular therapy in the treatment of vitamin D deficiency in the older population.

Previous studies have demonstrated that vitamin D deficiency may lead to impairment of vascular effects leading to abnormalities in central arterial stiffness [139]. Our randomised controlled trial noted a non- significant trend towards low vitamin D with raised PWV at baseline. I also noted a non-significant trend towards decreasing PWV as vitamin D status improved. The non-significance may be

explained by the underpowering of the study due to difficulty with recruitment. We noted a significant improvement in AIx from week 0 to week 8, with a mean difference of 3.803 ± 1.76 seen in the group who received the higher vitamin D dose ($p=0.033$). Our study demonstrated that the group that received 100,000IU vitamin D, median PWV decreased from $12.2(5.1-40.3)$ m/s to $11.5(4.3-14.9)$ m/s over the 8 week study period ($p=0.22$). Further research is needed to investigate whether sufficient supplementation of vitamin D by a reliable method could result in positive functional changes in arterial stiffness. ALT, total cholesterol and LDL all increased over the 8 week period. A recent meta-analysis confirms an increase in LDL and total cholesterol post vitamin D supplementation[189]. These potential positive effects on lipids using vitamin D treatment should be investigated through large scale RCTs. We were unable to find any correlation between PWV and hsCRP, MMP-9 or OPG as had been demonstrated in a number of previous studies[192-194]. This may be due to our small sample size.

Recommendations of this study

Intramuscular methods of vitamin D supplementation may not be adequate to achieve optimal vitamin D levels in this older population. If IM vitamin D is utilised, levels should be checked subsequently to ensure that sufficient serum 25OHD levels have been achieved. A trend towards improvement in arterial stiffness as vitamin D status improved was noted. Further research is needed to expand our knowledge of the pathogenesis of arterial stiffness and its association with vitamin D deficiency in the older population.

Ultimately, the question of whether vitamin D supplementation improves vascular health can only be determined by performing large randomised controlled trials, specifically designed to answer this question. Until appropriate trial data are

available, extending the prescribing indications for vitamin D beyond its current use in osteomalacia and osteoporosis cannot be justified.

Bibliography

1. Johnell, O. and J.A. Kanis, *An estimate of the worldwide prevalence and disability associated with osteoporotic fractures*. *Osteoporos Int*, 2006. **17**(12): p. 1726-33.
2. Ahmed, S.F. and M. Elmantaser, *Secondary osteoporosis*. *Endocr Dev*, 2009. **16**: p. 170-90.
3. McGreevy, C., Williams D, *Safety of drugs used in the treatment of osteoporosis*. *Therapeutic Advances in Drug Safety*, 2011. **2**(4): p. 159-172.
4. Burge RT, W.D., Johansen A, Bhattacharyya S, Bose U., *The cost of osteoporotic fractures in the UK: projections for 2000–2020*. *J Med Econ*, 2001. **4**: p. 51-62.
5. Blair, H.C., et al., *Osteoclastic bone resorption by a polarized vacuolar proton pump*. *Science*, 1989. **245**(4920): p. 855-7.
6. Zallone, A., *Direct and indirect estrogen actions on osteoblasts and osteoclasts*. *Ann N Y Acad Sci*, 2006. **1068**: p. 173-9.
7. Eriksen, E.F., et al., *Hormone replacement therapy prevents osteoclastic hyperactivity: A histomorphometric study in early postmenopausal women*. *J Bone Miner Res*, 1999. **14**(7): p. 1217-21.
8. Hughes, D.E., et al., *Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-beta*. *Nat Med*, 1996. **2**(10): p. 1132-6.
9. Yasuda, H., et al., *Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL*. *Proc Natl Acad Sci U S A*, 1998. **95**(7): p. 3597-602.
10. Clunie Gavin, K.R., *Osteoporosis*. 2008, United Kingdom Oxford University Press.
11. Adams, J.S. and M. Hewison, *Update in vitamin D*. *J Clin Endocrinol Metab*, 2010. **95**(2): p. 471-8.
12. DeLuca, H.F. and C. Zierold, *Mechanisms and functions of vitamin D*. *Nutr Rev*, 1998. **56**(2 Pt 2): p. S4-10; discussion S 54-75.
13. Pilz, S., et al., *Vitamin D status and arterial hypertension: a systematic review*. *Nat Rev Cardiol*, 2009. **6**(10): p. 621-30.
14. Mathieu, C., et al., *Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3*. *Diabetologia*, 1994. **37**(6): p. 552-8.
15. Tishkoff, D.X., et al., *Functional vitamin D receptor (VDR) in the t-tubules of cardiac myocytes: VDR knockout cardiomyocyte contractility*. *Endocrinology*, 2008. **149**(2): p. 558-64.
16. Aggarwal N, R.J.P., Michos E.D, *Vitamin D Deficiency and Its Implications on Cardiovascular Disease*. *Curr Cardiol Rep*, 2010. **4**(1): p. 68-75.
17. Mithal, A., et al., *Global vitamin D status and determinants of hypovitaminosis D*. *Osteoporos Int*, 2009. **20**(11): p. 1807-20.
18. Ashwell, M., et al., *UK Food Standards Agency Workshop Report: an investigation of the relative contributions of diet and sunlight to vitamin D status*. *Br J Nutr*, 2010. **104**(4): p. 603-11.
19. McGreevy, C. and D. Williams, *New insights about vitamin D and cardiovascular disease: a narrative review*. *Ann Intern Med*, 2011. **155**(12): p. 820-6.
20. DeLuca, H.F., *Overview of general physiologic features and functions of vitamin D*. *Am J Clin Nutr*, 2004. **80**(6 Suppl): p. 1689S-96S.

21. Dawson-Hughes, B., et al., *IOF position statement: vitamin D recommendations for older adults*. *Osteoporos Int*, 2010. **21**(7): p. 1151-4.
22. (IOM), I.o.M. *Dietary Reference Intakes for Calcium and Vitamin D*. 2010 [cited 2011 May 11th]; Available from: <http://www.iom.edu/Activities/Nutrition/DRIVitDCalcium/2010-NOV-30.aspx>.
23. Hill, T.R., et al., *Vitamin D status of 51-75-year-old Irish women: its determinants and impact on biochemical indices of bone turnover*. *Public Health Nutr*, 2006. **9**(2): p. 225-33.
24. Hill, T., et al., *Vitamin D intake and status in Irish postmenopausal women*. *Eur J Clin Nutr*, 2005. **59**(3): p. 404-10.
25. DeLappe, E., et al., *Vitamin D insufficiency in older female community-dwelling acute hospital admissions and the response to supplementation*. *Eur J Clin Nutr*, 2006. **60**(8): p. 1009-15.
26. Romero-Ortuno, R., et al., *Seasonal variation of serum vitamin D and the effect of vitamin D supplementation in Irish community-dwelling older people*. *Age Ageing*, 2011. **40**(2): p. 168-74.
27. Heaney, R.P., et al., *Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol*. *Am J Clin Nutr*, 2003. **77**(1): p. 204-10.
28. Hypponen, E. and C. Power, *Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors*. *Am J Clin Nutr*, 2007. **85**(3): p. 860-8.
29. Lips, P., *Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications*. *Endocr Rev*, 2001. **22**(4): p. 477-501.
30. Chapuy, M.C., et al., *Prevalence of vitamin D insufficiency in an adult normal population*. *Osteoporos Int*, 1997. **7**(5): p. 439-43.
31. Snijder, M.B., et al., *Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women*. *J Clin Endocrinol Metab*, 2005. **90**(7): p. 4119-23.
32. van der Wielen, R.P., et al., *Serum vitamin D concentrations among elderly people in Europe*. *Lancet*, 1995. **346**(8969): p. 207-10.
33. Ovesen, L., R. Andersen, and J. Jakobsen, *Geographical differences in vitamin D status, with particular reference to European countries*. *Proc Nutr Soc*, 2003. **62**(4): p. 813-21.
34. Ginde, A.A., M.C. Liu, and C.A. Camargo, Jr., *Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004*. *Arch Intern Med*, 2009. **169**(6): p. 626-32.
35. Sachan, A., et al., *High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India*. *Am J Clin Nutr*, 2005. **81**(5): p. 1060-4.
36. Islam, M.Z., et al., *Vitamin D deficiency: a concern in premenopausal Bangladeshi women of two socio-economic groups in rural and urban region*. *Eur J Clin Nutr*, 2002. **56**(1): p. 51-6.
37. Lips, P., et al., *The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation*. *J Intern Med*, 2006. **260**(3): p. 245-54.

38. Siddiqui, A.M. and H.Z. Kamfar, *Prevalence of vitamin D deficiency rickets in adolescent school girls in Western region, Saudi Arabia*. Saudi Med J, 2007. **28**(3): p. 441-4.
39. Arabi, A., et al., *Hypovitaminosis D osteopathy: is it mediated through PTH, lean mass, or is it a direct effect?* Bone, 2006. **39**(2): p. 268-75.
40. Sambrook, P.N., et al., *Vitamin D deficiency is common in frail institutionalised older people in northern Sydney*. Med J Aust, 2002. **176**(11): p. 560.
41. Brock, K., et al., *Associations with Vitamin D deficiency in "at risk" Australians*. J Steroid Biochem Mol Biol, 2004. **89-90**(1-5): p. 581-8.
42. Robinson, P.D., et al., *The re-emerging burden of rickets: a decade of experience from Sydney*. Arch Dis Child, 2006. **91**(7): p. 564-8.
43. Holick, M.F., *Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease*. Am J Clin Nutr, 2004. **80**(6 Suppl): p. 1678S-88S.
44. MacLaughlin, J. and M.F. Holick, *Aging decreases the capacity of human skin to produce vitamin D3*. J Clin Invest, 1985. **76**(4): p. 1536-8.
45. Pearce, S.H. and T.D. Cheetham, *Diagnosis and management of vitamin D deficiency*. BMJ, 2010. **340**: p. b5664.
46. Matsuoka, L.Y., et al., *Sunscreens suppress cutaneous vitamin D3 synthesis*. J Clin Endocrinol Metab, 1987. **64**(6): p. 1165-8.
47. Wortsman, J., et al., *Decreased bioavailability of vitamin D in obesity*. Am J Clin Nutr, 2000. **72**(3): p. 690-3.
48. Holick, M.F., *Vitamin D for health and in chronic kidney disease*. Semin Dial, 2005. **18**(4): p. 266-75.
49. Zhou, C., et al., *Steroid and xenobiotic receptor and vitamin D receptor crosstalk mediates CYP24 expression and drug-induced osteomalacia*. J Clin Invest, 2006. **116**(6): p. 1703-12.
50. Van Den Bout-Van Den Beukel, C.J., et al., *Vitamin D deficiency among HIV type 1-infected individuals in the Netherlands: effects of antiretroviral therapy*. AIDS Res Hum Retroviruses, 2008. **24**(11): p. 1375-82.
51. Foundation, N.O., *National Osteoporosis Foundation. Clinician's Guide to Prevention and Treatment of Osteoporosis*. Washington, DC. 2010: Washington, DC.
52. WHO, *Assessment of fracture risk and its application to screening for postmenopausal osteoporosis*, in 843. 2006: Geneve.
53. Kanis, J.A., et al., *FRAX and the assessment of fracture probability in men and women from the UK*. Osteoporos Int, 2008. **19**(4): p. 385-97.
54. Klotzbuecher, C.M., et al., *Patients with prior fractures have an increased risk of future fractures: a summary of the literature and statistical synthesis*. J Bone Miner Res, 2000. **15**(4): p. 721-39.
55. *Guideline for the prevention of falls in older persons*. American Geriatrics Society, British Geriatrics Society, and American Academy of Orthopaedic Surgeons Panel on Falls Prevention. J Am Geriatr Soc, 2001. **49**(5): p. 664-72.

56. Kelley, G.A., K.S. Kelley, and Z.V. Tran, *Resistance training and bone mineral density in women: a meta-analysis of controlled trials*. Am J Phys Med Rehabil, 2001. **80**(1): p. 65-77.
57. Nikander, R., et al., *Targeted exercise against osteoporosis: A systematic review and meta-analysis for optimising bone strength throughout life*. BMC Med, 2010. **8**: p. 47.
58. Howe, T.E., et al., *Exercise for preventing and treating osteoporosis in postmenopausal women*. Cochrane Database Syst Rev, 2011(7): p. CD000333.
59. Nowson, C.A., *Prevention of fractures in older people with calcium and vitamin D*. Nutrients, 2010. **2**(9): p. 975-84.
60. Bischoff-Ferrari, H.A., et al., *Is fall prevention by vitamin D mediated by a change in postural or dynamic balance?* Osteoporos Int, 2006. **17**(5): p. 656-63.
61. Bischoff-Ferrari, H.A., et al., *Prevention of nonvertebral fractures with oral vitamin D and dose dependency: a meta-analysis of randomized controlled trials*. Arch Intern Med, 2009. **169**(6): p. 551-61.
62. *Patient level pooled analysis of 68 500 patients from seven major vitamin D fracture trials in US and Europe*. BMJ, 2010. **340**: p. b5463.
63. Tang, B.M., et al., *Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis*. Lancet, 2007. **370**(9588): p. 657-66.
64. Berry, S.D., et al., *Application of the National Osteoporosis Foundation Guidelines to postmenopausal women and men: the Framingham Osteoporosis Study*. Osteoporos Int, 2010. **21**(1): p. 53-60.
65. Bolland, M.J., et al., *Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis*. BMJ, 2010. **341**: p. c3691.
66. Bolland, M.J., et al., *Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis*. BMJ, 2011. **342**: p. d2040.
67. Cummings, S.R., et al., *Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures: results from the Fracture Intervention Trial*. JAMA, 1998. **280**(24): p. 2077-82.
68. Black, D.M., et al., *Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis*. N Engl J Med, 2007. **356**(18): p. 1809-22.
69. Pols, H.A., et al., *Multinational, placebo-controlled, randomized trial of the effects of alendronate on bone density and fracture risk in postmenopausal women with low bone mass: results of the FOSIT study*. Fosamax International Trial Study Group. Osteoporos Int, 1999. **9**(5): p. 461-8.
70. Rosen, C.J., et al., *Treatment with once-weekly alendronate 70 mg compared with once-weekly risedronate 35 mg in women with postmenopausal osteoporosis: a randomized double-blind study*. J Bone Miner Res, 2005. **20**(1): p. 141-51.
71. Chesnut, I.C., et al., *Effects of oral ibandronate administered daily or intermittently on fracture risk in postmenopausal osteoporosis*. J Bone Miner Res, 2004. **19**(8): p. 1241-9.
72. Reid, I.R., et al., *Intravenous zoledronic acid in postmenopausal women with low bone mineral density*. N Engl J Med, 2002. **346**(9): p. 653-61.
73. Greenspan, S.L., et al., *Effect of recombinant human parathyroid hormone (1-84) on vertebral fracture and bone mineral density in postmenopausal women with osteoporosis: a randomized trial*. Ann Intern Med, 2007. **146**(5): p. 326-39.

74. Seeman, E., et al., *Five years treatment with strontium ranelate reduces vertebral and nonvertebral fractures and increases the number and quality of remaining life-years in women over 80 years of age*. *Bone*, 2010. **46**(4): p. 1038-42.
75. Agency., E.M., *Meeting highlights from the Pharmacovigilance Risk Assessment Committee (PRAC) 8-11 April 2013: Outcome of periodic safety update report assessment leads to recommendation to restrict use of a medicine*. April 12, 2013.
76. Anderson, G.L., et al., *Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial*. *JAMA*, 2004. **291**(14): p. 1701-12.
77. Rossouw, J.E., et al., *Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial*. *JAMA*, 2002. **288**(3): p. 321-33.
78. Dias, A.R., Jr., et al., *Effects of conjugated equine estrogens or raloxifene on lipid profile, coagulation and fibrinolysis factors in postmenopausal women*. *Climacteric*, 2005. **8**(1): p. 63-70.
79. McClung, M.R., et al., *Prevention of bone loss in postmenopausal women treated with lasofoxifene compared with raloxifene*. *Menopause*, 2006. **13**(3): p. 377-86.
80. Prestwood, K.M., et al., *A comparison of the effects of raloxifene and estrogen on bone in postmenopausal women*. *J Clin Endocrinol Metab*, 2000. **85**(6): p. 2197-202.
81. Gennari, L., et al., *Selective estrogen receptor modulators for postmenopausal osteoporosis: current state of development*. *Drugs Aging*, 2007. **24**(5): p. 361-79.
82. *European Medicines Agency recommends limiting long-term use of calcitonin medicines 2012* [cited 2012 September 19th]; Press Release]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2012/07/news_detail_001573.jsp&mid=WC0b01ac058004d5c1.
83. Cummings, S.R., et al., *Denosumab for prevention of fractures in postmenopausal women with osteoporosis*. *N Engl J Med*, 2009. **361**(8): p. 756-65.
84. McCormick, B.B., J. Davis, and K.D. Burns, *Severe hypocalcemia following denosumab injection in a hemodialysis patient*. *Am J Kidney Dis*, 2012. **60**(4): p. 626-8.
85. Jachna, C.M., et al., *Differing patterns of antiresorptive pharmacotherapy in nursing facility residents and community dwellers*. *J Am Geriatr Soc*, 2005. **53**(8): p. 1275-81.
86. Colon-Emeric, C., et al., *Prevalence and predictors of osteoporosis treatment in nursing home residents with known osteoporosis or recent fracture*. *Osteoporos Int*, 2007. **18**(4): p. 553-9.
87. Wright, R.M., *Use of osteoporosis medications in older nursing facility residents*. *J Am Med Dir Assoc*, 2007. **8**(7): p. 453-7.
88. Rojas-Fernandez, C.H., et al., *Undertreatment of osteoporosis in residents of nursing homes: population-based study with use of the Systematic Assessment of Geriatric Drug Use via Epidemiology (SAGE) database*. *Endocr Pract*, 2002. **8**(5): p. 335-42.
89. Warriner, A.H., et al., *Management of osteoporosis among home health and long-term care patients with a prior fracture*. *South Med J*, 2009. **102**(4): p. 397-404.
90. Manek, N., *Missed opportunities for osteoporosis management in older patients: a literature review*. *Primary Healthcare Research and Development*, 2010. **11**(2): p. 155-165.
91. Zittermann, A., S.S. Schleithoff, and R. Koerfer, *Putting cardiovascular disease and vitamin D insufficiency into perspective*. *Br J Nutr*, 2005. **94**(4): p. 483-92.

92. Zittermann, A., K. Scheld, and P. Stehle, *Seasonal variations in vitamin D status and calcium absorption do not influence bone turnover in young women*. Eur J Clin Nutr, 1998. **52**(7): p. 501-6.
93. Douglas, A.S., et al., *Seasonal variation in coronary heart disease in Scotland*. J Epidemiol Community Health, 1995. **49**(6): p. 575-82.
94. Scragg, R., *Seasonality of cardiovascular disease mortality and the possible protective effect of ultra-violet radiation*. Int J Epidemiol, 1981. **10**(4): p. 337-41.
95. Xiang, W., et al., *Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems*. Am J Physiol Endocrinol Metab, 2005. **288**(1): p. E125-32.
96. Young, K.A., et al., *Vitamin D deficiency and coronary artery calcification in subjects with type 1 diabetes*. Diabetes Care, 2011. **34**(2): p. 454-8.
97. Forman, J.P., et al., *Plasma 25-hydroxyvitamin D levels and risk of incident hypertension*. Hypertension, 2007. **49**(5): p. 1063-9.
98. Martins, D., et al., *Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey*. Arch Intern Med, 2007. **167**(11): p. 1159-65.
99. Grimes, D.S., E. Hindle, and T. Dyer, *Sunlight, cholesterol and coronary heart disease*. QJM, 1996. **89**(8): p. 579-89.
100. Rostand, S.G., *Ultraviolet light may contribute to geographic and racial blood pressure differences*. Hypertension, 1997. **30**(2 Pt 1): p. 150-6.
101. Wang, T.J., et al., *Vitamin D deficiency and risk of cardiovascular disease*. Circulation, 2008. **117**(4): p. 503-11.
102. Giovannucci, E., et al., *25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study*. Arch Intern Med, 2008. **168**(11): p. 1174-80.
103. Poole, K.E., et al., *Reduced vitamin D in acute stroke*. Stroke, 2006. **37**(1): p. 243-5.
104. Scragg, R., et al., *Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: a community-based study*. Int J Epidemiol, 1990. **19**(3): p. 559-63.
105. Zittermann, A., et al., *Low vitamin D status: a contributing factor in the pathogenesis of congestive heart failure?* J Am Coll Cardiol, 2003. **41**(1): p. 105-12.
106. Cigolini, M., et al., *Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among type 2 diabetic patients*. Diabetes Care, 2006. **29**(3): p. 722-4.
107. Belz, G.G., *Elastic properties and Windkessel function of the human aorta*. Cardiovasc Drugs Ther, 1995. **9**(1): p. 73-83.
108. Nichols, W., O'Rourke, MF, *Mc Donalds blood flow in arteries: Theoretical experimental and Clinical Principles*. 4th Edition ed. 2006, London, UK: Edward Arnold.
109. Lakatta, E.G. and D. Levy, *Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part II: the aging heart in health: links to heart disease*. Circulation, 2003. **107**(2): p. 346-54.
110. Reriani, M.K., L.O. Lerman, and A. Lerman, *Endothelial function as a functional expression of cardiovascular risk factors*. Biomark Med, 2010. **4**(3): p. 351-60.
111. Endemann, D.H. and E.L. Schiffrin, *Endothelial dysfunction*. J Am Soc Nephrol, 2004. **15**(8): p. 1983-92.

112. McEniery, C.M., et al., *Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans*. Hypertension, 2006. **48**(4): p. 602-8.
113. Joannides, R., et al., *Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo*. Circulation, 1995. **91**(5): p. 1314-9.
114. Celermajer, D.S., *Reliable endothelial function testing: at our fingertips?* Circulation, 2008. **117**(19): p. 2428-30.
115. Laurent, S., et al., *Flow-dependent vasodilation of brachial artery in essential hypertension*. Am J Physiol, 1990. **258**(4 Pt 2): p. H1004-11.
116. Mackenzie, I.S., I.B. Wilkinson, and J.R. Cockcroft, *Assessment of arterial stiffness in clinical practice*. QJM, 2002. **95**(2): p. 67-74.
117. Safar, M., Frohlich ED, *Atherosclerosis, large arteries and cardiovascular risk*. Vol. 44. 2007, Basel: Karger. 1-18.
118. Laurent, S., et al., *Expert consensus document on arterial stiffness: methodological issues and clinical applications*. Eur Heart J, 2006. **27**(21): p. 2588-605.
119. Cho, S.W., et al., *Non-invasively measured aortic wave reflection and pulse pressure amplification are related to the severity of coronary artery disease*. J Cardiol, 2013. **62**(2): p. 131-7.
120. Chirinos, J.A., et al., *Aortic pressure augmentation predicts adverse cardiovascular events in patients with established coronary artery disease*. Hypertension, 2005. **45**(5): p. 980-5.
121. Vlachopoulos, C., et al., *Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals*. Circulation, 2005. **112**(14): p. 2193-200.
122. Karamanoglu, M., et al., *Pressure wave propagation in a multibranched model of the human upper limb*. Am J Physiol, 1995. **269**(4 Pt 2): p. H1363-9.
123. Stone, I.S., et al., *Reproducibility of arterial stiffness and wave reflections in chronic obstructive pulmonary disease: The contribution of lung hyperinflation and a comparison of techniques*. Respir Med, 2013.
124. Hickson, S.S., et al., *Validity and repeatability of the Vicorder apparatus: a comparison with the SphygmoCor device*. Hypertens Res, 2009. **32**(12): p. 1079-85.
125. Weber, T., et al., *Arterial stiffness, wave reflections, and the risk of coronary artery disease*. Circulation, 2004. **109**(2): p. 184-9.
126. Dong, Y., et al., *A 16-week randomized clinical trial of 2000 international units daily vitamin D3 supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial stiffness*. J Clin Endocrinol Metab, 2010. **95**(10): p. 4584-91.
127. Al Mheid, I., et al., *Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans*. J Am Coll Cardiol, 2011. **58**(2): p. 186-92.
128. Momiyama, Y., et al., *High plasma levels of matrix metalloproteinase-8 in patients with unstable angina*. Atherosclerosis, 2010. **209**(1): p. 206-10.
129. Kai, H., et al., *Peripheral blood levels of matrix metalloproteinases-2 and -9 are elevated in patients with acute coronary syndromes*. J Am Coll Cardiol, 1998. **32**(2): p. 368-72.
130. Timms, P.M., et al., *Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders?* QJM, 2002. **95**(12): p. 787-96.

131. Targher, G., et al., *Serum 25-hydroxyvitamin D3 concentrations and carotid artery intima-media thickness among type 2 diabetic patients*. Clin Endocrinol (Oxf), 2006. **65**(5): p. 593-7.
132. de Boer, I.H., et al., *25-hydroxyvitamin D levels inversely associate with risk for developing coronary artery calcification*. J Am Soc Nephrol, 2009. **20**(8): p. 1805-12.
133. Van Campenhout, A. and J. Golledge, *Osteoprotegerin, vascular calcification and atherosclerosis*. Atherosclerosis, 2009. **204**(2): p. 321-9.
134. Bucay, N., et al., *osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification*. Genes Dev, 1998. **12**(9): p. 1260-8.
135. Kiechl, S., et al., *The osteoprotegerin/RANK/RANKL system: a bone key to vascular disease*. Expert Rev Cardiovasc Ther, 2006. **4**(6): p. 801-11.
136. Secchiero, P., et al., *An increased osteoprotegerin serum release characterizes the early onset of diabetes mellitus and may contribute to endothelial cell dysfunction*. Am J Pathol, 2006. **169**(6): p. 2236-44.
137. Zauli, G., et al., *Osteoprotegerin increases leukocyte adhesion to endothelial cells both in vitro and in vivo*. Blood, 2007. **110**(2): p. 536-43.
138. Molines, L., P. Darmon, and D. Raccah, *Charcot's foot: newest findings on its pathophysiology, diagnosis and treatment*. Diabetes Metab, 2010. **36**(4): p. 251-5.
139. Melamed, M.L., et al., *Serum 25-hydroxyvitamin D levels and the prevalence of peripheral arterial disease: results from NHANES 2001 to 2004*. Arterioscler Thromb Vasc Biol, 2008. **28**(6): p. 1179-85.
140. Auwerx, J., R. Bouillon, and H. Kesteloot, *Relation between 25-hydroxyvitamin D3, apolipoprotein A-I, and high density lipoprotein cholesterol*. Arterioscler Thromb, 1992. **12**(6): p. 671-4.
141. Braam, L.A., et al., *Beneficial effects of vitamins D and K on the elastic properties of the vessel wall in postmenopausal women: a follow-up study*. Thromb Haemost, 2004. **91**(2): p. 373-80.
142. Burgaz, A., et al., *Confirmed hypertension and plasma 25(OH)D concentrations amongst elderly men*. J Intern Med, 2011. **269**(2): p. 211-8.
143. Major, G.C., et al., *Supplementation with calcium + vitamin D enhances the beneficial effect of weight loss on plasma lipid and lipoprotein concentrations*. Am J Clin Nutr, 2007. **85**(1): p. 54-9.
144. Scragg, R., K.T. Khaw, and S. Murphy, *Effect of winter oral vitamin D3 supplementation on cardiovascular risk factors in elderly adults*. Eur J Clin Nutr, 1995. **49**(9): p. 640-6.
145. Pan, W.H., et al., *No significant effect of calcium and vitamin D supplementation on blood pressure and calcium metabolism in elderly Chinese*. Chin J Physiol, 1993. **36**(2): p. 85-94.
146. Li, Y.C., et al., *1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system*. J Clin Invest, 2002. **110**(2): p. 229-38.
147. Zhou, C., et al., *Calcium-independent and 1,25(OH)2D3-dependent regulation of the renin-angiotensin system in 1alpha-hydroxylase knockout mice*. Kidney Int, 2008. **74**(2): p. 170-9.
148. Burgess, E.D., R.G. Hawkins, and M. Watanabe, *Interaction of 1,25-dihydroxyvitamin D and plasma renin activity in high renin essential hypertension*. Am J Hypertens, 1990. **3**(12 Pt 1): p. 903-5.

149. Snijder, M.B., et al., *Vitamin D status and parathyroid hormone levels in relation to blood pressure: a population-based study in older men and women.* J Intern Med, 2007. **261**(6): p. 558-65.
150. Hulter, H.N., et al., *Chronic continuous PTH infusion results in hypertension in normal subjects.* J Clin Hypertens, 1986. **2**(4): p. 360-70.
151. Fitzpatrick, L.A., et al., *Parathyroid hormone and the cardiovascular system.* Curr Osteoporos Rep, 2008. **6**(2): p. 77-83.
152. Pfeifer, M., et al., *Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women.* J Clin Endocrinol Metab, 2001. **86**(4): p. 1633-7.
153. Kristal-Boneh, E., et al., *Association of calcitriol and blood pressure in normotensive men.* Hypertension, 1997. **30**(5): p. 1289-94.
154. Sugden, J.A., et al., *Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels.* Diabet Med, 2008. **25**(3): p. 320-5.
155. Llewellyn, D.J., et al., *Vitamin D and risk of cognitive decline in elderly persons.* Arch Intern Med, 2010. **170**(13): p. 1135-41.
156. Grey, A. and M. Bolland, *Vitamin D: a place in the sun?* Arch Intern Med, 2010. **170**(13): p. 1099-100.
157. Blacher, J., et al., *Aortic pulse wave velocity index and mortality in end-stage renal disease.* Kidney Int, 2003. **63**(5): p. 1852-60.
158. Laurent, S., et al., *Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients.* Hypertension, 2001. **37**(5): p. 1236-41.
159. Cohn, J.N., *Arterial stiffness, vascular disease, and risk of cardiovascular events.* Circulation, 2006. **113**(5): p. 601-3.
160. Cruickshank, K., et al., *Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function?* Circulation, 2002. **106**(16): p. 2085-90.
161. Kracht, D., et al., *Validating a new oscillometric device for aortic pulse wave velocity measurements in children and adolescents.* Am J Hypertens, 2011. **24**(12): p. 1294-9.
162. Sedgwick, P., *Sample size calculations I.* BMJ, 2010. **340**: p. c3104.
163. Bland, J.M. and D.G. Altman, *Statistical methods for assessing agreement between two methods of clinical measurement.* Lancet, 1986. **1**(8476): p. 307-10.
164. van Leeuwen-Segarceanu, E.M., et al., *Comparison of two instruments measuring carotid-femoral pulse wave velocity: Vicorder versus SphygmoCor.* J Hypertens, 2010. **28**(8): p. 1687-91.
165. Sugawara, J., et al., *Carotid-Femoral Pulse Wave Velocity: Impact of Different Arterial Path Length Measurements.* Artery Res, 2010. **4**(1): p. 27-31.
166. Wilkinson, I.B., et al., *Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis.* J Hypertens, 1998. **16**(12 Pt 2): p. 2079-84.
167. Nemeth, Z.K., et al., *The method of distance measurement and torso length influences the relationship of pulse wave velocity to cardiovascular mortality.* Am J Hypertens, 2011. **24**(2): p. 155-61.
168. Muller, J., et al., *Oscillometric carotid to femoral pulse wave velocity estimated with the Vicorder device.* J Clin Hypertens (Greenwich), 2013. **15**(3): p. 176-9.

169. Crilly, M., et al., *Repeatability of the measurement of augmentation index in the clinical assessment of arterial stiffness using radial applanation tonometry*. Scand J Clin Lab Invest, 2007. **67**(4): p. 413-22.
170. Shahin, Y., et al., *The Vicorder device compared with SphygmoCor in the assessment of carotid-femoral pulse wave velocity in patients with peripheral arterial disease*. Hypertens Res, 2012.
171. Andrade, J., et al., *Exploration of association of 1,25-OH₂D₃ with augmentation index, a composite measure of arterial stiffness*. Clin J Am Soc Nephrol, 2008. **3**(6): p. 1800-6.
172. Sanders, K.M., et al, *Is high dose vitamin D harmful?* Calcif Tissue Int, 2013. **92**(2): p. 191-206.
173. Heaney, R.P., et al., *Vitamin D(3) is more potent than vitamin D(2) in humans*. J Clin Endocrinol Metab, 2011. **96**(3): p. E447-52.
174. Houghton, L.A. et al, *The case against ergocalciferol (vitamin D₂) as a vitamin supplement*. Am J Clin Nutr, 2006. **84**(4): p. 694-7.
175. Khazai, N.B., et al., *Treatment and prevention of vitamin D insufficiency in cystic fibrosis patients: comparative efficacy of ergocalciferol, cholecalciferol, and UV light*. J Clin Endocrinol Metab, 2009. **94**(6): p. 2037-43.
176. Malham, M., et al., *The effect of a single oral megadose of vitamin D provided as either ergocalciferol (D₂) or cholecalciferol (D₃) in alcoholic liver cirrhosis*. Eur J Gastroenterol Hepatol, 2012. **24**(2): p. 172-8.
177. Moher, D., et al., *CONSORT 2010 Explanation and Elaboration: Updated guidelines for reporting parallel group randomised trials*. J Clin Epidemiol, 2010. **63**(8): p. e1-37.
178. Cashman, K.D., et al., *Vitamin D status of Irish adults: findings from the National Adult Nutrition Survey*. Br J Nutr, 2013. **109**(7): p. 1248-56.
179. Dawson-Hughes, B., et al., *Estimates of optimal vitamin D status*. Osteoporos Int, 2005. **16**(7): p. 713-6.
180. Zabihyeganeh, M., et al, *Treatment of hypovitaminosis D with pharmacologic doses of cholecalciferol, oral vs intramuscular; an open labeled RCT*. Clin Endocrinol (Oxf), 2013. **78**(2): p. 210-6.
181. Khan, A.H., et al., *Response of a single 'mega intramuscular dose' of vitamin D on serum 25OHD and parathyroid hormone levels*. J Coll Physicians Surg Pak, 2012. **22**(4): p. 207-12.
182. Leventis, P. et al., *The tolerability and biochemical effects of high-dose bolus vitamin D₂ and D₃ supplementation in patients with vitamin D insufficiency*. Scand J Rheumatol, 2009. **38**(2): p. 149-53.
183. Romagnoli, E., et al., *Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D₂) or cholecalciferol (vitamin D₃) in the elderly*. J Clin Endocrinol Metab, 2008. **93**(8): p. 3015-20.
184. Cipriani, C., et al., *Long-term bioavailability after a single oral or intramuscular administration of 600,000 IU of ergocalciferol or cholecalciferol: implications for treatment and prophylaxis*. J Clin Endocrinol Metab, 2013. **98**(7): p. 2709-15.
185. Tellioglu, A., et al., *Efficacy and safety of high dose intramuscular or oral cholecalciferol in vitamin D deficient/insufficient elderly*. Maturitas, 2012. **72**(4): p. 332-8.

186. Whyte, M.P., et al., *Vitamin D bioavailability: serum 25-hydroxyvitamin D levels in man after oral, subcutaneous, intramuscular, and intravenous vitamin D administration.* J Clin Endocrinol Metab, 1979. **48**(6): p. 906-11.
187. Giusti, A., et al., *Heterogeneity in serum 25-hydroxy-vitamin D response to cholecalciferol in elderly women with secondary hyperparathyroidism and vitamin D deficiency.* J Am Geriatr Soc, 2010. **58**(8): p. 1489-95.
188. Yendt, E.R., K.A. Kovacs, and G. Jones, *Secondary hyperparathyroidism in primary osteoporosis and osteopenia: optimizing calcium and vitamin D intakes to levels recommended by expert panels may not be sufficient for correction.* Clin Endocrinol (Oxf), 2008. **69**(6): p. 855-63.
189. Wang, H., et al., *Influence of vitamin D supplementation on plasma lipid profiles: a meta-analysis of randomized controlled trials.* Lipids Health Dis, 2012. **11**: p. 42.
190. Sokol, S.I., et al., *The effects of vitamin D repletion on endothelial function and inflammation in patients with coronary artery disease.* Vasc Med, 2012. **17**(6): p. 394-404.
191. Stricker, H., et al., *Effect of a single, oral, high-dose vitamin D supplementation on endothelial function in patients with peripheral arterial disease: a randomised controlled pilot study.* Eur J Vasc Endovasc Surg, 2012. **44**(3): p. 307-12.
192. Kullo, I.J., et al., *C-reactive protein is related to arterial wave reflection and stiffness in asymptomatic subjects from the community.* Am J Hypertens, 2005. **18**(8): p. 1123-9.
193. Pietri, P., et al., *Relationship between low-grade inflammation and arterial stiffness in patients with essential hypertension.* J Hypertens, 2006. **24**(11): p. 2231-8.
194. Tousoulis, D., et al., *Serum osteoprotegerin and osteopontin levels are associated with arterial stiffness and the presence and severity of coronary artery disease.* Int J Cardiol, 2012.
195. Roth, H.J., et al., *Accuracy and clinical implications of seven 25-hydroxyvitamin D methods compared with liquid chromatography-tandem mass spectrometry as a reference.* Ann Clin Biochem, 2008. **45**(Pt 2): p. 153-9.
196. Zimmerman, S.I., et al., *The prevalence of osteoporosis in nursing home residents.* Osteoporos Int, 1999. **9**(2): p. 151-7.
197. Sugarman, JR. et al., *Hip fracture incidence in nursing home residents and community dwelling older people. Washington State. 1993-1995.* J Am Geriatr Soc. 2002 Oct;50(10):1638-43
198. Rodondi, A, et al. *Prevalence of vertebral fracture in oldest old nursing home residents.* Osteoporosis Int. 2012 Nov;23(11):2601-6
199. Rapp K, et al. *Fractures after nursing home admission: incidence and potential consequences.* Osteoporos Int 2009 Oct;(10):1775-1783
200. Parikh S et al. *Pharmacological management of osteoporosis in nursing home populations: a systematic review.* J Am Geriatr Soc 2009 Feb;57(2):327-34
201. Collin, C et al. *The Barthel Index: a reliability study.* Int Disabil Stud. 1988;10(2):61-3
202. Beaupre LA et al. *Diagnosis and treatment of osteoporosis before and after admission to longterm care institutions.* Osteoporos Int 2012 Feb;23(2):573-80
203. Giangregorio, LM et al. *Osteoporosis management among residents living in long-term care.* Osteoporos Int 2009 Sep(20):1471-1478

- 204 Gupta G et al. *Underuse of procedures for diagnosing osteoporosis and of therapies for osteoporosis in older nursing home residents.* J Am Med Dir Assoc. 2003 Jul-Aug;4(4):200-2
- 205 Kamel HK et al. *Failure to diagnose and treat osteoporosis in elderly patients hospitalized with hip fracture.* Am J Med 109:326-328
- 206 Brauner DJ et al. *Treating non dementia illnesses in patients with dementia.* JAMA 2000;283(24):3230-3235
207. Redelmeier, D.A., S.H. Tan, and G.L. Booth, *The treatment of unrelated disorders in patients with chronic medical diseases.* N Engl J Med, 1998. **338**(21): p. 1516-20.
208. Berry SD et al. *Second hip fracture in older men and women: the Framingham study.* Arch Intern Med 2007 Oct 8;167(18):1971-6
209. Gannon, B., E. O'Shea, and E. Hudson, *Economic consequences of falls and fractures among older people.* Ir Med J, 2008. **101**(6): p. 170-3.

APPENDIX A

Beaumont Hospital Ethics (Medical Research) Committee

Chairperson: Professor Gerry McElvaney
Convenor: Professor Alice Stanton

Administrator: Gillian Vale

11th March 2011

REC reference: 11/14

EudraCT: 2010-2022401-17

Protocol: 123-David Williams

Prof. David Williams
Consultant in Geriatric Medicine
Beaumont Hospital

Dear Prof. Williams

RE: 11/14 – Prof. Williams – The effects of Vitamin D replacement on arterial stiffness in an elderly community based population

The Recognised Ethics Committee reviewed the above application at its meeting held on the 11th February 2011.

A Quorum was present at this meeting as outlined in S.I. 190 of 2004.

The Committee has given a favourable ethical opinion for the above clinical trial based on the application, protocol and supporting documentation (as listed in the attached document)

This study was given a favourable opinion on the 11th March 2011

This favourable opinion is extended to the site listed below only.

Chief Investigator & Principal Investigator	Site
Prof. David Williams	Beaumont Hospital

There are no conditions attached to this favourable opinion.

Yours sincerely



Prof. Alice Stanton
Convenor
Ethics (Medical Research) Committee

Clock Started: 21st January 2011
Clock Stopped: 21st February 2011
Clock Started: 7th March 2011
Clock Stopped: 11th March 2011

APPENDIX B



IRISH MEDICINES BOARD

21st February 2011

Department of Geriatric Medicine
Beaumont Hospital
Beaumont Road
Dublin 9

EUROPEAN COMMUNITIES (CLINICAL TRIALS ON MEDICINAL PRODUCTS FOR HUMAN USE) REGULATIONS, 2004

RE: CT Number: CT 900/502/2 – Vitamin D/Colecalciferol
Case number: 2094624
EudraCT number: 2010-024417-31
Protocol number: 2352
Title of trial: The effects of vitamin D replacement on arterial stiffness in an elderly community based population

Dear Sirs,

The Irish Medicines Board has considered the application dated 10th January 2011 seeking authorisation to conduct the above clinical trial.

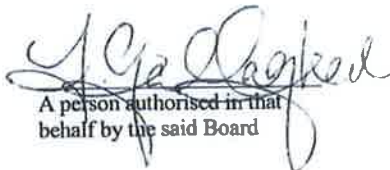
On the basis of the evidence available, the application is acceptable.

Please note that the date of this letter is the date of authorisation of the trial.

In accordance with Article 11 of Directive 2001/20/EC, confirmation of the authorisation of a clinical trial is mandatory for the updating of EudraCT and will be made public. Therefore, the Irish Medicines Board requires that you provide the following information for this clinical trial as soon as it is available:

The name of the responsible ethics committee, the opinion (favourable, not favourable, withdrawal) and the date of the opinion.

Yours sincerely,


A person authorised in that
behalf by the said Board

Bord Leigheasra na hÉireann
Kevin O'Malley House, Earlsfort Centre, Earlsfort Terrace, Dublin 2
Tel: 353-1-676 4971 Fax: 353-1-676 7836
Website: www.imb.ie

AUT-F0010-2

APPENDIX C

PROTOCOL Version 8 (15/1/13)

The effect of vitamin D replacement on arterial stiffness in an elderly community based population.

Protocol Code No: 2352
Eudra CT: 2010-024417-31

Protocol version: 8

Principal Investigator: Professor David Williams,
(Single centre trial) Associate Professor of Geriatric Medicine,
RCSI/Beaumont Hospital

Sponsors: N/A

Other relevant personnel: Dr Cora Mc Greevy,
Clinical Lecturer in Stroke & Geriatric
Medicine,
RCSI/ Beaumont Hospital

Ms. Miriam Barry
Clinical Research Nurse,
RCSI/Beaumont Hospital.

Study site(s); Beaumont Hospital
Dublin 9,
Ireland

TABLE OF CONTENTS

Title	Page 1
Contents	Page 2
Abbreviations	Page 3
Summary of Study	Page 4
Background Information	Page 5,6,7,8
Hypothesis	Page 8
Aim of Trial	Page 8,9
Study Design Diagram	Page 9
Study Design	Page 10
Randomisation & Blinding	Page 10
Medication	Page 10
Pilot Data & Power Calculation	Page 10,11
Participants	Page 11
Inclusion Criteria	Page 11
Exclusion Criteria	Page 11,12
Recruitment	Page 12
Initial visit	Page 12
Initial visit blood sampling	Page 12
Initial visit assessments	Page 13
8 week visit	Page 13
8 week blood sampling	Page 13
8 week assessments	Page 13
Concomitant medications	Page 13,14
Statistical analysis	Page 14,15
Collecting and storing of data	Page 15
Safety Monitoring	Page 15,16
Definitions of adverse events	Page 16
Adverse events monitoring	Page 17,18
Handling of data from subjects withdrawn	Page 18
Publication	Page 18
End of Trial	Page 18
References	Page 18,19,20

LIST OF ABBREVIATIONS

Hs-CRP-Highly sensitive C-reactive protein

IM-intramuscularly

LFT – Liver Function Tests

MMP-9-Matrix metalloproteinase 9

OPG – Osteoprotegerin

PAD – Peripheral Arterial Disease

PWA-Pulse Wave Analysis

PWV – Pulse Wave Velocity

RCT-Randomised Controlled Trial

Summary:

Vitamin D deficiency has been associated with hypertension, vascular disease and heart failure. Furthermore, previous studies have shown that vitamin D deficiency may lead to multiple dysregulatory vascular effects, eventually leading to abnormalities in central arterial stiffness. We hypothesise that vitamin D deficiency leads to increased arterial stiffness and that supplementation with vitamin D will lead to improved vascular compliance in an elderly cohort of patients.

We plan on randomising a group of elderly vitamin D deficient patients to receive a single dose of either 100,000 IU cholecalciferol IM or 50,000IU cholecalciferol IM, while assessing their arterial stiffness before and after vitamin D replacement.

Our study group will be elderly (aged 65 and over) consenting patients from a geriatric clinic at a tertiary care institution in Dublin with proven vitamin D deficiency, randomised into 2 groups receiving either 100,000 IU or 50,000U of cholecalciferol (vitamin D3). All patients will have brachial BP measurement, height and weight determination, pulse wave velocity and pulse wave analysis (measures of arterial compliance) performed at the time of initial assessment. Following vitamin D supplementation, the participants will be reassessed 2 months later to monitor for changes in these clinical measurements.

Background:

Vitamin D deficiency is highly prevalent in Ireland and worldwide particularly at higher latitudes. There has been a resurgence of interest in vitamin D synthesis, metabolism and action due to the worsening worldwide trend towards nutritional insufficiency and the emerging knowledge regarding the nonhormonal, intracrine and paracrine actions of vitamin D metabolites.¹ Vitamin D is a fat-soluble vitamin obtained by three routes: sunlight exposure, dietary intake, and pharmaceutical supplementation. It is produced endogenously when ultraviolet rays from sunlight strike the skin and trigger vitamin D synthesis. Vitamin D obtained from sun exposure, food, and supplements is biologically inert and must undergo two hydroxylations in the body for activation.² Serum concentration of 25 OHD is the best indicator of vitamin D status as it is the major circulating form of vitamin D and the precursor of the active form (1,25-dihydroxyvitamin D). Because of its long half-life, 25OHD measurements are useful for assessing vitamin D status in patients³. In conjunction with parathyroid hormone, vitamin D is largely responsible for the regulation of calcium and phosphate homeostasis. Vitamin D deficiency affects bone health and can lead to osteoporosis. Furthermore, there is increasing evidence that vitamin D plays a role in other regulatory mechanisms. Experimental data suggest that vitamin D affects cardiac muscle directly, regulates the renin-angiotensin-aldosterone system, and modulates the immune system. Because of

these biologic effects, vitamin D deficiency has been associated with hypertension, vascular disease and heart failure.⁴ Initial animal studies established a relationship between vitamin D deficiency and cardiovascular dysfunction including cardiac hypertrophy, fibrosis, hypertension, as well as alterations of serum calcium, parathyroid hormone, and renin levels.⁵ The vitamin D receptor is widely distributed throughout the body in several tissue types not involved in calcium metabolism such as lymphocytes, colonic cells, hepatocytes, and cardiac myocytes.⁶ Clinical studies have shown cross sectional associations between lower vitamin D levels and plasma renin activity, coronary artery calcification, blood pressure (BP) and cardiovascular disease. These studies therefore support a role for vitamin D in maintaining cardiovascular health through both a direct action of vitamin D on cardiomyocytes and indirect actions on circulating hormones and calcium.⁷

Endothelial dysfunction is characterized by a shift of the actions of the endothelium toward reduced vasodilation, a proinflammatory state, and prothrombic properties. It is associated with most forms of cardiovascular disease, such as hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes, and chronic renal failure.⁸ Endothelial dysfunction is an important early event in the pathogenesis of atherosclerosis, contributing to plaque initiation and progression and the severity of endothelial dysfunction has been shown to have prognostic value for future cardiovascular events. Arterial stiffness acts as a surrogate marker for endothelial dysfunction with increasing arterial stiffness being associated with worsening endothelial dysfunction.¹³ A number of studies which are described later have linked endothelial dysfunction with vitamin D deficiency.^{13,14}

Analysis of the NHANES (National Health and Nutrition Examination) study demonstrated that low serum 25OHD levels were found to be associated with a higher level of peripheral arterial disease (PAD) suggesting support for the theory that vitamin D may have potent anti-atherosclerotic properties.⁹ Hypovitaminosis D is also associated with decreased levels of HDL cholesterol-associated apolipoprotein A-I¹⁰, and vitamin D supplementation has been shown to have a beneficial effect on the elastic properties of the arterial wall in a randomized, placebo-controlled interventional study in postmenopausal women.¹¹

Pulse wave velocity (PWV) is generally accepted as the most simple, non invasive, validated, robust and reproducible method to determine arterial stiffness.¹² The SphygmoCor® system (ArtCor, Sydney, Australia) uses a single high fidelity applanation tonometer to obtain a proximal (i.e. carotid artery) and distal pulse (i.e. femoral) recorded sequentially a short time apart and calculates PWV from the transit time between the two arterial

sites.¹³ The arterial pressure waveform is a composite of the forward pressure wave created by ventricular contraction and a reflected wave. In the case of stiffened arteries, PWV rises and the reflected wave arrives back at the central arteries earlier, adding to the forward wave and augmenting the systolic pressure. This phenomenon can be quantified through the Augmentation index (Alx) which is a composite measure of central arterial stiffness and is measurable on the SphygmoCor® system. The more recent Vicorder® system (Skidmore Medical, Bristol, UK) measures simultaneous pressure waveforms by a volume displacement technique, using blood pressure cuffs placed around the sites of interest. It measures arterial stiffness by measuring carotid to femoral pulse wave velocity (PWV).¹⁴ The Augmentation index is calculated as part of the Pulse Wave analysis. Pulse Wave Velocity measurement is standardised according to the European Society of Cardiology expert consensus document published in 2006.¹²

Vitamin D supplementation and arterial function.

Previous studies have examined the association between hypovitaminosis D and endothelial dysfunction. Sugden et al found that a single large dose of vitamin D₂ improved endothelial function in an elderly group of patients with Type 2 diabetes mellitus¹⁵. Andrade et al looked at a possible association between vitamin D and Alx¹⁶ and found that patients with lower vitamin D levels were more likely to have higher Alx ($p=0.002$). A recently published study by Dong et al aimed to determine the effect of daily vitamin D supplementation on arterial stiffness as measured by pulse wave velocity (PWV).¹⁷ The experimental group received 2000IU cholecalciferol orally daily and the control group received 400IU cholecalciferol daily. Results showed that vitamin D supplementation with 2000IU daily led to a reduction in PWV (and therefore reduced arterial stiffness) from baseline to post-test [5.41 ± 0.73 m/sec to 5.33 ± 0.79 m/sec] ($P=0.031$).

Furthermore, vitamin D deficiency is associated with increased circulating concentrations of matrix metalloproteinase-9, which controls vascular wall remodelling and is increased in unstable angina, and vitamin D supplementation has been shown to lower serum matrix metalloproteinase-9 (MMP-9) concentrations.¹⁸

More recently, Osteoprotegerin (OPG) has become the subject of intense interest for its role in vascular disease and calcification. Studies in vitro and in animal models suggest that OPG inhibits vascular calcification.¹⁹ Paradoxically however, clinical studies suggest that serum OPG levels increase in association with vascular calcification, coronary artery disease, stroke and future cardiovascular events. This has led to an

extensive debate on the potential of OPG as a possible biomarker of vascular disease.

Vitamin D supplementation

Yearly intramuscular cholecalciferol (300,000U) has been used to treat vitamin D deficient patients.²² Patients who received this treatment showed a significant improvement in 25OHD levels, from 25.5 to 81 nmol/L with 11% remaining deficient. No patient became hypercalcaemic after treatment. There has been no previous study using doses of 100,000IU cholecalciferol previously, but we hypothesise that if 300,000IU sufficiently repletes vitamin D levels over 1 year, 100,000IU should replace levels over a 4 month period.

Furthermore, concern has been raised regarding the use of very large doses of vitamin D following the publication of a study by Sanders et al which found that that patients given a high-dose cholecalciferol (500 000 IU) orally once a year experienced 15% more falls and 26% more fractures than the placebo group, particularly in the first three months after administration²³. This study used the largest total annual dose of vitamin D (500 000 IU) reported in any large randomized controlled trial, raising the possibility that the adverse outcome is dose-related. However, a randomised double blind controlled trial of 100,000 IU oral cholecalciferol supplementation or matching placebo every four months over five years found that total fracture incidence was reduced by 22% and fractures in major osteoporotic sites by 33%.²⁴

The opposing outcomes of these two RCT's suggest that the dosing regimen (ie, 4 monthly versus annually) rather than the total dose may contribute to these differing outcomes.

Hypothesis

We hypothesise that vitamin D deficiency leads to increased arterial stiffness and that supplementation with vitamin D will lead to improved vascular compliance in an elderly cohort of patients. This work would have implications with regards to reducing cardiovascular events in this patient group.

Aims of Study:

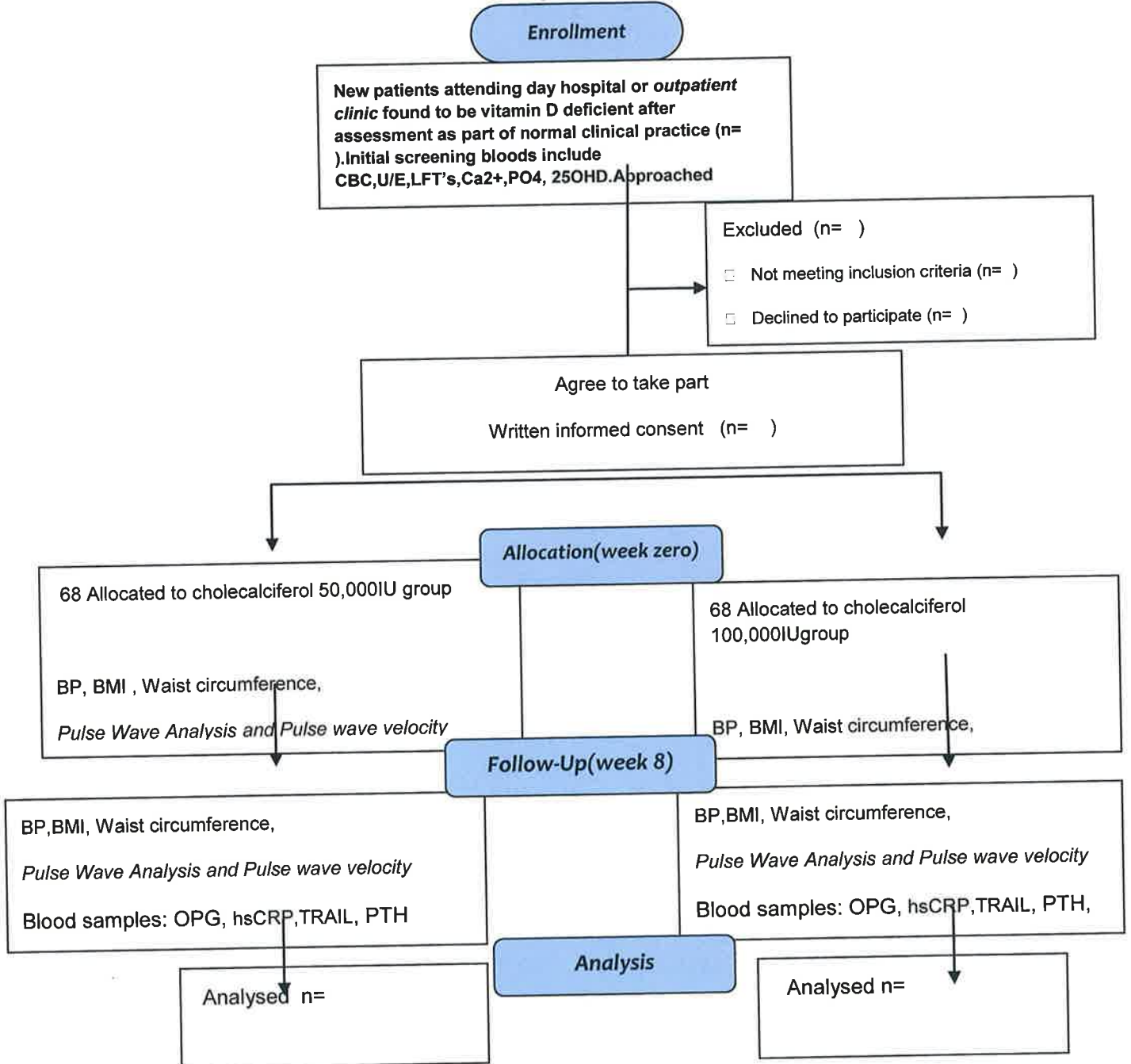
Primary Aim

The primary aim of this clinical trial is to determine whether vitamin D replacement leads to changes in arterial compliance in vitamin D deficient patients.

Secondary Aim

The secondary aim is to compare the efficacy of two different doses of intramuscular vitamin D in providing supplementation and whether there is a difference in their effect on arterial compliance.

Study Design



Study design

This is a randomised double blind study comparing the effects of two different doses on arterial compliance.

We aim to randomise 68 subjects in each treatment arm over a period of 1 year.

Participants will be allocated to either

- I. Cholecalciferol 100,000IU single dose intramuscularly.
- II. Cholecalciferol 50,000IU single dose intramuscularly.

Randomisation and blinding:

Subjects will be randomised using a restricted (stratified) random assignment of patients on a 1:1 basis into two parallel treatment arms (either 50,000IU IM cholecalciferol or 100,000IU IM cholecalciferol). Stratification will be made according to the standard 5-age or 10-age intervals (as appropriate). In particular, computer generated random lists of numbers within each age interval will be used where every first patient will be randomly assigned to the high dose arm while every second patient will be randomly assigned to the low dose arm. The injections will be prepared by a nurse independent of the study and placed in identical sealed brown envelopes. They will be administered on a blind basis by the research team (either Dr Mc Greevy or Ms Miriam Barry). Compliance is ensured by the once off dose.

Neither the participants nor the researchers will know which treatment they are on, until the code is broken at the end of the study, when result collection is complete.

Medication

D3 Vicotrat [Cholecalciferol injection 100,000IU (1ml ampoule) [Heyl Pharmaceuticals, Berlin, Germany] will be supplied by the pharmacy in Beaumont Hospital. The medication will be given by deep intramuscular injection.

Pilot Data and Power Calculation

The primary aim (outcome) is to determine whether the difference in PWV between group A (prior to and following 100,000 IU cholecalciferol administration) (Δ PWV1) and group B (prior to and following administration of 50,000IU cholecalciferol (Δ PWV2) is statistically significant assuming equal or similar baseline values of PWV.

Assuming an equal sample size and pooled SDs of 0.77 in the outcome variable, to have at least 81% power for a two-tailed p-value <0.05 by the t-test of equal means (having a difference) in the follow-up (post-test), we will require a total N=136 patients (2 equal groups of 68 patients at the end of the study).

Participants:

Older (aged 65 and over) patients from a geriatric clinic (either day hospital or outpatient clinic) at a tertiary care institution in Dublin will have vitamin D levels assessed along with CBC, U/E, LFT's, Ca²⁺ and PO₄ levels as part of standard care. At the initial screening stage, we will use a questionnaire regarding dietary vitamin D intake and sun exposure. Patients who are found to be vitamin D deficient (levels < 50nmol/L) will be recruited to join the study by the research team (either Dr Mc Greevy or Ms Miriam Barry). Once recruited, they will have baseline weight, height, waist circumference PWV and Pulse Wave Analysis (PWA) carried out. Subsequently they will be randomised into 2 groups- vitamin D replacement in the form of intramuscular (IM) cholecalciferol 100,000IU or 50,000IU. After 2 months, parameters including BP, PWV and PWA will be rechecked to assess for changes in these cardiovascular parameters and vitamin D levels and clinical biochemical parameters will be rechecked.

Inclusion Criteria:

- Age > 65 years
- Serum 25OHD levels < 50nmol/l
- Not receiving vitamin D preparations
- No change in medications in last 3 months

Exclusion Criteria:

- Age < 65 years
- Renal dysfunction, defined as a creatinine greater than 200µmol/l
- History of or current malignancy
- Known hypoparathyroidism
- Unable to read or give informed consent
- Hypercalcaemia (Total calcium > 2.6mmols)
- LFT's > 3 times the upper limit of normal
- History of renal stones
- Recent (3 months) change in medication
- Hypersensitivity to cholecalciferol or any of its excipients
- Hypervitaminosis D or evidence of vitamin D toxicity
- Contraindications to cholecalciferol as listed in the SPC

Recruitment:

We hope to recruit a total of 136 subjects from the Day Hospital and geriatric outpatient clinic in Beaumont Hospital. All new patients aged 65 and over attending the day hospital and geriatric *outpatient clinic* have screening blood tests including CBC (complete blood count), U/E (urea and electrolytes), LFT's (liver function tests), Ca²⁺ (calcium), PO₄ (phosphate), and 25OHD (vitamin D) levels as part of current clinical practice. If patients are diagnosed with vitamin D deficiency (25OHD levels <50nmol/L), they will be approached with regards to possible recruitment onto the study by an initial telephone call by a member of the research team (either Dr Mc Greevy or Ms Miriam Barry). The study will be explained to them and they will be sent a copy of the patient information leaflet.

A convenient time will be decided upon to ring the patient to ascertain if he/she is willing to participate in the study. If he/she is willing to be a subject in the study, a timeframe will be agreed upon for the investigations proposed to be performed at visits 0 weeks and 8 weeks.

Initial Study Visit

At the initial visit, a member of the research team (either Dr Mc Greevy or Ms Miriam Barry) will explain the study and the consent form. More time will be given to consider participation in the study if requested.

Three copies of the consent form, signed and dated by the participant, are required: one copy will be fixed into the hospital case record, one copy is given to the participant to keep, and one copy is retained by the researchers.

After recruitment and informed consent, venous blood samples will be taken by a member of the research team (either Dr Mc Greevy or Ms Miriam Barry) by careful venepuncture.

Initial Visit Bloods

- Parathyroid Hormone
- Osteoprotegrin, hsCRP, lipid profile, MMP-9 levels
- Serum 25OHD levels

Initial visit assessments:

- Blood pressure
- Body mass index
- Waist circumference
- Dietary vitamin D and sun exposure questionnaire

- Pulse wave velocity and Pulse-wave analysis(PWA) to include augmentation index (to assess degree of arterial stiffness). A member of the research team will measure the above parameters using a Vicorder apparatus. This involves placing a transducer (like an ultrasound probe) over the main artery in the leg and another one over the main artery in the neck and the process is entirely painless. The transit time of the pulse from the carotid artery to the femoral artery provides an estimate of the Pulse Wave Velocity (PWV), a measure of arterial compliance. PWA measurement is standardised as recommended by the European Society of Cardiology expert consensus document published in 2006¹². PWV will be determined in the same temperature controlled room at similar times of the day to ensure standardizations of measurements.

8 week visit

8 week visit bloods:

- Serum 25OHD levels
- Urea and electrolytes
- Calcium and phosphate levels
- OPG (Osteoprotegrin), hsCRP,
- **8 week visit assessments:**
 - Body mass index
 - Waist circumference
 - Pulse wave velocity and Pulse Wave analysis(as described above)

Patients will be followed up within six months as part of normal practice to ensure their vitamin levels remain sufficient as the doses being used in this study are likely to only be adequate for 4 months.

Concomitant medications

Concomitant medications are permitted but caution must be shown with the concomitant prescription of:

- Magnesium-containing antacids: hypermagnesaemia may develop in patients on chronic renal dialysis.
- Digitalis glycosides: hypercalcaemia in patients on digitalis may precipitate cardiac arrhythmias.

- Verapamil: atrial fibrillation has recurred when supplemental calcium and cholecalciferol have induced hypercalcaemia.
- Anti-convulsants: Vitamin D requirements may be increased in patients taking anti-convulsants (e.g. carbamazepine, phenobarbital, phenytoin and primidone).
- Thiazide diuretics: Thiazide diuretics can lead to hypercalcemia by the reduction of the renal calcium excretion. Serum calcium levels should be monitored during long-term therapy.

Statistical Analysis

The primary aim (outcome) is to determine whether the difference in PWV between group A (prior to and following 100,000 IU cholecalciferol administration) (Δ PWV1) and group B (prior to and following administration of 50,000IU cholecalciferol (Δ PWV2) is statistically significant assuming equal or similar baseline values of PWV. Assuming an equal sample size and pooled SDs of 0.77 in the outcome variable, to have at least 81% power for a two-tailed p-value <0.05 by the t-test of equal means (having a difference) in the follow-up (post-test), one will require to have in the analysis at least a total N=136 patients (2 equal groups of 68 patients at the end) - see below:

Two group t-test of equal means (equal n's)

	1
Test significance level, α	0.050
1 or 2 sided test?	2
Group 1 mean, m_1	5.330
Group 2 mean, m_2	5.710
Difference in means,	-0.380
$m_1 - m_2$	
Common standard	0.770
deviation, s	
Effect size, $d = m_1 - m_2 /$	0.494
s	
Power (%)	81.00
n per group	68

The best model is the full-factorial model, e.g. GLM(Generalised Linear Model) or repeated measure ANOVA (taking into account initial and end-visit values, providing the data are normally distributed) and any comparisons made and any difference found, will be adjusted, in one way or another, to the initial, baseline values.

Collecting and Storing Data

Personal data and health records that can be connected with the participant, and signed consent forms, will only be seen by the research team(Dr Mc Greevy and Ms Miriam Barry) and Professor Williams and will be kept in locked filing cabinet in a secure office in the hospital. Data generated in the study will be stored in linked anonymised form. Results will be entered into a secure database on University computers for analysis with only the above named having access. Professor Williams will be custodian of the research data. The data will be stored for a period of 7 years from the end of the study.

Safety Monitoring

An independent safety monitoring committee will be appointed to oversee the safety of the study. Reported or observed adverse events will be notified by the principal investigator (Prof David Williams) to the committee within 7 days, or immediately in the case of a serious adverse event. All serious adverse events will be reported in accordance with guidance provided by the European Commission.

Definition of adverse events

Adverse Event

An adverse event (AE) is defined as any unfavourable and unintended sign including an abnormal laboratory finding, symptom or disease associated with the use of a medical

treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure, that occurs during the course of the study.

Adverse reaction

A response to a medicinal product which is noxious and unintended and which occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease or for the restoration, correction or modification of physiological function.

Serious Adverse Events or Reactions

A serious adverse event or reaction is an adverse event or reaction which results in death, is life threatening, requires in-patient hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect. Life threatening in this context refers to a reaction in which the patient was at risk of death at the time of the reaction: it does not refer to a reaction which hypothetically might have caused death if more severe.

Unexpected adverse reaction

An adverse reaction, the nature, severity or outcome of which is not consistent with the Summary of Product Characteristics (SPC). This includes class-related reactions which are mentioned in the SPC but which are not specifically described as occurring with this product.

Suspected unexpected serious adverse reaction (SUSAR)

Any unexpected adverse reaction that:

- (a) results in death
- (b) is life-threatening
- (c) requires hospitalisation or prolongation of existing hospitalisation
- (d) results in persistent or significant disability or incapacity
- (e) consists of a congenital anomaly or birth defect.

Adverse Events Monitoring

Non serious adverse events

Prior to commencing the study, patients with serum creatinine > 200µmol/L, liver function tests (bilirubin, aminotransferases and alkaline phosphatase) > 3 times the upper limit of normal or corrected calcium >2.6 will be excluded from the study.

These blood tests will be repeated at 2 months to ensure no biochemical abnormality has occurred as a result of vitamin D supplementation. The participants will be advised to make contact with the principal investigator if they feel unwell in any way or if they have any concerns.

At the 2 month visit, the investigator will ask if there have been any adverse medical events since the last visit. Details of adverse medical events will be noted in the patient's medical records, together with a note of the date of starting, the duration, and any medical treatment received.

Serious Adverse Reactions

The principle investigator (Professor David Williams)) shall ensure that all relevant information about a suspected unexpected serious adverse reaction (SUSAR) which occurs during the course of the clinical trial and is **fatal or life-threatening** is reported **as soon as possible** to the IMB and the Research Ethics Committee. An emergency contact number for the principle investigator will be placed in the participant's hospital notes, to be alerted in case of hospital admission during the study. This needs to be done **not later than seven days** after the sponsor was first aware of the reaction. Any additional relevant information should be sent within eight days of the report. The sponsor shall ensure that a suspected unexpected serious adverse reaction (SUSAR) which is not fatal or life-threatening is reported as soon as possible, and in any event **not later than 15 days** after the Sponsor is first aware of the reaction.

In the event of an adverse event occurring during the study, the patient will be followed up until a full clinical and biochemical recovery has been made. In accordance with the normal practice, any adverse event involving a medication (vitamin D in this case) would be reported to the Irish Medicines Board. These arrangements are identical to normal clinical practice and would be followed for anyone commencing vitamin D therapy, even outside the setting of a clinical study. The subject's participation in the trial will be terminated if an adverse reaction is suspected.

After the study, all participants will continue to be followed up in outpatient clinics and treated in accordance with normal clinical practice. Patients will be informed of any abnormal test results during the study, and appropriate treatment and follow up of the patient will be instituted. Patients will be followed up within six months in the geriatric clinic in Beaumont Hospital as before as part of normal practice to ensure their vitamin levels remain sufficient as the doses being used in this study are likely to only be adequate for 4 months.

Handling of data from subjects withdrawn

This is an active comparator trial designed to assess if there is a response in arterial stiffness to vitamin D replacement therapy and not a measure of therapeutic efficacy. We will analyse any subjects who withdraw from the study on an intention to treat basis.

Publication

Dr Cora Mc Greevy , Ms Miriam Barry and Professor David Williams will prepare the findings for publication. An annual report will be sent to the ethics committee.

Findings will be submitted for presentation at national and international scientific conferences. At least one full scientific paper is expected and will be submitted to a high-impact factor peer-reviewed journal.

The end of the trial

The end of the trial will be defined as the last visit of the last participant.

APPENDIX D

Patient Information Leaflet

The effect of vitamin D replacement on arterial stiffness in an elderly community based population.

Principal Investigator's Name: Professor David Williams

Principal Investigator's Title: Associate Professor of Geriatric Medicine

Telephone No. of Principal Investigator: 01-7974731

You are being invited to take part in a clinical research study carried out at Beaumont Hospital. Before you decide whether or not you wish to take part, you should read the information provided below carefully and if you wish discuss it with your family, friends or GP. Take time to ask questions – do not feel rushed or under any obligation to make a hasty judgement. You should clearly understand the risks and benefits of participating in this study so that you can make a decision that is right for you – this process is known as Informed Consent.

You are not obliged to take part in this study and failure to participate will have no effect on your future care.

You may change your mind at any time (before the start of the study or even after you have commenced the study) for whatever reason without having to justify your decision and without any negative impact on the care you will receive from the medical staff.

WHY IS THIS STUDY BEING DONE?

Low vitamin D levels are very common in Ireland, particularly in those aged 65 and over. Vitamin D is known to play an important role in bone health, but recent research has suggested that it also has an important influence on cardiovascular (heart) health.

We aim to investigate the link between low vitamin D levels and cardiovascular health by checking a number of blood tests, blood pressure and stiffness of arteries before and after replacing vitamin D levels.

WHO IS ORGANISING AND FUNDING THIS STUDY?

This study is being organised by the Department of Geriatric Medicine in Beaumont Hospital. Professor David Williams is the principal investigator and sponsor. Dr Cora Mc Greevy is the co-investigator. This research is being done as part of an MD degree by Dr McGreevy. Miriam Barry, a research nurse will also be part of the research team.

HOW WILL IT BE CARRIED OUT?

This study is due to commence in August 2011 and will be 1 year in duration. We hope to enrol 136 participants. We will be screening all attendees aged 65 and over at our day hospital and outpatient clinics. Once a patient is found to have low vitamin D levels, they will be approached with a view to being recruited to the study by the research team(Dr Mc Greevy or Miriam Barry). If they choose not to be part of the study, they will be treated for vitamin D deficiency in any case.

WHAT WILL HAPPEN TO ME IF I AGREE TO TAKE PART?

If you agree to take part, we will already have taken blood tests and have established that your vitamin D levels are low. Prior to replacing your vitamin D levels, the research team will carry out a number of blood tests to assess your liver and kidney function along with calcium and other mineral levels. This will involve taking one blood sample which we will then divide into 5-6 smaller blood samples. We will also check your weight, height and blood pressure. We will assess the stiffness of your arteries using a machine called a Vicorder. This involves using an ultrasound device over the main artery in the leg and another one over the main artery in the neck. The ultrasound is similar to what is used on pregnant ladies and is entirely painless. The readings are inputted to a computer and a measure of the degree of stiffness of the blood vessels is calculated.

Once these tests are completed, a member of the research team will replace your low vitamin D levels with an injection of vitamin D called cholecalciferol .This is a medication which would be prescribed for you even if you were not participating in this study.The study participants will be divided into 2 groups, one group receiving 50,000IU cholecalciferol and one group receiving 100,000IU cholecalciferol.

IU stands for International Units and is a measure of the dosage of vitamin D.

The 100,000IU dose is the normally prescribed dose and the 50,000IU dose is a lower than normal dose. There is no danger in receiving a lower than normal dose as we will be checking your vitamin D levels after 2 months and if your levels are still low, we will be treating this with oral medication.

However, these injections should replenish your vitamin D stores for 4 months and you will be likely to need further vitamin D medication after this, which we will organise for you. The injection is usually given into the shoulder or buttock muscle and like any injection, can be locally painful for a very short period. Side effects are extremely rare but can include weakness, headache and dry mouth.

You will be required to attend the hospital on one further occasion, 8 weeks later in order for us to recheck your vitamin D levels. We will also repeat the blood pressure, height, weight and artery stiffness tests. This visit should take 30 minutes approximately.

WHAT ALTERNATIVE TREATMENTS ARE AVAILABLE TO ME?

It is important that your low vitamin D levels are replaced as low levels are associated with higher risks of breaking your bones in the event of a fall.

As already mentioned, we will replace your vitamin D levels as part of normal treatment even if you decline taking part in this study.

BENEFITS:

You will be less likely to break a bone if you fall, once your vitamin levels are normalised after the injection. Other research has shown improved muscle strength and balance with normalised vitamin D levels.

RISKS:

Any risks from taking this medication are extremely rare but may include weakness, headache, dry mouth, constipation and muscle pain.

CONFIDENTIALITY ISSUES

We will contact your GP to let them know you are taking part in the study. The above named investigators will be looking at your medical chart as part of this study.

At present, the blood test to check your vitamin D levels is carried out at St Vincents University Hospital in Dublin. However, vitamin D levels will be carried out in Beaumont Hospital from June 2011.

Data relating to you will be kept in a secure setting for 7 years after the study is completed and will then be destroyed.

IF YOU REQUIRE FURTHER INFORMATION

If you have any further questions about the study, or if you wish to withdraw from the study you may do so without justifying your decision and your future treatment will not be affected.

For additional information now or any future time please contact:

Dr Cora Mc Greevy,

Department of Geriatric Medicine,

Beaumont Hospital,

Dublin 9

Phone contact number between 9am-5pm Monday -Friday.-01-8092352

APPENDIX E

Information letter posted to participant's GP following recruitment to the clinical trial.

Participant's Name and address

Dear Dr.

I am conducting a study to investigate the prevalence of vitamin D deficiency in community dwelling older people and to investigate links between vitamin D deficiency and arterial compliance.

(Participant's name) has been recruited to the study. The details are as follows
Study title: The effects of vitamin D replacement on arterial stiffness in an elderly community based population.

Co-Investigators

Dr Cora Mc Greevy, Research Spr

Ms Miriam Barry, Research Nurse

Professor David Williams, Associate Professor of Geriatric Medicine, Beaumont Hospital.

What it involves for your patient?

This study group will be elderly (aged 65 and over) consenting patients from the geriatric outpatient clinics at Beaumont Hospital, Dublin. Detailed baseline characteristics, medications and co-morbidities will be ascertained by clinical and chart review. At this time, we will ask the participants to complete a screening questionnaire regarding their dietary intake of vitamin D and how much sunlight they are exposed to.

Subjects will be included in the study if their serum 25OHD levels are <50nmol/L.

If he/she is willing to be a subject in the study, a timeframe will be agreed upon for an initial visit and a follow up visit at 8 weeks. The investigations which will be carried out at each individual visit are listed below.

At baseline they will have height, weight and BP taken along with a number of blood samples and pulse wave velocity to assess arterial stiffness. Pulse wave velocity involves placing a transducer (like an ultrasound probe) over the main artery in the leg and another one over the main artery in the neck. The readings are inputted to a computer and a measure of the degree of stiffness of the blood vessels is calculated. We will also measure Pulse Wave Analysis which involves the same technique used for Pulse Wave Velocity. Subsequent to the above measurements the patients will be randomised to either receive a single dose of 100,000IU cholecalciferol(vitamin D3) or 50,000IU cholecalciferol intramuscularly at the initial assessment. We will reassess patients at 8 weeks to recheck vitamin D levels and pulse wave velocity. These vitamin D injections should adequately replace vitamin D levels for a 4 month period and we will advise patients on further therapy at the time of their second visit.

Should you have any queries, I can be contacted at 01-8092352 between 9am-5pm Monday –Friday.

Yours sincerely,

Cora Mc Greevy, MB BCH BAO, MRCPI

Clinical Lecturer and Research Spr

Department of Geriatric Medicine, Beaumont Hospital and RCSI.

APPENDIX F

Beaumont Hospital Ethics (Medical Research) Committee

Chairperson: Professor Gerry McElvaney
Convenor: Professor Alice Stanton

Administrator: Gillian Vale

11th March 2011

REC reference: 10/88

Dr. Cora McGreevy
Department of Geriatric Medicine
Beaumont Hospital

Dear Dr. McGreevy

10/88 – Prof. David Williams – A comparison between the prescription of bone health medications in general practitioner led nursing homes compared with geriatrician led nursing homes

Please find enclosed approval documentation in relation to the pilot phase of this study.

Please revert to the committee after the pilot stage with a statistical analysis plan / sample size calculation (justification of final sample size) for the main phase of this study.

With best regards

Yours sincerely



Ms. Gillian Vale
Administrator
Ethics (Medical Research) Committee

Ethics (Medical Research) Committee Beaumont Hospital Dublin 9
Tel: 353-1-809 2680 Email: gillianvale@beaumont.ie www.beaumontethics.ie

APPENDIX G

PROTOCOL

A comparison between the prescription of bone health medications in general practitioner led nursing homes compared with geriatrician led services.

Investigators

Principle Investigator: Professor David Williams

Co-investigators: Dr Cora Mc Greevy

Dr Alan Moore

Dr Kathleen Bennett

Miriam Barry

Study Sites:

Site:	Lead Investigator:
ST JOSEPHS COMMUNITY UNIT, RAHENY, DUBLIN 5. (GERIATRICIAN LED NURSING HOME)	PROF DAVID WILLIAMS, (CONSULTANT, BEAUMONT HOSPITAL)
RUSH NURSING HOME, RUSH, CO DUBLIN. (GP LED NURSING HOME)	PROF DAVID WILLIAMS, (CONSULTANT, BEAUMONT HOSPITAL)
SWORDS NURSING HOME, SWORDS, CO.DUBLIN (GP LED NURSING HOME)	PROF DAVID WILLIAMS, (CONSULTANT, BEAUMONT HOSPITAL)
TARA WINTHROP NURSING HOME, SWORDS, CO. DUBLIN. (GP LED NURSING HOME)	PROF DAVID WILLIAMS, (CONSULTANT, BEAUMONT HOSPITAL)

TABLE OF CONTENTS

Title, Investigator & Site details	Page 1
Table of Contents	Page 2
Background	Page 3,4
Aims and Objectives	Page 5
Study Design	Page 5
Study Design Diagram	Page 5,6
Pilot Data & Power Calculation	Page 6
Inclusion Criteria	Page 6
Exclusion Criteria	Page 6
Recruitment	Page 6,7
Statistical analysis	Page 7
Collecting and storing of data	Page 7
Publication	
End of Trial	Page 7
References	Page 7 Page 8

Background

Previous US (United States) epidemiologic studies have demonstrated that 70% to 85% of nursing home residents have osteoporosis. Despite the high burden of osteoporosis in nursing homes, several studies have suggested that osteoporosis screening and therapies are underutilized in the nursing home population¹. Colón-Emeric et al looked at osteoporosis treatment in 67 US nursing homes (895 residents) and found that residents with osteoporosis or recent fracture had moderate use of calcium (69%) and vitamin D (63%) but prescribing frequency was also low for other pharmacologic therapies² including bisphosphonates (19%) and

calcitonin(14%) with other osteoporosis medications being prescribed to less than 5% of the cohort.

A US study of >180,000 nursing home residents found that patients found that only 9.1% received anti-resorptive medications and/or supplements indicated for osteoporosis treatment despite the expected high prevalence of osteoporosis in this setting³.

The World Health Organization has developed a fracture risk assessment tool known as FRAX to identify individuals at high risk of osteoporotic fracture⁴. The current standard, which bases treatment decisions largely on bone mineral density measurement, has proven to be specific, but not sensitive, for the identification of patients at high risk of fracture. Because nearly 50% of postmenopausal women in the community over the age of 50 years who suffer an osteoporotic fracture do not have osteoporosis defined by a BMD test^{5,6}, and because of the limited availability of BMD in many countries, clinical risk factors were added to BMD to identify patients at high risk for osteoporotic fractures. A task force from the WHO evaluated the *clinical* risk factors that predict increased risk of *fracture* in nearly all of the 12 population cohorts evaluated worldwide. These are:

- Age
- Sex
- Prior fragility fracture after age 50
- History of corticosteroid use (5 mg or more for three months or more)
- Parental history of hip fracture
- Rheumatoid arthritis
- Secondary osteoporosis (e.g., type 1 diabetes, osteogenesis imperfecta in adults, longstanding hyperthyroidism, hypogonadism, premature menopause, chronic malabsorption and chronic liver disease)
- Current smoker
- Alcohol use of greater than 2 units daily
- Body Mass Index

FRAX integrates the future osteoporotic fracture risk associated with clinical risk factors with that associated with femoral neck BMD. The incident rates of fractures are country specific and provide the clinician the 10 year probability of hip fracture and the 10 year probability of major osteoporotic fracture (clinical vertebral, forearm, hip and shoulder).In the United Kingdom, osteoporosis treatment is calculated to be

cost-effective for a $\geq 3\%$ ten year risk of hip fracture .Once the relevant data is inputted into the FRAX algorithm, a graph is generated in order to assist with result interpretation and advises on the need to treat pharmacologically or to advise on lifestyle modification and reassure the patient.

The low level of fracture protection overall, combined with the high variability between facilities, suggests that there is significant room for improvement in the management of osteoporosis care in nursing homes. There has been no previous research on osteoporosis medication use in nursing homes in Ireland and we aim to study this in more detail.

Aims & Objectives

- 1) We aim to assess residents' suitability for osteoporosis treatment using the FRAX score which gives an estimate of 10 year risk of major osteoporotic fracture in both GP led and geriatrician led nursing homes.
- 2) We aim to estimate the prevalence of Osteoporosis treatment among those deemed suitable for treatment in nursing homes.
- 3) We hope to compare the use of secondary preventative osteoporosis medications between geriatrician-led nursing home patients with GP-led nursing home residents. Medication data for a 7 day period will be collected from medication charts with note taken of any monthly bisphosphonate prescription or yearly bisphosphonate infusion. Demographic information including the resident's age, gender, past medical history and comorbidities will be noted.

Study Design:

As there is no previous similar data, we plan on doing an initial pilot study of 30 patients in a GP led nursing home (Tara Winthrop NH) and 30 patients in a geriatrician led nursing home (St Joseph's Community Unit). From this data, an exact sample size and power calculation was estimated and the second phase of the study began. A total of 168 patients were calculated to be necessary in total-84 from the geriatrician led home and 84 from GP led nursing homes.

Inclusion criteria:

- Nursing Home resident
- Aged 65 and over
- Capacity to understand reason for study or if not, availability of next of kin to provide informed assent.

Exclusion criteria:

Patient or next of kin unable to give informed consent/assent.

Patient recruitment:

All residents of the previously named nursing homes will be approached for recruitment on to the study. Each resident will be given an information leaflet pertaining to the study. They will be given as much time as necessary to make a decision on whether they wish to take part in the study or not. If a resident is deemed not have to have the capacity to give consent due to cognitive impairment, their next of kin will be approached with an information leaflet and asked for assent after they have made a decision on whether they wish their next of kin to be part of the study. Once consent/assent has been given, medication data over a 7 day period will be collected from medication charts with note taken of any monthly bisphosphonate prescription or yearly bisphosphonate infusion. Demographic information including the resident's age, gender and past medical history will be noted. We will record patients for whom anti-resorptive drugs were considered but were subsequently found to be contraindicated due to a concurrent medical condition. We will also assess patients 10 year probability of fracture using the FRAX tool⁴. FRAX is a computer-based algorithm that provides models for the assessment of fracture probability in men and women. If an unsuitable bone health drug regime is found, the GP/geriatrician involved in the patients care will be informed.

Statistical Analysis:

An initial pilot study of a total of 60 patients (30 GP led nursing home and 30 geriatrician led nursing home) will be done in order to carry out a power calculation. Based on this, the numbers needed for phase 2 of the study will be ascertained.

Collecting and storing of data:

Data will be collected from resident's medical notes and kardexes in hard copy format and taken to Beaumont Hospital. Data collected will also be transferred to a spreadsheet (soft copy format) in Beaumont Hospital for the purposes of data analysis. Hard copy and soft copy data will be stored in a coded fashion. It is intended that it will be possible to break the code that identifies each resident as this will be necessary to inform their doctors in the case of inappropriate medications or a high FRAX score. Data will be stored on password protected computers and in locked filing cabinets in the Department of Geriatric Medicine Research Office in Beaumont Hospital

Publication

Dr Cora Mc Greevy and Professor David Williams will prepare the findings for publication. An annual report will be sent to the ethics committee.

Findings will be submitted for presentation at national and international scientific conferences. At least one full scientific paper is expected and will be submitted to a high-impact factor peer-reviewed journal.

The end of the trial

The end of the trial will be defined as the last data collection of the last participant.

References

- 1 Jachna C, Shireman T, Whittle J, et al. Differing patterns of antiresorptive pharmacotherapy among nursing facility residents and community dwellers. *J Am Geriatr Soc*. 2005; 53(8):1275–81
- 2 Colón-Emeric C, Lyles KW, Levine DA et al. Prevalence and predictors of osteoporosis treatment in nursing home residents with known osteoporosis or recent fracture. *Osteoporos Int*, 2007 April; 18(4):553-559
- 3 Wright RM. Use of osteoporosis medications in older nursing facility. *J Am Med Assoc* 2007 Sep; 8(7):453
- 4 Kanis JA, Johnell O, Oden A, Johansson H, McCloskey EV (2008) FRAX® and the assessment of fracture probability in men and women from the UK. *Osteoporos Int* 19:385–39
- 5 Siris ES, Miller PD, Barrett-Connor E, et al Identification and fracture outcomes of undiagnosed low bone mineral density in postmenopausal women: results from the National Osteoporosis Risk Assessment. *JAMA*. 2001 Dec 12; 286(22):2815-22.
- 6 Cummings SR, Nevitt MC, Browner WS, et al . Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. *N Engl J Med*. 1995 Mar 23; 332(12):767-73.

APPENDIX H

Resident Information Leaflet

Protocol Title:

A comparison between the prescription of bone health medications in general practitioner led nursing homes compared with geriatrician led services.

Principal Investigator's Name: Professor David Williams

Principal Investigator's Title: Associate Professor of Geriatric Medicine

Telephone No. of Principal Investigator: 01-7974731

You are being invited to take part in a clinical research study carried out at Beaumont Hospital. Before you decide whether or not you wish to take part, you should read the information provided below carefully and if you wish discuss it with your family, friends or doctor. Take time to ask questions – do not feel rushed or under any obligation to make a hasty judgement. You should clearly understand the risks and benefits of participating in this study so that you can make a decision that is right for you – this process is known as Informed Consent.

You are not obliged to take part in this study and failure to participate will have no effect on your future care.

You may change your mind at any time (before the start of the study or even after you have commenced the study) for whatever reason without having to justify your decision and without any negative impact on the care you will receive from the medical staff.

WHY IS THIS STUDY BEING DONE?

Osteoporosis (fragile bones) is very common in Ireland, particularly in those aged 65 and over. We plan on looking to see what types of medications are being used to treat osteoporosis in nursing homes. We also plan on analysing each resident's risk of fracture in the next 10 years using a tool called the FRAX tool, which involves inputting data into a computer and calculating that risk. If your FRAX score indicates that you should be on treatment for osteoporosis, we will write to your doctor with regards to starting that treatment.

WHO IS ORGANISING AND FUNDING THIS STUDY?

This study is being organised by the Department of Geriatric medicine in Beaumont Hospital. A pharmaceutical company called MSD have contributed towards funding this study. Professor David Williams is the principle investigator and sponsor. Dr Cora Mc Greevy is the co-investigator. This research is being done as part of an MD degree by Dr McGreevy.

HOW WILL IT BE CARRIED OUT?

This study is due to commence in January 2011 and will be 1 year in duration. We hope to enrol 280 participants in total. We will be including nursing home residents aged 65 and over in a number of facilities in North Dublin.

WHAT WILL HAPPEN TO ME IF I AGREE TO TAKE PART?

We will look through your medical records to see what medications you are on currently. Using information gained from your medical records, we will calculate your 10 year risk of fracture. If you are found to be at high risk of fracture and are not on appropriate treatment, we will contact your doctor so that you can be started on appropriate medication. If you are found to be on an unsuitable medication regime for your bones, we will inform your doctor.

BENEFITS:

You will be less likely to break a bone in the event of a fall if you are on appropriate osteoporosis medications.

RISKS:

There are no risks associated with participation in this study as it involves collection of data from medical charts only.

CONFIDENTIALITY ISSUES

We will contact your doctor to let them know you are taking part in the study. The above named investigators will be looking at your medical chart as part of this study. Data relating to you will be kept in a secure setting for 5 years after the study is completed and will then be destroyed.

IF YOU REQUIRE FURTHER INFORMATION

If you have any further questions about the study, or if you wish to withdraw from the study you may do so without justifying your decision and your future treatment will not be affected.

For additional information now or any future time please contact me between 9am-5pm
Monday -Friday:

Dr C Mc Greevy
Dept of Geriatric Medicine,
Beaumont Hospital

Phone No-01-8092352

APPENDIX I

Next of Kin Information Leaflet

Protocol Title:

A comparison between the prescription of bone health medications in general practitioner led nursing homes compared with geriatrician led services.

Principal Investigator's Name: Professor David Williams

Principal Investigator's Title: Associate Professor of Geriatric Medicine

Telephone No. of Principal Investigator: 01-7974731

Your next of kin is being invited to take part in a clinical research study carried out at Beaumont Hospital. We are asking you to read this information on behalf of your next of kin, as we are aware that unfortunately, your next of kin may not be able to understand the information contained in this leaflet due to cognitive impairment, and may not have the capacity to decide whether to take part in this research project or not.

Before you decide whether or not you wish your next of kin to take part, you should read the information provided below carefully and if you wish discuss it with your family, friends or doctor. Take time to ask questions – do not feel rushed or under any obligation to make a hasty judgement. You should clearly understand the risks and benefits of participating in this study so that you can make a decision that is right for you – this process is known as Informed Assent.

Your next of kin is not obliged to take part in this study and failure to participate will have no effect on their future care.

You may change your mind at any time (before the start of the study or even after the study has been commenced) for whatever reason without having to justify your decision and without any negative impact on the care your next of kin will receive from the medical and nursing staff.

WHY IS THIS STUDY BEING DONE?

Osteoporosis (fragile bones) is very common in Ireland, particularly in those aged 65 and over. We plan on looking to see what types of medications are being used to treat osteoporosis in nursing homes. We also plan on analysing each resident's risk of fracture in the next 10 years using a tool called the FRAX tool, which involves inputting data into a computer and calculating that risk. If your next of kin's FRAX score indicates that they should be on treatment for osteoporosis, we will write to their doctor with regards to starting that treatment.

WHO IS ORGANISING AND FUNDING THIS STUDY?

This study is being organised by the Department of Geriatric medicine in Beaumont Hospital. A pharmaceutical company called MSD have contributed towards funding this study. Professor David Williams is the principle investigator and sponsor. Dr Cora Mc Greevy is the co-investigator. This research is being done as part of an MD degree by Dr McGreevy.

HOW WILL IT BE CARRIED OUT?

This study is due to commence in January 2011 and will be 1 year in duration. We hope to enrol 280 participants in total. We will be including nursing home residents aged 65 and over in a number of facilities in North Dublin.

WHAT WILL HAPPEN TO ME IF I AGREE TO TAKE PART?

We will look through your next of kin's medical records to see what medications they are on currently.

Using information gained from your next of kin's medical records, we will calculate their 10 year risk of fracture. If they are found to be at high risk of fracture and are not on appropriate treatment, we will contact their doctor so that they can be started on appropriate medication. If your next of kin is found to be on an unsuitable medication regime, we will inform their doctor.

BENEFITS:

Your next of kin will be less likely to break a bone in the event of a fall if they are on appropriate osteoporosis medications.

RISKS:

None

CONFIDENTIALITY ISSUES

We will contact your next of kin's doctor to let them know he/she is taking part in the study. The above named investigators will be looking at your next of kin's medical chart as part of this study.

Data relating to your next of kin will be kept in a secure setting for 5 years after the study is completed and will then be destroyed.

IF YOU REQUIRE FURTHER INFORMATION

If you have any further questions about the study, or if you wish to withdraw your next of kin from the study, you may do so without justifying your decision and their future treatment will not be affected.

For additional information now or any future time please contact me between 9am-5pm
Monday -Friday:
Dr C Mc Greevy
Dept of Geriatric Medicine,
Beaumont Hospital

Phone No-01-8092352

APPENDIX J

A comparison between the prescription of bone health medications in general practitioner led nursing homes compared with geriatrician led services
Data collection sheet: & Barthel score

Code: _____

Male Female

Age:

Weight (kg):

Height(cm):

Date of admission to NH:

Past medical history:

Allergies:

Current osteoporosis medications:

Calcium/Vitamin D	Yes	No	Name/ Dosage/Date started
PO Bisphosphonate	Yes	No	Name/ Dosage/Date started
IV Bisphosphonate	Yes	No	Name/ Dosage/Date started
Strontium	Yes	No	Name/ Dosage/Date started
Teriparatide	Yes	No	Name/ Dosage/Date started
Denosomab	Yes	No	Name/ Dosage/Date started

Other: _____

Hx of osteoporosis medications in past and reason for stopping?

Unable to swallow:

Poor compliance:

