LEABHARLANN CHOLÁISTE NA TRÍONÓIDE, BAILE ÁTHA CLIATH | TRINITY COLLEGE LIBRARY DUBLIN
Ollscoil Átha Cliath | The University of Dublin

Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

VITAMIN D IN OLDER ADULTS AND ITS RELATIONSHIP TO COGNITION, BLOOD PRESSURE, MOOD AND FALLS.

Kevin Gerald McCarroll

BA, MB, BCh, BAO, MRCPI

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Medicine

UNIVERSITY OF DUBLIN

TRINITY COLLEGE

AUGUST, 2014.

TRINITY COLLEGE
5 JAN 2015
LIBRARY DUBLIN

IX-2-127

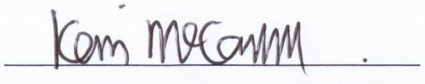
DECLARATION

I declare that the work in this thesis is entirely my own, except where credit is given in the acknowledgements.

All subjects participating in the studies gave full and informed consent and ethical approval was granted by St James's Hospital Research Ethnics committees.

I have contributed to the study from which this thesis is based by recruiting over two hundred study participants and supervising and assisting several study recruiters over a two year period.

This thesis has not been submitted as an exercise at this or any other University. I agree that the Library of the University of Dublin may lend or copy this thesis upon request.



Kevin Mc Carroll, August 2014

SUMMARY

Introduction

An increasing body of evidence supports a role for vitamin D in cognition, blood pressure, mood and falls. Vitamin D deficiency is prevalent in older adults and might provide an alternative novel treatment strategy for some non-skeletal diseases. However, studies to date have given conflicting results and the role of vitamin D beyond bone health remains unclear.

Aims

This study aimed to explore the relationship between vitamin D and cognition, blood pressure, falls and mood in community dwelling older Irish adults. It also aimed to investigate the correlates of serum vitamin D in the same population, with particular regard to the effect of season and supplement use.

Methods

The study population comprised community dwelling adults aged over sixty years who were participants of a large cross sectional (Trinity, University of Ulster, Dept of Agriculture) study. Study subjects were recruited into three disease defined cohorts which included those with hypertension, cognitive impairment or osteopaenia / osteoporosis. Detailed psychosocial, medical, biophysical and neuropsychological assessments were performed. Vitamin D and other blood tests were also taken.

Results

Analysis was performed in 1568, 1199 and 1330 subjects in the hypertensive, bone and cognitive cohorts respectively. When the vitamin D by cohort interaction term was significant ($P < 0.05$) the cohorts were analysed separately.

The most important correlates of vitamin D were season and supplement use, though other predictors included physical frailty, sun holiday travel, preference for sun exposure, smoking, oily fish consumption and depression. Vitamin D levels were lowest in the months of February/March and highest in August/September. Vitamin D deficiency (< 50 nmol/l) was common, especially in non-supplemented older adults where prevalence ranged between 40.2% – 73.6%

SUMMARY

Serum vitamin D was positively associated with global cognitive performance, executive function and language tests but only in 'younger' old participants. The findings were independent of several confounding factors.

Likewise, an inverse relationship between vitamin D and depressive symptoms was identified in adults at the younger end of the older age spectrum independent of a prior history of depression and other covariates.

In a pooled analysis including subjects in all three cohorts, lower vitamin D status was associated with a diagnosis of hypertension but not diastolic or systolic blood pressure. The relationship was significant after adjustment for several confounds including serum parathyroid hormone.

Finally, in 'older' old adults in the cognitive cohort, lower vitamin D status was associated with an increased risk of falling but not recurrent falls, and only in those who were not supplemented. The effect was independent of physical frailty as assessed with the Timed Up and Go.

Conclusion

Results emphasize the high prevalence of vitamin D deficiency in older adults and the importance of factoring in season when interpreting vitamin D levels. Study findings support a role for vitamin D in mood and cognition, in particular executive function and language. However, results suggest that the benefit may be confined to 'younger' old adults and raises the possibility that longer-term deficiency may cause negative effects that may not be amenable to treatment with vitamin D in later life. Results also suggest that lower vitamin D status may be important in the development of a hypertensive disease state. Finally, vitamin D may play a role in falls in older adults but it remains unclear which target group benefits most and the specific mechanistic actions that mediates any potential reduction.

PUBLICATIONS RELATED TO THESIS

The following are publications, presentations or submissions related to my work in preparing this thesis:

Abstract

1. Correlates of Vitamin D in Older Irish Adults

McCarroll K, Beirne A, Molloy A, Healy M, Mc Nulty H, Cunningham C.

Ir Jr Med Sc, 182 Suppl 6: 2013: S179-296

2. Vitamin D and Blood Pressure in Older Irish Adults

McCarroll K, Casey M, Walsh JB, McNulty H, Cunningham C.

Ir Jr Med Sc, 182 Suppl 6: 2013: S179-296

Presentation

1. Correlates of Vitamin D in Older Irish Adults

Platform Presentation - Irish Gerontological Society Annual Meeting – Sept 2013.

McCarroll K, Beirne A, Molloy A, Healy M, Mc Nulty H, Cunningham C.

Journal Paper Submission

1. Determinants of Vitamin D in Older Irish Adults

McCarroll K, Beirne A, Casey M, Molloy A, Healy M, Laird E, Mc Nulty H, Ward M, Strain S, Cunningham C.

Submitted to Age Ageing (under review)

ACKNOWLEDGEMENTS

I would like to thank my supervisor Conal Cunningham for his mentorship, constructive criticism and unfailing support. I would like to acknowledge all the staff involved in the recruitment of subjects for this study, particularly Helen Toohey and Sinead McNiffe. Special thanks also to laboratory staff Regina Dempsey and Tracey Claxton for processing of study bloods and in particular to Martin Healy for his expertise and analysis of serum vitamin D samples; to colleagues in the University of Ulster, Coleraine through which this study was made possible and to all participants who volunteered to be recruited. Finally, I would like to thanks friends and family for their support during the time I worked on this thesis.

This study was funded by the Mercer's Institute for Research on Ageing in conjunction with grant support from the Dept. of Agriculture, Ireland and the Dept for Employment and Learning, Northern Ireland.

TABLES

Table 2.1 Vitamin D deficiency in population studies.....17

Table 2.2 Vitamin D deficiency in Ireland..... 18

Table 2.41 Vitamin D content of food.....25

Table 2.42 Major causes of vitamin D deficiency..... 29

Table 3.2 Vitamin D Target Gene Products in the Brain.....42

Table 3.81 Cross sectional studies of 25(OH)D and Cognition.....62

Table 3.82 Longitudinal studies of 25(OH)D and Cognition..... 64

Table 3.83 Randomised Controlled Trials of Vitamin D and Cognition.....65

Table 4.83 Cross sectional studies of 25(OH)D and Blood Pressure..... 83

Table 4.83 Cross sectional studies of 25(OH)D and Blood Pressure..... 84

Table 4.84 Longitudinal studies of 25(OH)D and Blood Pressure.....88

Table 4.85 Intervention Trials of Vitamin D and Blood Pressure..... 96

Table 4.85 Intervention Trials of Vitamin D and Blood Pressure.....97

Table 5.53 Meta-analyses of RCT's of vitamin D and Falls.....111

Table 6.52 Cross sectional studies of 25(OH)D and Mood..... 123

Table 6.52 Cross sectional studies of 25(OH)D and Mood..... 124

Table 6.53 Longitudinal studies of 25(OH)D and Mood.....127

Table 6.54 Intervention trials of 25(OH)D and Mood.....131

Table 8.1 Study Cohort Characteristics..... 140

Table 12.1 Comparison of serum 25(OH)D across cohorts..... 150

Table 12.2 Comparison of serum 25(OH)D across cohorts by category 150

Table 12.3 Seasonal variation in serum 25(OH)D by cohort.....151

Table 12.4 Mean Seasonal Global Solar Radiation by cohort..... 151

Table 12.5 Correlates of serum 25(OH)D in Cognitive cohort.....155

TABLES

Table 12.6 Correlates of serum 25(OH)D in Hypertensive cohort..... 156

Table 12.7 Correlates of serum 25(OH)D in Bone cohort..... 156

Table 13.1 Factors associated with supplement use in each cohort.....159

Table 17.1 Relationship between 25(OH)D and Cognition in Hypertensive cohort..... 181

Table 17.2 Relationship between 25(OH)D and Cognition in Cognitive cohort.182

Table 17.3 Relationship between 25(OH)D and Cognition in Bone cohort..... 183

Table 18.1 Relationship between 25(OH)D and RBANS Index Subtests in Hypertensive cohort.....185

Table 18.2 Relationship between 25(OH)D and RBANS Index Subtests in Bone cohort.....186

Table 18.3 Relationship between 25(OH)D and RBANS Index Subtests in Cognitive cohort.....186

Table 22.1 Characteristics of participants by diagnosis of Hypertension..... 202

Table 22.2 Serum 25(OH)D and Hypertension 203

Table 22.3 Serum 25(OH)D and Systolic/Diastolic Blood pressure.....204

Table 27.1 Serum 25(OH)D and Depression in the Hypertensive cohort..... 213

Table 27.2 Serum 25(OH)D and Depression in the Cognitive cohort..... 214

Table 28.1 Mood versus GSR/Season in Hypertensive & Bone cohort.....217

Table 32.1 Serum 25(OH)D and Falls in the Hypertensive cohort..... 225

Table 32.2 Serum 25(OH)D and Falls in the Cognitive cohort..... 225

Table 32.3 Serum 25(OH)D and Falls in the Bone cohort..... 226

Table 33.1 Characteristics of Fallers in Cognitive cohort (non-supplemented).. 228

Table 33.2 Vitamin D and Falls in Non-supplemented Cognitive cohort..... 229

FIGURES

Fig 1.1 Rickets.....4

Fig 1.12 Francis Glisson's Treatise on Rickets.....4

Fig 1.13 Adolf Windaus.....7

Fig 1.24 Vitamin D3.....8

Fig 1.25 Vitamin D2.....8

Fig 1.40 25-hydroxyvitamin D 9

Fig 1.41 1,25 Dihydroxyvitamin D3.....10

Fig 12.1 Seasonal Variation in serum 25(OH)D in Cognitive Cohort 152

Fig 12.12 Seasonal Variation in serum 25(OH)D in Cognitive Cohort (supplemented).....152

Fig 12.13 Seasonal Variation in serum 25(OH)D in Cognitive Cohort (non-supplemented)..... 152

Fig 12.2 Seasonal Variation in serum 25(OH)D in Hypertensive Cohort..... 153

Fig 12.21 Seasonal Variation in serum 25(OH)D in Hypertensive Cohort (non-supplemented)..... 153

Fig 12.22 Seasonal Variation in serum 25(OH)D in Hypertensive Cohort (supplemented).....153

Fig 12.3 Seasonal Variation in serum 25(OH)D in Bone Cohort..... 154

Fig 12.31 Seasonal Variation in serum 25(OH)D in Bone Cohort (non-supplemented)..... 154

Fig 12.32 Seasonal Variation in serum 25(OH)D in Bone Cohort (supplemented).....154

Fig 13.1 Monthly Variation in serum 25(OH)D and Global Solar Radiation (GSR) in the Hypertensive Cohort..... 160

Fig 13.11 Monthly Variation in serum 25(OH)D and Global Solar Radiation (GSR) in the Hypertensive Cohort (non-supplemented).....160

FIGURES

Fig 13.12 Monthly Variation in serum 25(OH)D and Global Solar Radiation (GSR) in the Hypertensive Cohort (supplemented).....160

Fig 13.2 Monthly Variation in serum 25(OH)D and Global Solar Radiation (GSR) in the Cognitive Cohort (supplemented).....161

Fig 13.21 Monthly Variation in serum 25(OH)D and Global Solar Radiation (GSR) in the Cognitive Cohort (non -supplemented).....161

Fig 13.3 Monthly Variation in serum 25(OH)D and Global Solar Radiation (GSR) in the Bone Cohort (supplemented) 161

Fig 13.31 Monthly Variation in serum 25(OH)D and Global Solar Radiation (GSR) in the Bone Cohort (non-supplemented).....161

Fig 13.4 Timed Up and Go vs. serum 25(OH)D in Cognitive cohort 163

Fig 13.41 Timed Up and Go vs. serum 25(OH)D in Cognitive cohort (non-supplemented).....163

Fig 13.42 Timed Up and Go vs. serum 25(OH)D in Cognitive cohort (supplemented).....164

Fig 13.5 Timed Up and Go vs. serum 25(OH)D in Hypertensive cohort.....165

Fig 13.51 Timed Up and Go vs. serum 25(OH)D in Hypertensive cohort (non- supplemented)..... 165

Fig 13.52 Timed Up and Go vs. serum 25(OH)D in Hypertensive cohort (supplemented)..... 165

Fig 13.6 Timed Up and Go vs. serum 25(OH)D in Bone cohort..... 165

Fig 13.61 Timed Up and Go vs. serum 25(OH)D in Bone cohort (supplemented).....165

Fig 13.62 Timed Up and Go vs. serum 25(OH)D in Bone cohort (non-supplemented)..... 165

FIGURES

Fig 18.1 Total Scale (RBANS) vs. quintiles of 25(OH)D in the Hypertensive Cohort..... 189

Fig 18.2 FAB score vs. quintiles of 25(OH)D in the Hypertensive Cohort.... 189

Fig 18.3 Index III score vs. quintiles of 25(OH)D in the Hypertensive Cohort..... 190

Fig 18.31 Category fluency (Index III) score vs. quintiles of 25(OH)D in the Hypertensive Cohort..... 190

Fig 18.32 Picture Naming (Index III) score vs. quintiles of 25(OH)D in the Hypertensive Cohort..... 191

Fig 28.1 CES-D Score vs. quintiles of 25(OH)D in the Hypertensive Cohort 216

Fig 28.2 CES-D Score vs. quintiles of 25(OH)D in the Cognitive Cohort.... 217

LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
AMT	Abbreviated Mental Test
BMI`	Body mass index
BP	Blood Pressure
BDI	Beck Depression Inventory
CANTAB	Cambridge Neuropsychological Testing Automated Battery
CDR	Clinical Dementia Rating Scale
CDT	Clock Drawing Test
CES-D	Centre of Epidemiologic Studies Depression Scale
CNS	Central Nervous System
COX	Cyclo-oxygenase
CI	Confidence Interval
CSI	Cognitive Style Index
CTRM	Camden Topographical Recognition Memory
DBP	Diastolic Blood Pressure
DEQAS	Vitamin D External Quality Assessment Scheme
DSMT	Digit Symbol Matching Test
DSST	Digit Symbol Substitution Test
EBMT	Eastern Boston Memory Test
FAB	Frontal Assessment Battery
GCS	Global Composite Score
GDNF	Glial Derived Nerve Factor
GDS	Geriatric Depression Scale
GSR	Global Solar Radiation

LIST OF ABBREVIATIONS

HAD	Hospital Anxiety Depression Scale
HPA	Hypothalamic Pituitary Axis
HTN	Hypertension
IOM	Institute of Medicine
LCMS	Liquid Chromatography Mass Spectroscopy
MCS	Memory Composite Score
MFQ	Mood and Feelings Questionnaire
MHI	Mental Heath Inventory
MI	Myocardial Infarction
MMSE	Mini Mental State Examination
NGF	Nerve Growth Factor
NHANES	National Health and Nutrition Examination Survey
NS	Non-significant
NOS	Nitric Oxide Synthetase
NT-3	Neurotrophin-3
PMS	Premenstrual Syndrome
PTH	Paraythyroid Hormone
RAAS	Renin Angiotensin Aldosterone System
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
RDA	Recommended Daily Allowance
RCT	Randomised Controlled Trial
ROCF	Rey Osteireth Complex Figure
SAD	Seasonal Affective Disorder
SBP	Systolic Blood Pressure

LIST OF ABBREVIATIONS

SBT	Short Blessed Test
SHR	Spontaneous Hypertensive Rats
SPMSQ	Short Portable Mental State Questionnaire
TMA&B	Trail making tests A & B
TUI	Tolerable Upper Intake
25(OH)D	25-hydroxyvitamin
1,25(OH)$_2$D	1,25 dihydroxyvitamin D
TUG	Timed Up and Go
TUDA	Trinity, University of Ulster, Dept of Agriculture
VDR	Vitamin D Receptor
VDBP	Vitamin D binding protein
WMS	Weschler Memory Scale

TABLE OF CONTENTS

SECTION ONE: TITLE: I
DECLARATION II
SUMMARY. III
PUBLICATIONS RELATED TO THESIS V
ACKNOWLEGDEMENTS VI
LIST OF TABLES VII
LIST OF FIGURES IX
LIST OF ABBREVIATIONS XII

SECTION TWO: VITAMIN D: GENERAL INTRODUCTION 3
CHAPTER 1: HISTORY AND BACKGROUND 4
CHAPTER 2: VITAMIN D DEFICIENCY 14
CHAPTER 3: VITAMIN D AND COGNITION 40
CHAPTER 4: VITAMIN D AND BLOOD PRESSURE 67
CHAPTER 5: VITAMIN D AND FALLS 99
CHAPTER 6: VITAMIN D AND MOOD 113

SECTION THREE: STUDY POPULATION 133
CHAPTER 7: STUDY DESIGN 134
CHAPTER 8: PARTICIPANT CHARACTERISTICS 138
CHAPTER 9: DISCUSSION 141

SECTION FOUR: VITAMIN D CORRELATES IN OLDER IRISH ADULTS 145
CHAPTER 10: INTRODUCTION 146
CHAPTER 11: METHODOLOGY 147
CHAPTER 12: RESULTS 149
CHAPTER 13: POST HOC ANALYSIS & RESULTS 158
CHAPTER 14: DISCUSSION 166

SECTION FIVE: VITAMIN D AND COGNITION 175
CHAPTER 15: INTRODUCTION 176
CHAPTER 16: METHODOLOGY 178
CHAPTER 17: RESULTS 180
CHAPTER 18: POST HOC ANALYSIS & RESULTS 184
CHAPTER 19: DISCUSSION 192

SECTION SIX: VITAMIN D AND BLOOD PRESSURE 197
CHAPTER 20: INTRODUCTION 198
CHAPTER 21: METHODOLOGY 199
CHAPTER 22: RESULTS 202
CHAPTER 24: DISCUSSION 205

SECTION SEVEN: VITAMIN D AND MOOD 209
CHAPTER 25: INTRODUCTION 210
CHAPTER 26: METHODOLOGY 211
CHAPTER 27: RESULTS 213
CHAPTER 28: POST HOC ANALYSIS & RESULTS 216
CHAPTER 29: DISCUSSION 218

TABLE OF CONTENTS

SECTION EIGHT: VITAMIN D AND FALLS.....221
CHAPTER 30: INTRODUCTION..... 222
CHAPTER 31: METHODOLOGY..... 223
CHAPTER 32: RESULTS..... 225
CHAPTER 33: POST-HOC ANALYSIS & RESULTS..... 227
CHAPTER 34: DISCUSSION 230

SECTION NINE: CONCLUSION..... 233
CHAPTER 35: CONCLUSIONS..... 236

BIBLIOGRAPHY.....237

APPENDICES.....309
NEUROPYSCHOLOGICAL, MOOD AND FUNCTIONAL SCALES.....310

SECTION 2: VITAMIN D: GENERAL INTRODUCTION

CHAPTER 1: VITAMIN D – HISTORY AND BACKGROUND.

1.1 History of Vitamin D

The discovery of Vitamin D in the 1920's came from the search for a cure for rickets at the turn of the last century. The finding that both cod liver oil (which contains vitamin D) and sunlight could be used in the prevention and treatment of rickets led to a unifying theory underpinning our current understanding of the "sunshine vitamin"

Rickets

Rickets is a disease of children giving rise to the classical deformity of the lower limbs called 'bowed' or 'knock knees'. It occurs when calcium, phosphate and vitamin D are not present in sufficient amounts to allow growing bone calcify normally (Weick, 1967). In the 17th and 18th century, rickets was quite prevalent in Europe and also in the New England Colonies (Weick, 1967). By the early 20th century it reached epidemic proportions with an estimated 80-90% of children in Northern Europe and some US cities having evidence of rickets (Holick, 2006). Whilst the bony deformities of rickets were noted as far back as the 2nd century AD, the earliest scientific description was made much later by the English physician Daniel Whistler in 1645 (Smerdon, 2007). A treatise on rickets in 1650 by Francis Glisson outlined in detail the observations of the disease based on clinical and post-mortem experience (Dunn et al., 1998) though added little to our current understanding of its aetiopathology. It was nearly two centuries later before any advances were made in the study of rickets. Two theories began to emerge, centred on the proposed effect of sunlight exposure and/or cod liver oil as a prevention or cure.

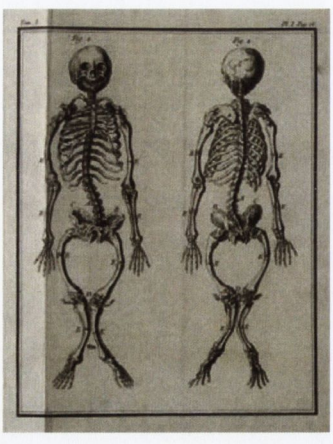


Fig 1.1: Rachitic skeleton of child

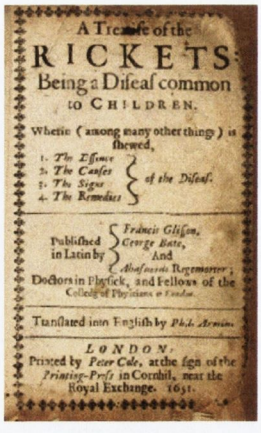


Fig 1.12: Francis Glisson's Treatise on Rickets

Rickets and Sunlight Exposure

The hypothesis that differences in sunlight exposure may a play a part in rickets was first made by Sniadecki in 1822. He discovered that children in Poland who lived in rural areas had a lower incidence of rickets in comparison to those living in the city of Warsaw (Mozolowski et al., 1939). In 1890, Theodore Palm found that children living in equatorial countries did not develop rickets (Palm, 1890). He noted that while children living in London and Glasgow had rickets, those in Asia and India were free of the disease (Holick, 2006). He concluded that the common factor for the development of rickets in children was lack of sunlight exposure and suggested sunbathing as a means of preventing and curing rickets (Holick et al., 2006). Studies reporting a seasonal variation in rickets also suggested a role for sunlight. The influence of season on the incidence of rickets was first noted in 1884 by Max Kassowitz who identified that it was greater in the winter with a decline in the Summer and Autumn (Kassowitz et al., 1897). This was also supported by autopsy studies that identified more rickets in the months between November and May (Schmorl, 1909).

This theory led scientists to directly study the effects of light treatment on rickets. In 1919 the effect of UV light in curing rickets was reported by Huldschinsky. He showed that exposure of four children with rickets to a mercury vapour light over a four month period resulted in cure of their rickets as shown by x-ray analysis (Huldschinsky et al., 1919). Similar studies by Hess and Unger using x-ray analysis in 1921 found an improvement in rickets in children in response to sunlight (Hess et al., 1921; Hess et al., 1922).

Rickets and Cod Liver Oil

The use of cod liver oil as a specific remedy for rickets was noted as early as 1824 (Guy, 1923). In the mid 1800's, Bretonneau reported that the treatment of a 15 month old child (who had acute rickets) with cod liver oil led to a quick recovery. The use of cod liver oil in treating rickets was subsequently recommended by Trousseau in 1865. He had used a variety of fish oils to treat rickets and advocated their use preferably with exposure to sunlight as a cure (Holick et al., 1990; Dunn, 1999). In 1889, it was proposed by Bland-Suttton that rickets might be caused by a deficiency of dietary fat (Chesney et al., 2009). He found that addition of cod liver oil and crushed bones to

the diet of rachitic lion cubs (previously fed only boneless lean meat) helped them recovery fully.

These findings had strongly suggested that rickets was due to a 'nutritional deficiency' though this was not widely accepted until 1918 when Sir Edward Mellanby discovered cod liver as a cure for rachitic dogs. He proposed that cod liver oil contained a fat soluble factor that he called the 'antirachitic factor' (Mellanby, 1918). This was later identified as a heat stable component of cod liver oil by Mc Collum in 1922 (McCollum et al., 1922). He concluded that it was distinct from fat soluble vitamin A as it was not destroyed by oxidation under heat. As three vitamins had already been named and this was the fourth in order it was called vitamin D (Mc Collum, 1957). By the 1930's the use of cod liver oil in the prevention and treatment of rickets had become common place (Weick, 1967).

A Unifying Theory

The finding that UV irradiation of foods or skin produced an antirachitic factor greatly improved our understanding of vitamin D biochemistry and supported the dictum the " light equals vitamin D."

Hess and Weinstock showed that an antirachitic factor was produced by UV irradiation of food (Hess et al., 1924). This ultimately led to the discovery of precursor molecules of vitamin D, the first of which was ergosterol. The product from this irradiation was initially called vitamin D1 but was mistakenly believed to be a single product and when purified was later named ergocalciferol or vitamin D2 (Fieser et al., 1959). Steenbock also confirmed this in his experiments and recommended that the irradiation of milk (with added ergosterol) might be a good way to provide vitamin D to children and prevent rickets (Steenbock, 1924). By 1934, the FDA had approved the vitamin D fortification of milk in several US states (FDA, 1934). This addition of vitamin D2 to milk led to the eradication of rickets as a health problem in the US within a few years (Centers for Disease Control, 1999).

The discovery of the structure of vitamin D3 came in 1936 following the isolation of a form of cholesterol called 7-DHC (7-dehydrocholesterol) in the skin as a precursor of Vitamin D by Windaus and colleagues (Windaus et al., 1937). Their experiments

showed that when 7-DHC isolated from pig skin was exposed to UV irradiation it induced the formation of Vitamin D which was named Vitamin D3 or cholecalciferol (Wolf et al., 2004). 7-DHC was later identified in human cadaver skin (Rauschkolb et al., 1969) confirming the role of UV light in vitamin D synthesis in humans.

These experimental findings ultimately unified the two competing concepts regarding the use of cod liver oil or UV light in treating rickets, by showing that exposure to UV light was responsible for Vitamin D synthesis.

Fig 1.13: Adolf Windhaus (1876-1959)

Adolf Windaus who with his colleagues discovered the structure of vitamin D3 in 1936 and was awarded the Nobel prize in chemistry in 1928 for his studies on the constitution of sterols and their connection with vitamins (Wolf et al., 2004).

1.2 Biochemistry

Vitamin D is a fat soluble secosteroid (steroid with a broken ring). It exists in two major physiological forms, Vitamin D2 (ergocalciferol) and Vitamin D3 (cholecalciferol). The structural difference between both forms exist in their side chains, with vitamin D2 having a methyl group on carbon 24 and the presence of a double bond between carbon 22 and 23. Vitamin D2 is produced in invertebrates, plants, yeasts and fungi in response to ultraviolet B (UVB) irradiation of ergosterol. Vitamin D3 is synthesised photochemically in the skin of vertebrates in response to UVB irradiation (wavelength 270 - 310 nm) of 7-dehydrocholesterol (7-DHC), (Holick, 2007: Holick et al., 2009).

Fig 1.24: Vitamin D3

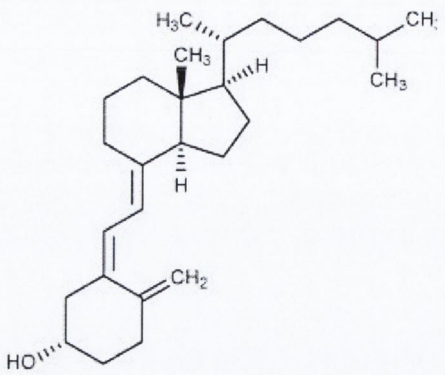
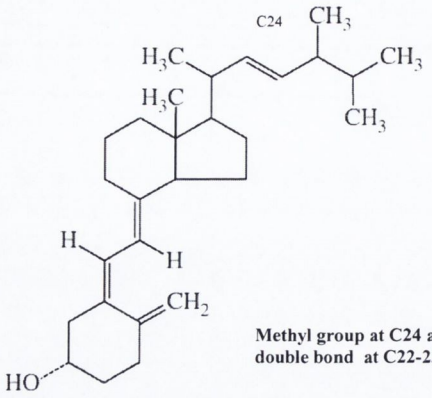


Fig 1.25: Vitamin D2



Methyl group at C24 and double bond at C22-23

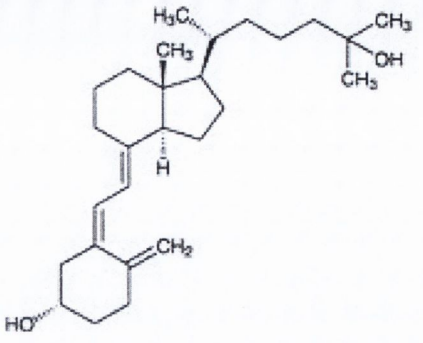
1.3 Photobiology

The action of UVB radiation (270-310 nm) on 7-DHC results in it's photolytic conversion to previtamin D3 through cleavage of the C9-C10 bond (Holick et al., 200). About 95% of previtamin D3 is produced in the epidermis where most UVB radiation is absorbed (Holick et al., 1981). This synthesis peaks at a wavelength of 295-297 nm. Previtamin D3 is transformed rapidly to vitamin D3 within the skin cell plasma membrane by a thermally induced isomerisation. It is then translocated to the dermal capillary bed by an unknown mechanism which may involve Vitamin D binding protein (DBP) (Holick et al., 1990). Prolonged exposure to sunlight degrades vitamin D3 into inactive photo products including suprasterols, thereby preventing excess vitamin D synthesis (Webb et al., 1989).

1.4 Metabolism

Vitamin D2 and D3 from dietary sources are absorbed in the small bowel and incorporated into chylomicrons and transported in the lymphatic system into the circulation. Vitamin D3 synthesised in the skin is also released into the circulation (Holick, 2006) . The majority of both forms of circulating vitamin D are bound to vitamin D binding protein (VDBP) (80-90%) and albumin (10-20%), with a small fraction being free (Zerwekh et al., 2008). Vitamin D in circulation is transported to the liver where it is converted by vitamin D-25 hydroxylase to 25-hydroxyvitamin D [25(OH)D] (Holick, 2007). Liver production of 25(OH)D is primarily dependant on the availability of vitamin D (Zerwekh et al., 2008).

Fig 1.4: 25-Hydroxyvitamin D3 [25(OH)D]



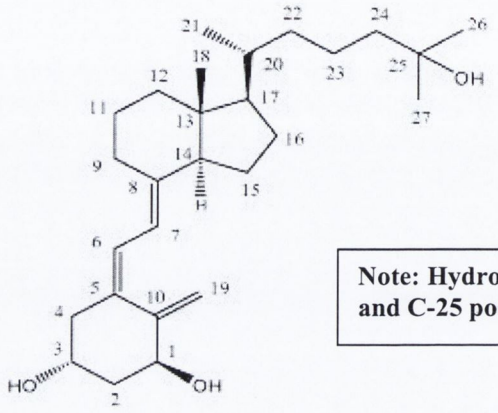
Hydroxyl group at C25

Activation of Vitamin D

Inactive 25(OH)D is converted in the kidneys (in the mitochondria of the proximal renal tubules) by a 25-hydroxyvitamin D-1ά hydroxylase into its biologically active form 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] (Plum & De Luca, 2010). The enzyme responsible for this is part of the cytochrome system and is known as CYP27B1 (Prosser et al., 2004).

Renal production of active vitamin D is tightly regulated and dependant on the activity of 25-hydroxyvitamin D-1ά hydroxylase which is controlled by serum levels of PTH, calcium and phosphorus. When serum 25(OH)D or calcium levels drop, increased release of PTH leads to upregulation of 25-hydroxyvitamin D-1ά hydroxylase in the kidney and increased synthesis of active $1,25(OH)_2D$ to maintain it at a constant level. Conversely, $1,25(OH)_2D$ is a potent down regulator of PTH which provides a negative feedback loop to 1ά-hydroxylation. $1,25(OH)_2D$ also suppresses it's own activity by inducing a 24-OHase which converts active vitamin D into a metabolically inactive metabolite ($1,24,25(OH)_3D$). It also increases the level of fibroblast growth factor 23 (FGF-23) which causes phosphate diuresis and suppresses 1,25(OH)D synthesis. In addition, 24-OHase activity is also suppressed by PTH. In this way the active form of vitamin D ($1,25(OH)_2D$) is maintained at a homeostatic level (Plum & De Luca, 2010).

Fig 1.41: 1,25 Dihydroxyvitamin D3 [1,25(OH)$_2$D]



Note: Hydroxylation at C-1 and C-25 positions

Extra-renal activation of Vitamin D

It is now known that 1-α hydroxylase (CYP27B1) which is responsible for conversion of vitamin D to its active form is found in several tissues outside the kidney suggesting a role for vitamin D beyond bone health. As far back as 1979, studies suggested that the placenta could produce 1,25(OH)$_2$D (Wiesman et al., 1979) and soon after osteoblasts were identified as having CYP27B1 (Turner et al., 1980). Expression of 1-α hydroxylase has since been shown in numerous tissues following the cloning of the enzyme (Fu et al., 1997) and development of antibodies to it (Zehnder et al., 2001). It is often expressed in conjunction with the vitamin D receptor (VDR) and has been found in breast, colon, cervix, endometrium, ovary, testis, thyroid, liver, brain, pancreas, parathyroid, adrenals, monoctyes and lung tissue (Holick et al., 2007).

1.5 Mechanism of Action

Directly or indirectly 1,25(OH)D has been reported to regulate over 200 genes (Holick et al., 2007). Wang et al., reported that vitamin D controls the expression of at least 913 genes (Wang et al., 2005). More recently, it has been noted that vitamin D may regulate up to 3% of the human genome (Pilz et al., 2009).

Vitamin D Receptor

The discovery of the nuclear vitamin D receptor (VDR) for $1,25(OH)_2D$ was made in 1969 (Haussler et al., 1969). This had followed the discovery of $1,25(OH)_2D$ as a new steroid hormone in the late 1960's (Norman, 2006). However, it wasn't until 1987 that it was cloned and sequenced (Baker et al., 1988). Vitamin D exerts its genomic effect through binding to the nuclear vitamin D receptor (VDR). This is a member of the steroid/thyroid hormone super-family of transcription regulation factors that is widely distributed throughout the body and found in over 50 tissues (Stumpf et al., 2005). VDR are present on a large variety of cell types including myocytes, cardiomyoctes, pancreatic beta cells, vascular endothelial cells, neurons and osteoblasts (Holick et al., 2007).

Classical Action

The classical action of $1,25(OH)_2$ D begins when it binds to the nuclear VDR, which after phosphorylation heterodimerises with nuclear receptors of the retinoic X receptor (RXR) family. VDR is the only nuclear protein that binds $1,25(OH)_2D$ with high affinity. The $1,25(OH)_2D$/VDR/RXR complexes recognise specific genomic sequences named vitamin D Response Elements (VDRE's) within the promoter regions of hundreds of genes. Binding of co-repressive or co-activator factors to these complexes modulates gene expression by both directly activating or down regulating gene transcription (Fernandes de Abreu et al., 2009). Two major co-activator complexes have been identified, the steroid receptor activator complex (SRC) and the vitamin D receptor interacting protein complex (DRIP), (Rachez et al, 2000; Leo et al., 2000). Activation of genes involves the binding of at least one VDR molecule but often more to VDREs. Co-pressors that block VDR mediated transcription include nuclear compressor (NcoR) and Silencing Mediator of Retoinic acid and Thyroid receptor (SMRT), (Bikle et al., 2009).

Non Classical Action

$1,25(OH)_2D$ is believed to mediate more rapid non classical (non-genomic) actions through binding to the putative MARRS (Membrane Associated Rapid Response Steroid Binding) receptor at the cell surface (Jia et al., 1999). VDR is however, necessary for some of these non-genomic pathways (Jia et al., 1999). Vitamin D by this mechanism is known to activate a variety of signal transduction systems including

calcium influx, release of calcium from intracellular stores, modulation of adenylate cyclase, PLC. protein kinases C and D as well as MAP and Raf kinase pathways (Fernandes de Abreu et al., 2009). Some rapid responses include the intestinal absorption of calcium, secretion of insulin by pancreatic cells, opening of voltage gated Ca^{2+} and Cl^- channels in osteoblasts, and the rapid migration of endothelial cells (Norman, 2006).

1.6 Vitamin D and Bone Health

The classical role of vitamin D is to maintain adequate levels of serum calcium and phosphorus for bone mineralisation. In this way, vitamin D prevents the development of rickets in children and osteomalacia in adults.

Activated vitamin D [$1,25(OH)_2D$] stimulates the active transport of calcium and phosphate from the lumen of the intestine into the blood (Schachter et al., 1963; Walling et al., 1977). It mediates this by increasing the expression of the epithelial calcium channel (EcaC) and the calcium binding protein Calbindin 9K (Plum & DeLuca, 2010). Correcting vitamin D deficiency increases intestinal absorption of calcium from about 10-15% to 30-40%. It also increases phosphorus absorption from about 60% to 80% (Holick , 2007).

$1,25(OH)_2D$ also increases the expression of RANKL (receptor activator nuclear kappa ligand) by osteoblasts which on binding to RANK receptors on pre-osteoclasts induces their maturation to osteoclasts. Mature osteoclasts remove calcium and phosphorus from bone maintaining homeostatic serum calcium and phosphorus (Holick, 2007).

Vitamin D deficiency by causing impaired calcium absorption leads to reduced serum ionized calcium. This causes an increase in PTH secretion (secondary hyperparathyroidism) to maintain normal serum calcium levels. PTH increases urinary calcium absorption and bone breakdown releasing calcium in to the bloodstream. In this way, vitamin D deficiency may help to cause or exacerbate osteopaenia or osteoporosis. In addition, inadequate calcium and phosphate may lead

to poor bone mineralisation which will cause osteomalacia in adults and rickets in children (Holick, 2007).

1.7 Vitamin D and Non-Skeletal Health Outcomes

Vitamin D and Cancer

In the early 1980's it was proposed that low vitamin D status from UVB exposure might explain the association established in the 1930's between higher latitudes and greater mortality from several different cancers (Garland et al., 1980). Numerous laboratory based and populations studies (cross sectional, longitudinal and randomised controlled trials) have since been performed linking vitamin D to over a dozen cancers (Garland et al., 2009). The role of vitamin D in cancer prevention still however, remains unproven (Rosen et al., 2012).

Vitamin D, Cardiovascular Disease and Mortality

Several studies and large meta-analyses have found an inverse relationship between vitamin D status and mortality (Bjelakovic et al., 2011; Schottker et al., 2012; Saliba et al., 2012; Zittermann et al., 2012). There are multiple potential mechanisms by which this could be mediated including cardiovascular disease, cancer and dementia. Vitamin D has been inversely associated with atrial fibrillation (Demir et al., 2012), heart failure (Pourdjabbar et al., 2013), stroke (Sun et al., 2012), coronary heart disease (Robinson-Cohen et al., 2013), peripheral arterial disease (Chua et al., 2011) and cerebrovascular disease (Chowdury et al., 2012). It is also associated with vascular risk factors including diabetes (Forouhi et al., 2012), hypertension (Tamez et al., 2012), dyslipidaemia (Jorde et al., 2011) and obesity (Earthman et al., 2012).

Vitamin D and other Diseases

Studies point to a potential role for vitamin D in Parkinson's disease, (Newmark et al., 2007), Alzheimer's disease (Lu'o'ng, K. V. et al., 2011) and multiple sclerosis (Simon et al., 2012). In addition, vitamin D has also been associated with COPD and several autoimmune diseases (Székely et al., 2012) and has been implicated in cognitive dysfunction and depression. For a summary of the evidence linking vitamin D to cognition, blood pressure, falls and mood (see sections five, six and seven).

CHAPTER 2: VITAMIN D DEFICIENCY

2.1 Vitamin D Deficiency

No consensus has been established regarding the optimum level of serum 25(OH)D. In 2011, the US Institute of Medicine (IOM) concluded after a comprehensive literature review that most people had replete vitamin D status when the serum level was greater than or equal to 50 nmol/l. Their findings considered that 25(OH)D levels of 40 nmol/l and 50 nmol/l met the requirements of over half and 97.5% of the North American population respectively (Institute of Medicine, 2011).

These recommendations were based on a detailed review of vitamin D and several health outcomes. This included markers of bone health but also cancer and site specific neoplasms, cardiovascular disease, hypertension, diabetes, metabolic syndrome, autoimmune disorders, infectious diseases, falls and physical performance, neurospsychological function (cognition, depression and autism) and disorders of pregnancy (Ross et al., 2011).

Data available at the time only supported a causal role for vitamin D in skeletal disease and hence recommendations were based on the vitamin D level required for optimal bone health (including calcium absorption, PTH levels, bone mineral density and fracture risk). A small number of randomised controlled trials, lack of confounding and reverse causality were cited as factors in studies of vitamin D and non skeletal outcomes in their conclusion. They also found evidence of a 'U' shaped relationship for all cause mortality, fractures, falls, certain cancers and cardiovascular disease. While several studies have established that vitamin D is important in bone health the evidence for non-skeletal benefits is less clear.

The IOM did not find a beneficial role for higher vitamin D level in falls prevention. However, this conflicts with a meta-analysis of studies looking at the affect of vitamin D supplementation on falls in older adults which supported a minimum level of 60 nmol/l in falls reduction (Bischoff et al., 2010).

2.11 Vitamin D deficiency

It is generally considered that a 25(OH)D level below 50 nmol/l represents deficiency (Holick., 2007). This is based upon the fact that most studies show that secondary hyperparathyroidism and markers of bone disease are minimized when the level is above this (Chapuy et al., 1997; Thomas et al., 1998; Heaney et al., 2003). Maintaining a level of up to 50 nmol/l is also consistent with the most recent IOM recommendations. Serum 25(OH)D levels below 25nmol/l are known to cause rickets in children and osteomalacia in adults and so this is often considered to represent severe deficiency (Holick, 2007)

2.2 Vitamin D Insufficiency

As studies suggest a beneficial affect of higher 25(OH)D levels on falls prevention, bone and other health outcomes, many experts have defined vitamin D insufficiency as less than 75 nmol/l (Holick et al., 2007). This remains controversial though such a level does not appear to be associated with an increased risk of adverse health outcomes (Heaney et al., 2011; Holick et al., 2011). In addition, the statistical methodology used by the IOM in calculating the optimum level for bone health and falls reduction has been questioned (Maxmen 2011; Holick et al. 2012).

Serum vitamin D levels up to 75 nmol/l have been associated with more optimal PTH suppression, intestinal calcium absorption and bone mineral density in some studies. In a review of studies looking at fracture risk and vitamin D in 2012, it was suggested that a 25(OH)D level of at least 65 nmol/l is needed to reduce non-vertebral fractures and 75 nmol/l may be needed to lower hip fracture risk (Dawson-Hughes B, 2013). In 2005, a review of the literature by six experts stated that the optimal vitamin D level for bone health was greater than 75 nmol/l (Dawson Hughes et al., 2005).

A higher 25(OH)D level has been advocated by other experts. Heaney et al report that the distinction between deficiency and insufficiency is not useful or necessary and argues that levels below 120 nmol/l are associated with preventable disease and could be classified as deficient (Heaney et al., 2011). A risk review of vitamin D supplementation also suggested that a vitamin D level of about 75 –110 nmol/l should be attained for optimal health outcomes (Bichoff Ferrari et al., 2009). Similarly, a target range of 75 –100 nmol/l has also been recommended for multiple health

outcomes (Souberbielle et al., 2010). Levels above 75 nmol/l and up to 90-100 nmol/l were also found to be optimal in relation to various outcomes including falls, fractures, physical functioning, dental health, and colorectal cancer (Bischoff-Ferrari et al., 2006). However, in a recent large study of 422,822 subjects aged over 45 and followed up for 54 months, a 25(OH)D level between approximately 50-90 nmol/l was associated with the lowest risk of all cause mortality and acute coronary syndrome (Dror et al., 2013).

The concept of 'Vitamin D insufficiency' has been adopted by the US Endocrine Society which it defines as less than 75 nmol/l (Holick et al., 2012). The ESCEO (European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis) also recommends a minimum level of 75 nmol/l in fragile elderly who are increased risk of falls and fracture (Rizzoli et al., 2013).

2.3 Prevalence of Vitamin D Deficiency

It is estimated that one billion people worldwide have Vitamin D deficiency or insufficiency (Holick, 2007). Prevalence rates vary widely depending on the definition used and time of year when levels are checked (see Table 2.1). In a recent systematic review of 75 studies which included over 168,000 subjects in 44 countries, mean population 25(OH)D levels varied between 4.9-136.2 nmol/l. In addition, in 37.3% of studies mean level was in the deficient range (<50 nmol/l), (Hilger et al., 2013). Deficiency has been reported in healthy children, adolescents and adults. A high prevalence has been found in postmenopausal women, those with a history of osteoporosis or fracture, institutionalised elderly and in ethnic groups with increased skin pigmentation (Holick, 2007).

Some studies suggest that vitamin D insufficiency/deficiency is on the rise. The prevalence of vitamin D levels below 25 and 75 nmol/l trebled and nearly doubled respectively, in participants of the NHANES study comparing results in 1988-1994 with that in 2001-2004 (Ginde et al., 2009). In a survey of over 20,000 Americans also as part of the NHANES study, approximately 75% of adults had serum 25(OH)D levels below 75 nmol/l (Looker et al., 2008). Despite this, based on IOM criteria, the majority of the North American population have adequate vitamin D levels and vitamin D inadequacy could therefore be overestimated (Ross et al., 2011).

Table 2.1: Prevalence of vitamin D deficiency (< 50 nmol/l) in population studies.

Prevalence of vitamin D deficiency (< 50 nmol/l) in population studies				
Author	**Size**	**Population**	**Prevalence (%)**	**Other comments**
Choi et al, 2011	6925	Subjects in KNHANES study in Korea aged ≥ 10yrs	47.3 (M), 64.5 (F)	
Thuesen et al., 2012	6416	General population in Denmark aged 30-60 years	52.2	
McCullough et al., 2010	4723	Cancer free men and women from 10 cohorts	29 -82	Subjects in US, Asia
Forrest et al., 2011	4496	Subjects in NHANES study in US	41.6	
Hintzpeter et al., 2008	4030	Community dwelling in Germany aged 18-79 years	57.0 (M), 58.0 (F)	75% in those aged 65-79 yrs.
Rockell et al., 2006	2946	New Zealand aged ≥ 15 yrs	48.0	
Shea et al., 2011	2581	Community dwelling in US aged 70 – 81 years.	18 (whites), 54 (blacks)	
Hirani et al., 2010	2070	Community dwelling in England aged ≥ 65.	49.0 (M), 57.0 (F)	9-13% (< 25 nmol)
Klenk et al., 2013	1418	Community dwelling in Germany (aged ≥ 65 yrs).	78.8[a], 16.1[b]	Not taking supplements
Absoud et al., 2011	1102	Aged 4 –18 years in Great Britain	35.0	
Gonzalez Gross et al., 2012	1006	Healthy European adolescents	42.0	
Janssen et al., 2012	802	Subjects in Netherlands aged 40-80 years	36 (M), 51(F)	
Vierucci et al., 2013	652	Children and adolescents aged 2-21years in Italy	45.9	
Nanri et al., 2010	529	Community dwelling aged 21-67 years in Japan	46.7[c] 9.3[d]	
Menant et al., 2012	463	Community dwelling aged 70-90 years in Australia	21.0 (M), 44(F)	≤ 50 nmol/l

(M)- Male, (F) –Female, [a]Month of March, [b]Month of August , [c]Month of November, [d]Month of July, KNHANES – Korean National Health & Nutrition Examination Survey, NHANES - National Health & Nutrition Examination Survey (US).

Table 2.2: Prevalence of Vitamin D deficiency (< 50 nmol/l) in Ireland.

Prevalence of Vitamin D deficiency (< 50 nmol/l) in Ireland				
Author	**Size**	**Population**	**Prevalence (%)**	**Other Comments**
Cashman et al., 2013	1132	Adults aged ≥ 18 years	40.1	Year round figure
Hill et al., 2008	1015	12-15 years old in Northern Ireland	36.0	Adolescent group
Lardner et al., 2011	143	Community dwelling middle aged females	47.0	Not on supplements
Hill et al., 2006	95	Healthy postmenopausal females (51-75 years)	48.0	In Winter, not on supplements

2.4 Causes of Vitamin D deficiency

As approximately 90% of vitamin D is derived from cutaneous synthesis, lack of UVB exposure is attributed as the major cause (Holick, 2007). Several other factors are also associated with Vitamin D deficiency / insufficiency (See Table 2.4).

Cutaneous synthesis is affected by age, skin pigmentation, sunscreen use and UVB exposure which is determined by time spent outdoors, clothing, time of year, cloud cover, latitude, altitude and sun tanning practises. Factors associated with reduced bioavailability include malabsorption states, liver impairment and the use of medications which alter vitamin D metabolism or absorption. 25(OH)D levels are also strongly affected by diet and supplement use, whilst renal impairment will affect $1,25(OH)_2D$ levels. In addition, genetic factors appear to have a small influence on vitamin D status (Berry et al., 2011).

The relative importance of different factors may vary by season, location and ethnicity. For example, in the Winter dietary intake may play a more important role than sun exposure particularly in locations above 30° North Latitude and in those with more pigmented skin. The effect of other lifestyle factors on serum 25(OH)D also appears to be different across seasons (Nanri et al., 2011).

Listed below are the main determinants of vitamin D status and the proposed mechanisms by which they influence serum 25(OH)D.

2.41 Geophysical Factors

Several factors influence the amount of UVB light that is absorbed in the atmosphere and hence reaches the earth. These include the solar zenith angle, latitude, altitude and cloud cover (Webb et al., 1988). The zenith angle depends on the time of the day and season. It is more oblique in the early morning and late afternoon resulting in more attenuation of UVB and reduction in vitamin D synthesis by up to 20%. It is also estimated that cloud cover can block up to 99% of vitamin D production (Engelsen et al., 2005).

Season

Season has a significant affect on vitamin D levels, as during the Winter months the zenith angle is very low and nearly all UVB radiation is absorbed in the atmosphere. It is estimated that in locations which are above 35°N in latitude, there is little or no cutaneous production of vitamin D during the Winter months (Holick, 2007). This has given rise to the notion of a 'Vitamin D winter' with ineffective vitamin D synthesis between the months of November to March at locations of similar latitude to Ireland (Webb et al., 1988). Changes in UVB irradiation reaching the earth's surface leads to changes in serum 25(OH)D with a lag period of about 6-8 weeks. This may relate to the time required to reach steady state post UVB exposure This may explain why in most studies peak and trough vitamin D levels occur in Autumn and Spring respectively. The prevalence of deficiency is also greater in the Spring and Winter months (Klenk et al., 2013).

Other factors related to season also influence vitamin D status. For example, during the Winter in the Northern hemisphere people also spend less time outside and wear more clothing which prevents skin exposure to UVB light (Matsuoko et al., 1992). Seasonal changes in cloud cover and hence atmospheric absorption of UVB radiation may also account for lower levels in the Winter (Bolland et al., 2010). The mean seasonal variation in serum 25(OH)D in studies ranges from approximately 10-20 nmol/l (van der Mei et al., 2007). This variation may be attenuated in those who are supplemented (Romano Oturno et al., 2011; Whiting et al., 2011).

Altitude / Latitude

Increasing altitude has been associated with increased production of vitamin D in the skin. This may be explained by the reduced attenuation of UVB as it passes through a shorter distance of atmosphere (Chen et al., 2010).

Higher latitude is associated with lower vitamin D status, reflecting the greater distance UVB has to travel to reach the earth's surface. A 1° increase in latitude has been associated with a 1 nmol/l reduction in 25(OH)D in subjects living between 38° – 48° S in the southern hemisphere (van der Mei et al., 2007). In the UK, the odds of vitamin D insufficiency were greater in Northern versus Southern England after adjustment for several covariates (OR 2.13, P <0.001) (Hypponnen et al., 2007). In a

meta-analysis of several studies, greater latitude was also associated with lower vitamin D though only in Caucasians (Hagenau et al., 2009).

2.42 Lifestyle Factors

Sun Exposure

Lack of adequate sun exposure is attributed as the main cause of vitamin D deficiency. The dose equivalent of vitamin D for those receiving abundant sun exposure is estimated to be up to 4000 IU - 20,000 IU per day (Barger Lux et al., 2002). Even exposure of the hands, arms and legs to UVB for approximately 20 minutes or a time sufficient to cause the skin go pink (one erythemal dose) is equivalent to consuming about 4000 IU of vitamin D (Holick et al., 2007).

Sunscreen has been shown to reduce vitamin D production by up to 95% and clothing also blocks UVB irradiation (Matsuoko et al., 1992). The nature and type of clothing worn will also affect the sun protection factor (SBF) provided. An SPF ranging between two and one thousand has been identified between different clothing items (Robson et al., 1990). Clothing practises in some countries may also affect vitamin D status. For example, veiled woman have been found to have lower vitamin D status than their peers in the same country (Al Attia et al., 2012). Conversely, those who use tanning beds are more likely to have higher serum 25(OH)D levels (Tangpricha et al., 2004; Van der Meer et al 2008; Wallingford et al., 2013) on account of direct UVB exposure. Time spent outdoors and hours of UVB exposure are both associated with higher vitamin D status (Brustad et al., 2004; Hyponnen et al., 2007). Similarly, sun holiday travel and sun avoidance is associated with higher and lower vitamin D status respectively (Al Anouti et al., 2011; Mavroeidi et al., 2013).

Dietary Intake

The overall contribution of diet to vitamin D status is small as up to 95% of the body's vitamin D requirement comes from the synthesis in the epidermis from sun exposure (NIH, 2007). Dietary sources of vitamin D are limited (Holick, 2007) with most foods containing little or no vitamin D. Naturally occurring sources of vitamin D2 (ergosterol) include yeast products and mushrooms and of vitamin D3

(cholecalciferol), oily fish and egg yolks (See Table 2.41 for Vitamin D content of foods). Vitamin D2 and D3 are also found in fortified foods and supplements.

Several studies show a positive relationship between dietary intake and/or supplement use on vitamin D status (Hyppönen et al., 2007; Scragg et al., 2008; Pasco et al., 2009; Brock et al., 2010; Shea et al., 2011). The total average adult dietary consumption ranges from about 150-300 IU per day in population studies (Moore et al. 2004). Intake has been found to vary with gender, age and national fortification and supplementation policies (Calvos et al., 2005).

Dietary Intake from food

In Ireland, the intake of vitamin D has been reported to be 136 IU /day from food (Hill et al., 2006). This is substantially less than the current RDA requirement (400 - 600 IU daily) set by the Institute of Medicine in the US, highlighting the small contribution that diet makes to overall vitamin D status. Data on vitamin D intakes from two national nutrition surveys in Ireland have suggested that meat and meat products, fish and fish products, and egg and egg-dish foods account for 30%, 12–14%, and 9% of the respective mean daily intake of vitamin D in 18 – 64 year olds (Cashman et al., 2012).

Differences in dietary food intake have been correlated with serum vitamin D status. For example, a vegetarian diet has been identified as a risk factor for vitamin D deficiency in the UK, which may relate to reduced intake of fish and/or eggs (Davey et al., 2003). Conversely, oily fish intake has been positively correlated with vitamin D in several cross sectional studies (Hyponnen et al., 2007; van Dam et al., 2007; van der Meer et al., 2008; Nanri et al., 2011) Some of the difference in dietary intake can be explained by differences in cultural eating practises. For example, fish accounts for about 60-72 IU of the daily intake in Norway (Jorde et al., 2000) but about 256 IU in older Japanese women (Nakamura et al., 2002).

Fortified Foods

In come countries such as Canada, fortification of certain foods including milk is mandatory (Calvos et al., 2004) though in general fortification is less widespread in Western Europe aside from margarine (Laird et al., 2010). In Ireland, vitamin D

fortified milk is available (Supermilk©) and contains approximately 80 IU per 200 ml. Even in countries where there is fortification, vitamin D intake has been found to be low in certain groups (Calvos et al., 2005). However, vitamin D food fortification has been shown to increase serum 25(OH)D), (O'Donnell et al., 2008; Black et al., 2012).

The contribution of supplements to overall vitamin D intake varies widely and was found to be between 6 - 47% of the average vitamin D intake in a global review that included several countries (Calvos et al., 2005). Supplement use added about 160-200 IU to the total daily intake of vitamin D for women and men over aged 50 in the NHANES 1999-2000 study (Whitig et al., 2007). It also added about 68 IU to the daily intake of vitamin D in Ireland in a small study (Hill et al., 2006).

Many over the counter supplements have small amounts of vitamin D, However, prescribed supplements used in the treatment of osteopaenia/osteoporosis (Calcichew D3 forte©, Ideas©, Osteofos©, Desunin©) contain greater amounts (400IU-800 IU per tablet) and therefore could contribute to most or all of the daily vitamin D need depending on compliance and the target serum level desirable.

Medication use

Medications that alter vitamin D metabolism or impair absorption can affect vitamin D levels. Few medications are known to impair vitamin D absorption, though both cholesytramine and orlistat have been implicated as cause of low vitamin D status via this mechanism (Compston et al., 1978; McDuffie et al., 2002).

Vitamin D metabolism may be altered by several medications, particularly those that are ligands for the Pregnane X Receptor (PXR) (Grober et al., 2011). PXR is found in renal, hepatic and gastrointestinal cells and plays an important role in detoxifying drugs. It has a 60% homology with DNA binding domains on the VDR which means that it can bind to VDRE's and can affect the expression of genes that can be otherwise regulated by vitamin D. Drugs that are ligands for PXR can up regulate expression of 24-hydroxylase that leads to the degradation of 25(OH)D and $1,25(OH)_2D$. PXR ligands also induce other enzymes such as CYP2C9 and CYP3A4 which alter vitamin D metabolism. Drugs that activate the RXR receptor and lower

25(OH)D levels include anti-epileptic medications (phenytoin, carbamazepine), steroids, some anti-hypertensives and antiretroviral drugs. Phenobarbitone is also known to suppress 25 hydroxylation (Hosseinpour et al., 2007) while oestrogens increases activity of 1-α-hydroxylase responsible for conversion of 25(OH)D to the biologically active $1,25(OH)_2D$ (Grober et al., 2011).

Smoking

Vitamin D levels have been found to be lower in smokers in some studies (Brot et al., 1999; van Dam et al., 2007; Pasco et al., 2009; Nanri et al., 2011;Yoshimura et al., 2013) but no clear causation has been established. It has been proposed that altered hepatic metabolism of vitamin D may explain this finding, though there is little evidence to support this (Brot et al., 1999). Smoking may simply be a surrogate marker for other factors that negatively influence vitamin D.

2.43 Biological Factors

Age

Skin thickness reduces with age and this is correlated with a decrease in epidermal levels of 7-dehydrocholesterol and the capacity of the skin to produce vitamin D3 (MacLaughlin et al., 1985). Older adults may be three times less affective at increasing 25(OH)D levels following UVB exposure (Holick et al., 1989). At the age of 70 years, despite similar sunlight exposure vitamin D synthesis has been reported to be approximately 75% less than that of a twenty year old (Mithal a et al., 2009). Numerous studies have also shown lower serum vitamin D with increasing age (van Dam et al., 2007). In addition, there are several lifestyle factors in older age that may independently affect vitamin D levels. These include sun exposure, diet, medication use and medical illness such as liver or renal disease. Physical frailty which is more prevalent with increasing age is also associated with lower vitamin D status (Wilhelm-Leen et al, 2010; Wong et al., 2013). Conversely, several studies show increased vitamin D in those who are more physically active (Scragg et al., 2008: Pasco et al., 2009: Brock et al., 2010),

Table 2.41: Vitamin D content of Foods

Source	Vitamin D content (IU)
Fish /Fish oils	
Cod liver oil	400-1000 IU / teaspoon
Cod	100 IU / 100g
Salmon (wild)	600-1000 IU / 100g
Salmon (farmed)	100-250 IU / 100g
Salmon (canned)	300-600 IU / 100g
Trout (farmed)	250 IU / 100g
Sardines (canned)	300 IU / 100g
Mackerel (canned)	250 IU / 100g
Tuna (canned)	236 IU / 100g
Mushrooms	
Shiitake (fresh)	100 IU / 100g
Shiitake (sun dried)	1600 IU / 100g
Portabella	400 IU / 100g
Egg yolk	20-40 IU / yolk
Meat	
Liver (lamb)	36 IU / 100g
Kidney (lamb)	24 IU / 100g
Pork	30 IU / 100g
Turkey	100 IU / 100g
Fortified Foods	
Supermilk©	80 IU / 200 ml
Low fat spread (Flora©)	300 IU / 100g
Breakfast cereals (Kelloggs Special K©)	100 IU / 35g
Supplements	
Calcichew D3 Forte / Osteofos D3 / Ideas	400 IU / tablet
Desunin©	800 IU / tablet
References: *Vitamin D Deficiency*, Holick MT, 2007, NEJM; *Vitamin D, Physiology, Molecular Biology and Clinical Applications* (2nd edition, published 2010): Chapter 18, Determinants of vitamin D intake.	

Gender

There are conflicting results from many studies that have found gender differences in serum vitamin D levels. Lower vitamin D status has been associated with female gender in some (Hyponnen et al., 2007; Scragg et al., 2008) but not all studies (Hintzpeter et al., 2008; Brock et al., 2010). In addition, a recent systematic review of

vitamin D status in several populations across the world found no gender difference (Hilger et al., 2013). The observed findings in some studies may reflect lifestyle and biophysical differences between both genders. For example, lower serum vitamin D in females was largely explained by their greater body fat in one study (van Dam et al., 2007)

Skin Pigmentation

Skin pigmentation can reduce cutaneous synthesis by as much 99.9% (Clemens et al., 1982). This is because melanin absorbs solar radiation from 290 –700nm (in the range of UVB) thereby reducing cutaneous synthesis of previtamin D3. Those with skin type III-IV require twice the UVB exposure per vitamin D synthesised (Clemens et al., 1982).

Adiposity

As vitamin D is lipophilic, it is postulated that sequestration in fat cells may accounts for lower serum levels (Holick et al., 2006). Storage of vitamin D in adipose tissues in humans has been reported to be as high as 5000 IU per kilogram (Lawson et al., 1986). Several studies show that different measures of adiposity are negatively correlated with serum 25(OH)D including body mass index (BMI) (Jorde et al., 2010), waist circumference (Snidjer et al., 2005), percentage body fat (Arunabh et al., 2003) and visceral and cutaneous fat (Kremer et al., 2009; Dong et al., 2010; Caron-Jobin et al., 2011),

Recent meta-analysis of 34 studies which included healthy adults revealed an overall negative correlation between BMI and 25(OH)D except for women living in developing countries (Saneei et al., 2013). In one of the largest studies to date that identified an inverse relationship (N=10,229), changes in BMI were found to be a significant negative predictor of change in vitamin D status at several years follow up (Jorde et al.,2010). Studies have also shown a negative correlation between vitamin D levels post irradiation or supplement use and BMI (Wortsman et al., 2000; Dong et al., 2010; Forsythe et al., 2012).

Malabsorption States

As vitamin D absorption occurs in the ileum and jejunum (Bikle et al., 2007) disorders of the small bowel including coeliac and Crohn's disease can lead to malabsorption and lower vitamin D status (Avioli et al., 1969; Driscoll et al., 1982). Coeliac disease is in fact considered to be a silent cause of vitamin D deficiency (Holick, 2010) and in particular active Crohn's disease is associated with lower 25(OH)D (Jorgensen et al., 2013).

Liver and Renal Disease

Severe liver disease will impair 25-hydroxlyation preventing the formation of 25(OH)D and renal impairment may lead to reduced activation of 25(OH)D to $1,25(OH)_2D$ (Holick et al., 2007). Several studies have also found lower 25(OH)D levels in subjects with renal disease though it unclear as to how this may be mediated and it could be due to other surrogate factors (Liu et al., 2013).

2.44 Genetic Factors and Vitamin D

There is some evidence to suggest that genetics may explain a small variation in serum 25(OH)D. Studies have investigated for associations between serum vitamin D and single nuclear polmymorphisms (SNP's) in common vitamin D pathway genes. These include genes that encode Vitamin D Binding Protein (VDP), 25-Hydroxylase (CYP2B1) and 1α hydroxylase (CYP27B).

Genetic variations in the VDP gene have been associated with serum 25(OH)D (Lauridsen et al., 2005; Engelman et al., 2008; Sinnotte et al., 2009; Fu et al., 2009) and response to supplementation (Nimitphong et al., 2013).

Variants in SNP's in the gene encoding VDP and 25-Hydroxylase (CYP2R1) were also associated with serum 25(OH)D levels and clinical deficiency (Ahn et al., 2010). Likewise, variants in three genetic loci near genes involved in hydroxylation and vitamin D transport were linked to an increased risk of having vitamin D insufficiency (Wang et al., 2010). A genetic variant in the VDBP was associated with vitamin D insufficiency in 712 southern Chinese women (Cheung et al., 2013).

The overall affect of genetic factors on serum vitamin D is small compared with environmental exposure (Berry et al., 2011). For example, approximately 5% of the variation in 25(OH)D in Afro-Americans has been linked to a genotype score representing risk alleles across three SNP's for the gene encoding VDP and CYP27B1 (Signorello et al., 2011).

Table 2.42: Major causes of Vitamin D Deficiency

Major Causes of Vitamin Deficiency	
Reduced Skin Synthesis	**Effect**
Sunscreen Use - absorption of UVB radiation	Reduces vitamin D3 synthesis in skin: SPF 8 by 92.5%
Skin pigment - absorption of UVB	Reduces vitamin D3 synthesis by as much as 99%
Aging – reduction of 7-dehydrocholesterol in the skin	Reduces vitamin D3 synthesis by about 75% in 70 year old
Season, latitude and time of day	Above 35° N latitude, little vitamin D3 produced from Nov to Feb
Skin graft or burns - reduction in 7-DHC in skin	Reduced synthesis of vitamin D3
Decreased Bioavailability	
Malabsorption – Coeliac disease, Whipples disease, Crohn's disease, Cystic Fibrosis, bypass surgery, medications that reduce cholesterol absorption	Impairs absorption of vitamin D from bowel
Obesity – sequestration of vitamin D in fat	Reduced availability of vitamin D
Increased Catabolism	
Medications - Anticonvulsants, glucocorticoids, antiretroviral and anti-rejection medications	Activates the destruction of 25(OH)D and $1,25(OH)_2D$
Decreased synthesis of 25-hydroxyvitamin D	
Liver failure Mild to moderate dysfunction	Malabsorption of vitamin D but not production of 25(OH)D
Dysfunction of 90% or more	Inability to make 25(OH)D
Increased urinary loss of 25-hydroxyvitamin D	
Nephrotic Syndrome Loss of 25- hydoxyvitamin D bound to vitamin D binding protein in urine	Substantial loss of 25(OH)D to urine
Decreased Synthesis of 1,25 dihdroxyvitamin D	
Chronic kidney disease Stage 2-3 (GFR 31-89 ml/min/1.73m^{-3}	Reduction in $1,25(OH)_2D$
Stage 4-5 (GFR <30 ml/min/1.73m^{-3}	Inability to produce adequate amounts of $1,25(OH)_2D$

Adapted from Vitamin D Deficiency; Holick MF., 2007, New England Journal Medicine.

2.5 Assessment of Vitamin D Status

Serum 25-hydroxyvitamin D is the only reliable measure of vitamin D status (Holick et al., 2007). Levels reflect both vitamin D obtained from ingestion and cutaneous synthesis. Serum $1,25(OH)_2D$ is not indicative of vitamin D status as it is tightly regulated such that the level usually remains within normal range. Indeed, serum 25(OH)D levels correlate directly with vitamin D but not 1,25(OH)D. This is because when 25(OH)D levels drop, increases in PTH maintain it's level by increasing 1α-hydroxylation. In addition, it has a short half-life (4-6 hrs) making interpretation of levels difficult (Holick et al., 2009). For this reason, 25(OH)D and not $1,25(OH)_2D$ is recommended for measuring vitamin D status by the Food Nutrition Board of the Institute of Medicine.

25- Hydroxyvitamin D Measurement

The predominant mode of measurement of 25(OH)D is by immunoassay. Choice of assay used by labs may depend on cost, turn around time, convenience, specificity and accuracy. Some laboratories maintain quality assurance through the DEQAS (Vitamin D External Quality Assessment Scheme) which was founded in 1989 to compare vitamin D assay performance (Fraser et al., 2013).

The DiaSorin© radioimmunoassay is commonly used to determine 25(OH)D status. However, it also recognises 24,25(OH)D and to some extent other metabolites and can overestimate 25(OH)D by 10-20%. The IDS (a more recent radioimmunoassay) has been developed which has a specificity of 100% and 75% for 25(OH)D3 and 25(OH)D2 respectively (Holick, 2009).

The current gold standard for measurement of 25(OH)D is liquid chromatography tandem mass spectroscopy (LCMS). The removal of interfering Vitamin D metabolites by chromatography combined with spectroscopy allows for a direct and quantitative measurement of 25(OH)D2 and 25(OH)D3 (Holick et al., 2009).

2.6 Screening for Vitamin D deficiency

There are no guidelines that support universal screening for vitamin D deficiency / insufficiency. However, serum 25(OH)D should be checked in those with a diagnosis of osteoporosis or osteopaenia where a target level of 50nmol/l is recommended. Whilst no consensus has been established with regard to checking vitamin D status in other groups, some experts advise that it should be considered in those at high risk of vitamin D insufficiency/deficiency (Holick, 2010). This includes those with malabsorption states such as coeliac and inflammatory bowel disease, liver and renal disease, pregnant and lactating women, veiled, pigmented immigrant women, vegetarians and those with minimal sun exposure.

Predictive models for Vitamin D

No models to date have been created that reliably predict vitamin D status. The existence of a method to estimate serum 25(OH)D might be useful in identifying at risk groups without needing to formally do blood tests. Peiris et al attempted to create a predictive model for vitamin D deficiency but only correctly identified one third of those who were deficient (Peiris et al., 2011). However, it largely looked at blood parameters and basic demographics and failed to take account of the most important determinant ultraviolet B light exposure. A prediction model consisting of dietary and supplement intake, outdoor physical activity, tanning bed use, smoking and month of measurement was found to explain 40.1% of the variance in serum 25(OH)D in a study of 1494 pregnant women (Bjorn Jensen et al., 2013). Whilst the model was felt to be useful for examining associations between vitamin D and disease in the cohort, only 32.2% were placed in the same quintile when compared to measured serum levels.

Value of once-off Vitamin D levels

The extent to which a single 25(OH)D measurement reflects long-term vitamin D status is unclear. As 25(OH)D has a half life of about 3 weeks, levels may simply reflect recent sun exposure and dietary habits. In addition, changes in lifestyle factors over time could result in differences in serum vitamin D even when measured at the same time of year.

Some studies have reported on the measured change in vitamin D levels at between 3-5 year follow up. In a comparison analysis of 8881 subjects (not on long-term supplements) who had vitamin D levels taken in the same month of the year but on two different occasions between 2008-2011, levels were considered to be relatively stable with a small difference of 5 nmol/l at follow up ($r = 0.63$). The correlation did however, decrease with time ($r = 0.83$ after 1 year and $r = 0.55$ after 3 years), (Saliba et al., 2012).

In a smaller analysis of 672 post menopausal women in the Women's Health Initiative (WHI), a comparison of two measures of 25(OH)D status collected about 5 years apart revealed an mean increase by 7.8 nmol/l ($r = 0.61$). The difference was less for subjects with deficient versus sufficient vitamin D status at baseline (Meng et al., 2012) but still supported the use of once off measurement.

2.7 Prevention / Treatment of Vitamin D Deficiency

Recommended Daily Vitamin D (RDA) Intake

The RDA for vitamin D intake will depend on what is considered the most appropriate level desirable for optimal health outcomes. The first recommendation for vitamin D intake for Americans was in 1941 and advised on 400 IU daily for adults. This was based on the observation that a similar amount found in one teaspoon of cod liver oil could prevent rickets (Park, 1940). The most recent update in 2011, by the US Institute of Medicine (IOM) recommended an increase in the daily dietary vitamin D intake to 600 IU and 800 IU respectively for those aged between 51-70 and above 70 years of age. This assumed a setting of minimal sun exposure and was predicated on achieving a 25(OH)D level of 50 nmol/l. Guidelines were based on higher quality studies than 1997 leading to an increase in RDA. Dietary Reference Intakes were based on those required for optimal bone health outcomes (including calcium absorption, PTH levels, bone mineral density and fracture risk).

Tolerable Upper Intake

The Tolerable Upper Intake (TUI) is the highest daily intake that is likely to pose no risk. The IOM have recommended a TUI for vitamin D of 4000 IU per day. Whilst 10,000 IU was taken as a starting point the recommendation was based on

maintaining a blood levels of less than 150 nmol/l and factored in uncertain long term effects of higher intake on chronic disease and mortality. However, it is known that consumption of 10,000 IU daily for 5 months is associated with no toxicity (Holick et al, 2007).

Vitamin D Supplementation

It has been reported that for every 100 IU of vitamin D supplemented there is a corresponding mean rise in serum 25(OH)D of approximately 2.5 nmol/l (Heaney et al., 2005). However, in a recent meta-analysis of studies that included Caucasian subjects aged over 50 who were not taking calcium supplements, an equivalent mean rise of 4.9 nmol/l was calculated per 100 IU of daily vitamin D consumed (Autier et al., 2012). In addition, in a review of 41 studies the mean rise per 100 IU intake per day was 5.3 nmol/l (95% CI 4.4 –6.2), (Mc Kenna et al., 2013). As vitamin D has a half-life of about 3 weeks, supplementation with 2000 IU per day over a 3 month period would be expected to increase levels by at least 50 nmol/l. This corresponds to an approximate accumulative dose of 180,000 IU and should therefore be adequate to treat severe deficiency (<25 nmol/l). This however, assumes minimal sun exposure and so dosing requirements are likely to be less in the Summer months. Differences in recommendations for supplement dosage as advised by the IOM versus other bodies may be related to a greater focus on guidance for food fortification and general population use as opposed to tailored treatment for the individual.

Vitamin D Dosing Regimens

There are several different dosing regimens recommended by experts or expert organisations. Treatment dose will ultimately depend on the baseline serum vitamin D level and desired post treatment target range.

A regimen consisting of 50,000 IU per week for eight weeks followed by the same dose every 2 weeks after has been advocated by some experts (Holick et al., 2007). The US Endocrine Society's Clinical Practise Guideline suggest a dose of 1500-2000 IU per day in adults aged 19 or older to maintain serum 25(OH)D above 75 nmol/l (Pramyothin et al., 2012).

Others have suggested that an average older adult needs an oral intake of 800-1000 IU per day to achieve a serum 25(OH)D of 75 nmol/l (Dawson Hughes et al., 2005). A dose of between 1800-4000 IU /day has also been suggested to bring the majority of adults to a serum range above 75nmol/l (Bischoff-Ferrari et al., 2009). To maintain a level of above 50 nmol/l, a daily intake of 930 IU per day was predicted in a model that included a 95% range and accounted for inter-individual variability (Cashman et al., 2011).

However, to achieve a higher vitamin D status at a population level a higher daily dose may be required. For example, to maintain a 25(OH)D of 100 nmol/l in 97.5% of the population, a daily dose of 9600 IU has been proposed (Garland et al., 2011).

Factors Determining Effect of Vitamin D Supplementation

Several factors may influence the response to supplementation. These include baseline vitamin D level, preparation of supplement and type of vitamin D used, route and schedule of administration, body mass index, liver and renal function and medication use. Genetic factors may also affect response to treatment.

Baseline 25(OH)D status

Response to treatment may be greater in those with a lower baseline vitamin D but studies are limited. In one study of 10,229 subjects who were supplemented with vitamin D, the rise in 25(OH)D level was significantly and inversely related to baseline levels (Jorde et al., 2010). Similarly, in a review of three studies of vitamin D supplementation, those with the lowest baseline 25(OH)D had the highest increase (Didriksen et al., 2013). Aloia et al showed a nonlinear dose response relationship between vitamin D intake and serum 25(OH)D with a lower rate of increase at higher levels of intake (Aloia et al., 2008).

Supplement vehicle substance

The vehicle substance used in supplements may affect the bioavailability of vitamin D. In a review of four studies of supplementation of healthy subjects with vitamin D, an oil vehicle produced a greater serum 25(OH)D response than when used in a powder or ethanol form (Grossmann et al., 2010).

Adiposity

The effect of supplementation on plasma 25(OH)D concentrations has been found to be negatively modulated by adiposity as previously discussed (Dong et al., 2010; Didriksen A et al., 2013). It is proposed that vitamin D sequestration in the fatty tissue post supplementation may account for this.

Genetic Factors

Genetic variations in the gene encoding VDP may affect response to supplementation (Nimitphong et al., 2013;Didriksen et al., 2013). However, few studies have examined for the effects of genetics in this regard.

Vitamin D2 or D3

The effect of vitamin D2 (ergocalciferol) versus vitamin D3 (cholecalciferol) at increasing the levels of 25(OH)D appears to depend on the frequency of administration as opposed to the actual dose used (Holick et al., 2008). As vitamin D2 appears to have a higher rate of degradation, it requires more frequent dosing (Holick et al., 2008). However, it is unclear whether activated vitamin D3 [$1,25(OH)_2D3$] is more effective at a biological and receptor level than activated vitamin D2 [$1,25(OH)_2D2$]. As our bodies have evolved to synthesize vitamin D3, there is a biological plausibility that it is more potent.

Vitamin D2 was first produced in the early 1920's through ultraviolet exposure of food, a process that was patented and licensed by pharmaceutical companies in the US. Vitamin D2 is now widely used form of vitamin D available in the US today whereas vitamin D3 is more commonly used in Europe. Whilst it was initially thought that vitamin D2 and D3 were equipotent, as early as 1930 it was suggested that the activity of cod liver oil (D3) was different than commercially available D2 (Hess et al., 1930).

It was noted that one unit of cod liver oil was as effective at preventing rickets as four units of vitamin D2 (Visoterol). Whilst over 40 studies were conducted in the ten years following this, results were inconclusive (Park, 1940). In 1949, the World Health Organisation made no distinction between vitamin D2 or D3. However, it was

only in 1972, that one IU of vitamin D was defined in molar terms. This meant that although both molecules have different molecular weights, one IU of D3 and D2 now equated to the same molecular amount of vitamin D (Norman et al., 1972). This allowed for an equal comparison of vitamin D2 and D3 doses used in studies.

Most studies now suggest that provided dosing is regular or daily the efficacy of using vitamin D2 or D3 in raising 25(OH)D levels is similar. This is borne out by a recent meta-analysis of 10 randomised controlled trials. Though vitamin D3 was significantly better at raising 25(OH)D concentration than vitamin D2, the affect existed only for bolus dosing and was lost for daily supplementation (Tripkovic et al., 2012) .

The controversy of D2 versus D3 may stem from the faster clearance of D2 when given as a large dose (Armas et al., 2004). For example, a once off dose of vitamin D2 (50,000 IU) in as study of 20 healthy males was found to be about one third as potent as D3 in maintaining 25(OH)D levels based on area under curve analysis.. Though there was a similar initial increase in 25(OH)D compared with same dose D3, the concentration fell off rapidly after 3 days before returning to baseline at two weeks (Armas et al., 2004). A higher rate of degradation of ergocalciferol versus cholecalciferol was also noted following discontinuation of treatment after 12 weeks with 50,000 IU per week (Heaney et al., 2011).

There has however, been studies which have found daily supplementation with D3 to be more efficacious than D2 at raising 25(OH)D levels. Trang et al., found that supplementation with 4000 IU of oral D3 over a 2 week period was about twice as effective at increasing 25(OH)D levels than oral D2, though the study number was small (Trang et al., 1998). Mastaglia et al., also reported that 2.5 times the daily dose of vitamin D2 was required to achieve similar 25(OH)D levels compared to using D3 (Mastaglia et al., 2006). Similarly, vitamin D3 given at the same dose as D2 either daily or monthly also had a small but significantly greater affect at increasing 25(OH)D levels in 64 community dwelling adults aged 64 or older over a one year period (Binkley et al., 2011).

Biological Differences in Vitamin D3 & D2

Differences in hepatic hydroxylation, binding to VDP, and direct metabolisation of D2 to 24(OH)D may explain some of the differences in the effects of vitamin D2 versus vitamin D3 (Houghton et al., 2006). The weaker binding affinity of vitamin D2 metabolites to DBP may account for it's shorter circulating half life (Houghton et al., 2006) and may be potentially explained by the presence of the methyl group at carbon 24 (Hollis, 1984). Hepatic 25-hydroxylase may convert vitamin D3 to 25(OH)D at a faster rate than D2 which might account for D3 reputed increased potency (Holmberg et al., 1986). Further hydroxylation in the kidney leads to the formation of, $1,24,25(OH)_2D3$, though this may be a form in which D2 and not D3 is inactive (Horst et al., 1986). 1,24,25(OH)D3 retains its capacity to bind to the VDR (Houghton et al., 2006) and needs to be further oxidised for deactivation (Horst et al., 1986). In addition, it is believed that 24-hydroxlation of D2 but not D3 may also occur directly in the liver thereby leading downstream to $[1,24(OH)_2D]$ which has less affinity for the VDR (Houghton et al., 2006).

Method of Administration

The method of administration and dose used will affect the time required to reach a specific steady state serum level. Vitamin D2 or D3 can be given in tablet form or by intramuscular injection. Synthetic vitamin D analogues have also been administered intravenously in experimental studies. Dosing regimes vary from daily to weekly to monthly. It may be more optimal to give parenteral preparations to those who have disorders of malabsorption or where medication compliance may be an issue.

Vitamin D3 is available as a prescription drug (in a combined preparation with calcium carbonate) licensed for use as a phosphate binder and for the treatment of osteoporosis/osteopaenia (Calcichew©, Calcichew D3 forte©, Ideas©, Osteofos©, Calcipios©). It is also available in Ireland in prescribed tablet form (Denusin©) and as an unregulated over the counter supplement.

2.8 Vitamin D Toxicity

Vitamin D intoxication is observed when 25(OH)D levels are greater than 225-300 nmol/l (Holick, 2007) and is rare (Barne et al., 1957; Araki et al., 2011) Serum levels

of up to 2400 nmol/l have been reported in cases of extreme toxicity (Vieth et al., 2002). While circulating levels of $1,25(OH)_2D$ are not increased much in toxicity, it is likely that at high concentrations of 25(OH)D may bind to VDR contributing to the toxic effects (Lou et al., 2010). Toxicity is generally associated with the intake of large amounts of vitamin D. The toxic dose of vitamin D is estimated to be greater than 100,000 IU per day for a duration of at least one month (Vieth, 1999).

Vitamin D toxicity is not believed to result form sun exposure (Holick et al., 1981). In fact, vitamin D levels of up to 235 nmol/l without hypercalcaemia or hypercalcuria have been identified in subjects with sun exposure only (Vieth et al., 1999; Kimball et al., 2007) suggesting it may represent a normal and safe physiologic level.

The absence of any toxicity including hypercalcaemia at a serum 25(OH)D levels up to 250 nmol/l in trials of vitamin D supplementation also suggests that this may be considered a safe upper limit (Bishoff-Ferarri et al., 2010; Hathcock et al., 2007). However, the emergence of a 'U' shaped curve for vitamin D and health outcomes including cardiovascular disease, falls and bone health raises the possibility that long term levels above 125 nmol/l may be injurious. In addition, higher serum levels may increase the risk of renal calculi (IOM, 2011).

Signs and Symptoms of Vitamin D Toxicity

The clinical signs and symptoms of excess vitamin D are due to the effects of hypercalcaemia including nausea and polyuria (Araki et al., 2011).

Management of Vitamin D Toxicity

This involves treatment of hypercalcaemia with intravenous fluids, administration of bisphosphonates or calcitonin (to inhibit bone breakdown) and glucocorticoids which appear to suppress 1α hydroxylase conversion to the active vitamin D (Ozkan et al., 2012). After discontinuation of of vitamin D, the decline in serum levels occurs with a half life of about two monhts (Vieth et al., 2002) so high levels may take several months to normalise (Ozkan et al., 2012).

2.9 Conclusions

Vitamin D has a proven role in maintaining bone health and preventing rickets in children and osteomalacia in adults. Vitamin D is unique amongst vitamins in that approximately 90% is synthesized by our bodies in response to sunlight rather than obtained from our diet. Furthermore, vitamin D is a secosteroid which upon activation functions as a hormone that regulates the expression of hundreds of genes. The presence of the vitamin D receptors in most cells coupled with the metabolic pathways for it's activation strongly suggest a role for vitamin D in the pathophysiology of several diseases.

What constitutes an optimal serum vitamin D level remains controversial, though deficiency is generally defined as being below 50 nmol/l and severe deficiency below 25 nmol/l. This is based on what is considered optimal in studies for bone health outcomes. However, some studies point to beneficial effects on bone health at higher vitamin D levels and some experts define insufficiency as below 75 nmol/l. Higher levels may be deleterious and emerging evidence suggests a 'U' shaped relationship with some health outcomes.

The evidence for vitamin D in non-skeletal disease is inconclusive, though studies strongly support a role in falls prevention. In addition, vitamin D has been implicated in the pathogenesis of cardiovascular, neurological and autoimmune disease, as well as cancer and depression.

Vitamin D deficiency is widely prevalent and may be increasing at a population level. Given that supplementation is inexpensive, correction of low vitamin D status may have a significant global impact on several health outcomes but this remains to be proven. Well designed randomised controlled trials are needed to further establish the role of vitamin D beyond bone health.

CHAPTER 3: VITAMIN D & COGNITION

3.1 Introduction

An increasing body of evidence strongly suggests that vitamin D is involved in critical brain function and development and may play a role in cognition. Findings are based on studies that have looked at the:

1. The presence of metabolic pathways for vitamin D in the brain.
2. Affect of the loss of function of Vitamin D Receptor (in rodent models).
3. Affect of vitamin D on in the central nervous system in vitro and in vivo.
4. Association between serum vitamin D and cognition

Vitamin D may protect against cognitive decline through its affect on neuro-protection, vasculo-protection, modulation of vascular risk factors, anti-oxidation, neuronal calcium regulation, immunomodulation, enhanced nerve conduction and detoxification mechanisms (Buell et al. 2008). The evidence to date establishes a good biological plausibility for the role of vitamin D in cognition as supported by the widespread presence of VDR, vitamin D and 1ά- hydroxylase in the developing and adult brain and the known involvement of vitamin D target gene products in brain function and neuro-protection (McCann et al., 2008).

3.2 Vitamin D and the Brain

The first indirect clue that vitamin D may have some role in the brain was when its metabolites were discovered in the cerebrospinal fluid of healthy adults (Balabanova et al., 1984). This was supported by the earlier finding of $1,25(OH)_2D$ binding in the brain (Stumpf and O Brien, 1987) and later the presence of a vitamin D receptor (VDR) in the CNS of several species (Musiol et al., 1992; Stump et al.,1995)

3.21 Vitamin D Receptor in the CNS

VDR is expressed widely in the adult brain in temporal, orbital and cingulated cortices, cerebellum, mesopontine area, thamalus, hypothalamus, in the accumbens nuclei, parts of the stria terminalis and amygdala and widely throughout the olfactory system (McCann et al., 2008). It is also expressed in the pyramidal neurons of the hippocampal regions CA1 ,CA2, CA3, CA4 in rats (Stumpf et al., 1987) as well as in

humans and is most highly expressed in the subsantia nigra (Eyles et al., 2005). VDR has been reported in several cell types of the CNS including astrocytes, oligodendrocytes and Schwann cells (Cornet et al., 1998; Prufer et al., 1999; Baas et al., 2000). VDR is also densely expressed in the subventricular zone in neonatal rat brains, the area representing the active site for proliferation in the embryo and throughout life.

3.22 Vitamin D metabolites in the CNS

I,25$(OH)_2$D crosses the blood barrier (Kaleuff et al., 2006) and may also be synthesised in the brain (Eyles et al., 2005). In vitro and animal data confirm the presence of vitamin D in the brain. An early study of patients with Alzheimer's revealed the presence of VDR mRNA in humans (Sutherland et al., 1992). 1,25$(OH)_2$D injected into hamsters was found to be concentrated in nuclei of neurons in several brain areas important in memory and cognition (Musiol et al., 1992). The discovery of the 1ά-hydroxlase and biosynthetic / biodegradative pathways for vitamin D in neurones and glial cells (Neveu et al., 1994; Eyles et al., 2005) in co-location with the VDR supports a functional role for vitamin D in the human brain. Genes encoding 25-hydroxylase and 1ά-hydroxylase are expressed in neurons and glial cells within human brain structures critical for cognition (Neuveu et al, 1994; Zendher et al., 2001). This supports the theory that serum 25(OH)D levels may influence paracrine production of 1,25$(OH)_2$D directly in the central nervous system (Sutherland et al., 1992; Zehnder et al., 2001).

3.23 Vitamin D Gene Products in the Brain

Vitamin D is known to have multiple gene targets in the brain, whose products play an important role in neuro-protection, neuronal differentiation and brain function. Most evidence comes from studies looking at the direct effect of on specific gene expression while a small number have examined the effects of vitamin D deficiency. In vivo studies where effects on gene expression were observed used relatively high doses of 1,25$(OH)_2$D and in some it was directly injected into the brain (McCann et al., 2008). A list of gene products whose expression is altered by vitamin D is listed in Table 3.2.

Table 3.2: Vitamin D Target Gene Products in the Brain

$1,25(OH)_2D$ Gene Target Products in the Brain		
	Effect	**Role**
Neurotrophins		
Nerve Growth Factor (NGF)	Up regulates	Important for growth and survival of many cells including cholinergic basal forebrain neurons.
Neurotrophin –3 (NT-3)	Up regulates	Increases transmission in hippocampal cells, also found in neocortex.
Glial Derived Nerve Growth Factor (GDNF)	Up regulates	Reduces oxidative stress in Parkinson's Disease.
Calcium Binding Proteins		
Calmodulin, Parvalbumin, Calretinin	Up regulates	Calcium signalling and homeostasis.
Enzymes		
Gama Glutamyl Transpeptidase	Up regulates	Enhances innate antioxidant pathways by increasing production of glutathione – protects integrity of nerve conduction pathways.
Nitric Oxide Synthetase	Down regulates	Generates Nitric Oxide which can cause damage to neurons at high concentrations
Choline AcetyLtransferase	Up regulates	Responsible for neurotransmitter acetlycholine known to play a role in memory function.
Cell Signalling Proteins		
N-myc, c-myc, protein kinase C	Up regulates	Control of cell cycle, differentiation and proliferation.
L-Type Voltage Sensitive Channels	Up regulates	Regulation of neuronal calcium -role in neurotransmission, neurogenesis,, hippocampal cell loss.
Cytokines (l1-b, Il- 2)	Down regulates	Mediates inflammation and neuronal damage.

3.24 Vitamin D and Neurotrophins

Studies have shown that vitamin D may protect the structure and integrity of neurons through detoxification pathways and neutrophin synthesis. Vitamin D is known to effect the expression of three of the four neurotrophins in mammals including nerve growth (NGF), glial cell derived nerve factor (GDNF) and neurotrphin-3 (NT3) Neruotrophins are proteins necessary for neuronal survival in aging (McCann et al., 2008).

Nerve Growth Factor (NGF)

This is potently up regulated by vitamin D (Neveu et al., 1994) and is essential for the growth and survival of many cells in the brain but in particular the cholinergic basal forebrain neurons which project to the hippocampus (Korsching et al., 1985). It is also found in the hippocampus where it affects neurotransmission (Siegel, 1999). Decreased expression is observed in neonates when vitamin D is removed from the diet of pregnant rat females (Eyles et al., 2003). NGF is capable of binding to the pan-neurotrophin receptor $p75^{NTR}$ (Chao et al., 1995) both of which are essential factors in programmed cell death (Chao, 1994). Vitamin D has also been shown to positively regulate the expression of $p75^{NTR}$ in glioma cells (Naveilhan et al, 1996).

Neurotrophin-3 (NT-3)

This is found in the hippocampus and neocortex and is up regulated by vitamin D (Neveu et al., 1994). It increases transmission in hippocampal cells (Kang et al., 2003).

Glial cell Derived Neurotrophic Factor (GDNF)

Vitamin D has been shown to upregulate GDNF (Nalveilhan et al., 1996) which affects the survival and differentiation of dopaminergic cells (Christophersen et al., 2007). Recent evidence indicates a role in synaptogenesis (Ledda et al., 2007). Treatments with GDNF in rodent and primate models of Parkinson's rescued damaged dopamine neurons and associated functions (Tomac et al., 1995; Do Thi et al., 2007). In the Parkinson's model in rats, $1,25(OH)_2D$ treatment attenuated neurotoxicity (Wang et al., 2001). Studies show that the neuro-protective effects were

dose dependent and found at vitamin D levels that also increased GNDF (Wang et al., 2000).

3.25 Vitamin D and Enzyme Regulation

Nitic Oxide Synthetase

$1,25(OH)_2D$ inhibits inducible nitric oxide synthetase (Garcion et al., 1997) an enzyme that is up regulated during ischaemic events and in patients with Alzheimer's and Parkinson's Disease (Buell et al., 2008). It is responsible for generating nitric oxide which is known to cause damage to neurons and oligodendrocytes at high concentrations. In Alzheimer's and amyloid precursor protein (APP) transgenic mice, high NOS levels within astrocytes have been associated with amyloid β protein deposits (Luth et al., 2001). Nitric oxide snythetase activity has also been reported to increase significantly in leukoctyes (De Sevri et al., 2002), and brain micovessels of patients with AD (Dorheim et al., 1994). NOS deficiency protected AD-like mice from premature mortality, cerebral plaque formation, increased AB protein levels, astrocytosis and microgliosis (Nathan et al., 2005)

Gamma Glutamyl Transpeptidase

Vitamin D enhances innate antioxidant pathways. It is known to up regulate Gamma Glutamyl Transpeptidase (Baas et al. 2000), an enzyme that leads to increased production of glutathione (a potent antioxidant which protects oligodendrocytes and the integrity of nerve conduction pathways critical to mental processing) (Buell et al., 2009).

3.26 Vitamin D and Neuronal Calcium Regulation

Three calcium binding proteins have been shown to be modulated by vitamin D in brain tissues, calbindin, parvalbumin and calretinin (Alexianu et al., 1998; DeViragh et al., 1989). All three are widely and uniquely distributed in adult brain and are believed to serve a neuro-protective role as calcium buffers (Fierro et a., 1996) as well as being involved in critical brain functions (McCann et al., 2008)..

Calbindin D28K

This is required for normal signalling of synaptically evoked calcium transients (Airaksinen et al., 1997), synaptic plasticity (Chard et al, 1995), long term potentiation (Jouvenceau et al., 2002) and memory formation (Molinari et al., 1996 Dumas et al, 2004). It is also found in Purkinje cells in the cerebellum (where it comprises 15% of the total protein content) (Bambridge et al., 1992) and may have a direct involvement in motor control (Airaksinen et al., 1997).

Calmodulin

Calmodulin is involved in intracellular calcium homeostasis (Vazquez et al., 2000.) Calmodulin is also involved in neurotransmitter activity, NMDA induced synaptic plasticity and short term plasticity. It is also a cofactor for (CAM kinase II), the enzyme which is believed to have a central role in memory and learning and has been suggested as the basis of long-term synaptic memory. Calmodulin content has been found to be reduced in Alzheimer brain's versus controls (McLachlan et al., 1987)

L-Type Voltage Gated Channels

Vitamin D appears to regulate the expression of L-type gated calcium channels. This is important as intracellular calcium influx appears to play an important role in neurogenesis, synaptogenesis, myelination and neurotransmitter release (Berridge et al., 1998; Komuro et al., 1996). In addition, excessive calcium levels are deleterious for memory formation and cognitive function (Thibaukt et al., 2001; Veng et al., 2003). In particular, elevated intracellular calcium levels have been observed in neurons in Alzheimer's disease, as have increased L-type gated calcium channels (Coon et al., 1999). Hippocampal cell loss and neuronal aging (features of Alzheimer's) have been attributed to elevated L type voltage calcium channels and glucocorticoid neurotoxicity (Kimura et al., 1998). $1,25(OH)_2D$ is known to down regulate the expression of voltage sensitive calcium channel transcripts in rat hippocampal cells. Treatment of aged rats with $1,25(OH)_2D$ restores L-type voltage activity to that seen in younger animals (Brewer et al., 2006). In addition, amyloid β protein is known to trigger neurodegeneration by inducing L-type voltage gated calcium expression but also by suppressing VDR expression. Treatment with vitamin

D in this model protected neurones by downregulating LVSCC and upregulating VDR (Dursun et al., 2011).

3.27 Vitamin D and Cytokines

There is widespread agreement that vitamin D is a potent immunosuppressant and is considered an anti-inflammatory agent with profound affects on T cell function (Hayes et al., 2003; van Etten and Matthieu, 2008). One potential mechanism of action of neuroprotection may involve the suppression of pro-inflammatory cytokines in the brain (van Etten and Matthieu, 2008).

$1,25(OH)_2D$ is synthesized by microglial cells (Neveu et al., 1994) which are the primary mediators of pro-inflammatory immune responses in the brain. $1,25(OH)_2D$ is also known to inhibit the synthesis of pro-inflammatory cytokines in the microglial cell line (Lefbvre d'Hellencourt et al., 2003). It may decrease the production of Tumour Necrosis Factor-alpha (TNF-ά) in the brain, which is thought to play a pathogenic role in neurodegenerative disorders such as Alzheimer's and Parkinson's disease (van Etten., 2005). Oral treatment with 25(OH)D has also been shown to reduce production of the pro-inflammatory cytokine IL-1b in rat hippocampus. (Moore et al., 2005). In addition, $1,25(OH)_2D$ has been shown to down regulate NF-kB an important mediator of inflammation (Zhu et al., 2005) that has been associated with stress induced neuronal loss in rodents (Madrigal et al., 2002).

3.28 Vitamin D and Neuronal Function

Evidence suggests that, $1,25(OH)_2D$ may stimulate adult neurogenesis acting through some of its target gene proteins. Vitamin D is known to increase neurite outgrowth when added to embryonic hippocampal explant cultures in rodents, an effect which is most likely due to and induction of NGF in vitro (Brown et al., 2003).

Vitamin D has been shown to regulate the expression of a number of proteins important in maintaining the neuron cytoskeleton and plastic synapticity. It is known to upregulate the expression of microtubule-associated protein-2 (MAP-2), growth associated protein-43 (GAP43) and synapsin-ı in cultured rat cortical neurons (Taniura et al., 2006). Rat models of maternal hypovitaminosis D also show consistent down regulation of transcripts and proteins involved in cytoskeleton maintenance

(neruofilament, tubulin, actin, MAP2, glial fibrillary acidic protein), molecular transport of organelles (creatine kinase b, kinesin, Rhoa, dynactin) and synaptic plasticity (drebin, GAP43, connexion 43) (Almeras et al., 2007; Eyles et al., 2007).

3.3 Vitamin D Rodent Models

Direct evidence linking vitamin D to cognition has been obtained from rodent models where Vitamin D deficiency has been induced (through dietary or UV radiation restriction) or in models of defective vitamin D metabolism (VDR knockout mice or 1ά -hydroxylase knockout mice), (McCann et al., 2008).

VDR Knockout Mice

These mice have a defective VDR that can bind 1,25-dihydroxyvtiamin D, but cannot bind to DNA (Bula et al., 2005) and hence are not able to regulate gene expression. Such mice display several phenotypical and biochemical abnormalites as a result of loss of vitamin D function. They appear normal in growth rate until after weaning and usually die by 15 weeks of age unless fed a special calcium rich diet (Li et al., 1998). After 6 months of age, they show clear symptoms of premature aging, immunological deficiency, osteoporosis and ectopic calcification of the thalamic area of the brain (Tuohimaa et al., 2009). Behavioural studies suggest an effect on cerebral function with increased anxiety, abnormal grooming, pup cannibalism, impaired nest building and neophobia (Kalueff et al., 2004; Keisala et al., 2007: Minasyan et al., 2007).

Vitamin D deficient Mice

Models using gestational dietary and UV radiation restriction have resulted in greater than 90% reduction in serum 25(OH)D concentration in neonates (Eyles et al., 2003). Vitamin D deficient rats appear to have normal working memory but disrupted latent inhibition which is a measure of attentional processing (Becker at al., 2005). Several other abnormalities in models have been reported on specific tests including hyperlocomotion (Burne et al., 2004) and reduced habituation (Becker et al., 2006). In embryonic models, vitamin D deficiency during foetal development has resulted in morphological brain changes, motor impairments and memory and learning impairments. Vitamin D deficient mice had altered responses to heat, sound and shock, reduced levels of glutamate and glutamine and increased levels of GABA and

glycine (Groves et al., 2013). Vitamin D deprived rats also had significantly poorer performance on a spatial learning task (Taghizadeh et al., 2011).

Morphological changes identified in the newborn offspring of DVD-deficient rats include larger brains, increased volume of the lateral ventricles and a smaller neocortical width when corrected for overall larger brain (Eyles et al,., 2003). Addition of vitamin D into the diet from birth appears to ameliorate lateral ventricle changes (Feron et al., 2005).

Studies in vitamin D -deficient mice show significantly altered expression of 36 proteins and 74 genes involved with cytoskeleton maintenance, calcium homeostasis, synaptic plasticity and neurotransmission, oxidative phosphorylation, redox balance, protein transport, chaperoning, cell cycle control and post translational modifications. (Almeras et al, 2007; Eyles at al., 2007). Reduced concentration of brain calcium was also observed in Vitamin D restricted rats that resulted in a 50% reduction in serum calcium (Harris et al., 1981).

Limitations of Rodent Model Studies

Results from above the above studies appear to be inconsistent, including those reporting hyperlocomotion, altered grooming activity, impairment of tests of working memory and reduced habituation (McCann et al., 2008).

Possible reasons for this include differences in the sex and genetic background of the rodents used and different study methodologies employed such as the use of different dietary methods to rescue VDR knockout mice. In addition, few tests were targeted at learning and results in several spatial learning tests were mainly negative. Potential confounders including the effects of hypocalcaemia and the development of a phenotype similar to Type 2 Hereditary Rickets in humans. Some changes were consistent with reduced musculoskeletal development and motor impairment (reduced stride length, hyperlocomotion, and reduced habituation in the open field test.) (Mc Cann et al., 2008).

Given that mice are primarily nocturnal and that lithocholic acid can substitute for vitamin D in rodent deficiency states raises the possibility that other homeostatic

mechanisms might protect vital organs such as the brain from loss of calcitriol regulated functions.

3.4 Vitamin D, Alzheimer's

Alzheimer's disease is characterised pathologically by the presence of amyloid plaques and tangles in multiple brain areas important in cognition. Early in the disease the hippocampus is affected which is important in laying the trace for new memory As it progresses there is involvement of temporal, parietal and frontal lobes (Salawu et al., 2011).

Vitamin D may play a role in Alzheimer's by protecting against hippocampal atrophy, preventing or reducing amyloid accumulation, increasing acetylcholine levels, counteracting glutamate toxicity and by anti-inflammatory, antioxidant, vasculo-protective and neuro-protective affects. In addition, several risk factors (as identified in epidemiological studies) for Alzheimer's disease are also associated inversely with serum vitamin D. This includes diabetes, hyperlipidaemia, obesity, hypertension and depression. In a clinical trial of 43 subjects (mean age 84.7 ± 6.3 years) with a new diagnosis of AD, treatment with vitamin D and memantine for 6 months resulted in an improvement in MMSE scores versus vitamin D or memantine alone (Annweiler et al., 2012). Alzheimeric rats fed a diet lacking vitamin D had a lower performance on a spatial learning task versus Alzheimeric rats with or without supplementation with vitamin D (Taghizadeh et al., 2011).

Vitamin D & the Hippocampus

In human subjects with Alzheimer's, a reduction in VDR mRNA in specific regions of the hippocampus (CA1 and CA2) was found compared to controls (Sutherland et al., 1992). Hippocampal cell loss and neuronal aging have been attributed to elevated L type voltage calcium channels and glucocorticoid neurotoxicity (Kimura et al., 1998). Vitamin D (calcitriol) is known to down regulate the expression of voltage sensitive calcium channel transcripts in rat hippocampal cells. Treatment of aged rats with vitamin D restores L-type voltage activity to that seen in younger animals (Brewer et al., 2006). However, no association between vitamin D status and hippocampal volume was identifed in one study involving 318 older community

dwelling adults (mean age = 73.5, 72.6% female), though subjects did not have Alzheimer's (Buell et al., 2010)

Vitamin D & Amyloid

1,25-dihydroxyvitamin D may play a part in amyloid clearance, as it has been shown to stimulate β-amyloid phagocytosis while protecting against apoptosis (Masoumi et al., 2009). 1,25(OH)D also appears to enhance blood to brain amyloid efflux transport at the blood brain barrier through genomic and non genomic actions (Ito et al., 2011). Genomic and non-genomic signaling induced by $1,25(OH)_2D3$ has also been shown to promote the recovery of amyloid-β phagocytosis by Alzheimer's disease macrophages (Mizwikcki et al., 2012). VDR mRNA expression was found to be strongly upregulated in human microglia stimulated with aggregated Aβ-42 (Walker et al., 2006), suggesting that VDR activation by vitamin D could be a therapeutic anti-inflammatory target.

Vitamin D supplementation of aged rats was found to increase β-amyloid clearance and decrease amyloid burden (Briones et al., 2012). It also ameliorated the age related decline in learning and memory. In addition, a vitamin D enriched diet was correlated to a decrease in amyloid plaques and Aβ amyloid peptides in transgenic mice that develop amyloid plaques within 3-4 months of birth (Yu et al., 2011).

The level of vitamin D-binding protein (DBP) is increased in the cerebrospinal fluid of patients with Alzheimer's disease (AD), suggesting a relationship with its pathogenesis. DBP prevented Aβ-mediated death in cultured mouse hippocampal cells and decreased Aβ-induced synaptic loss in the hippocampus and rescued memory deficits in mice after injection of Aβ into the lateral ventricle. This suggests that DBP attenuates the harmful effects of Aβ by a direct interaction, and that it maybe a promising therapeutic agent for the treatment of AD (Moon et al., 2013).

Whilst in a cross sectional study of 1219 elderly subjects, no association was found between 25(OH)D and either serum Aβ-42 or Aβ-40, subjects were cognitively healthy (Gu et al., 2012).

Vitamin D, Prostaglandins and AD

Prostaglandins which are formed after the actions of COX-2 might play an inflammatory role in Alzheimer's as over expression of COX-2 has been identified in the hippocampus (Ho et al., 1999) and has been shown to potentiate Aβ protein production (Xiang et al., 2002). It has been reported that vitamin D may play a role in regulating the expression of several genes in the prostaglandin pathway, thereby reducing levels (Moreno et al, 2005). $1,25(OH)_2D$ and its analogs have been shown to inhibit the activity of COX-2 (Aparna et al., 2008).

Vitamin D Nitric Oxide and AD

Vitamin D is known to down regulate iNOS activity (Garcion et al., 1997) which may play a role in the pathogenesis of AD (Lu'ong et al., 2011). NOS activity has been found to be higher in leukocytes and microvessels of patients with AD (De Servi et al., 2002; Dorheim et al., 1994). In addition, in a model of Alzheimer like mice, iNOS deficiency protected against cerebral plaque formation, increased Aβ protein levels and microgliosis (Nathan et al., 2005).

Vitamin D, ACE activity and Alzheimer's

Studies point to a potential role of Angiotensin Converting Enzyme (ACE) in the pathogenesis of AD, though this remains controversial. ACE activity has been reported in post-mortem brain tissue from subjects with AD and was also associated with Aβ plaque load (Arregui et al., 1982). Increased ACE immunoreactivity and ACE activity has been identified, respectively in the parietal cortex (Savaskan et al, 2001) and peripheral blood of AD patients (Akatsu et al., 2011). ACE has also been shown to inhibit the aggregation and secretion of Aβ, an affect which was blocked by an ACE-I (Hu et al., 2001). Evidence though is conflicting and conversely in another study ACE-I's appeared to have no affect on levels of Aβ or plaque (Kolsch et al., 2005).

Vitamin D has been reported to reduce ACE activity in bovine endothelial cells (Higiwara et al., 1988). It is also known to down regulate the renin, angiotensin system, components of which have been indentified in the CNS in rat models (Ganten

et al., 1983). It is therefore possible that vitamin D might play a role through it's affect on ACE activity in the CNS.

Vitamin D & Glutamate Toxicity

Vitamin D has displayed neuro-protective properties against glutamate toxicity through antioxidant effects (Ibi et al., 2001). Glutamate toxicity is believed to play a role in Alzheimer's disease for which treatment with the glutamate antagonist memantine is used.

MHC II Class Antigen expression & Vitamin D

Vitamin D reduces the expression of MHC Class II antigens in humans (Tokuda et al., 1996) which are reported to be increased on microglial cells in AD brains (Styren et al., 1990).

VDR Polymorphisms and AD

Susceptibility to late onset Alzheimer's disease has been mapped in several studies to loci on chromosome 12q13, where the VDR gene is located (Podsuslo et al., 2001; Wang et al., 2012). Higher frequency of VDR polymorphisms have been found in Alzheimer's brains versus aged matched controls (Gezen-Ak et al., 2007).

In a study of 492 late onset cases of Alzheimer's and 496 controls an association was found between a specific single nucleotide polymorphism (SNP) and Alzheimer's (Wang et al., 2012). The presence of VDR genotypes Apa 1T and Taq1 G were associated with an increased risk of Alzheimer's disease in 255 subjects, especially in those under 75 years of age (OR ≥ 3.0, $p < 0.005$) (Lehmann et al., 2011). These polymorphisms were also associated with two other genes involved in the regulation of inflammation, interleukin –10 and dopamine B hydroxylase. A specific VDR polymorphism has been associated with a greater decline in category fluency (a test which is often impaired in AD) in females in one study (Beydoun et al., 2012

Clinical Studies of Vitamin D & AD

Three meta-analyses have looked at 25(OH)D levels and AD. In one which analysed seven case control studies, serum 25(OH)D levels were found to be 1.4 SD lower in

AD cases compared to cognitively healthy controls (Annweiler et al., 2013). Balion et al also performed a meta-analyis of 4 case control studies with AD and found a weighted mean difference of 25(OH)D of 6.2 nmol/l between AD subjects and controls (Balion et al., 2012). Finally, a meta-analyses of six studies (N=892) also found significantly lower vitamin D status in AD patients versus controls (Zhao et al., 2013). However, lack of sunlight exposure and dietary habits as a secondary consequence of AD might account for this association.

A 25(OH)D level of less than 50 nmol/l was associated with a higher prevalence of possible or probable Alzheimer's (Buell et al., 2010). Despite this, in a study of 45 high functioning females who had 25(OH)D levels below 25 nmol/l, no association was identified for those who developed Alzheimer's dementia at seven year follow up (Anweiller et al., 2011).

Lower dietary intake of vitamin D in community dwelling females (mean age 79.8 yrs) has also been associated with an increased risk of developing Alzheimer's dementia at 7 year follow up (Annweiller et al., 2012).

3.5 Vitamin D & Vascular Cognitive Impairment

Vitamin D may help reduce vascular related brain injury by mediating the effects of inflammation, calcium dysregulation and increased oxidative stress. During transient ischaemic events TGF and GDNF are up regulated in hippocampal cells to promote survival. Vitamin D increases innate antioxidant defences by upregulating glutathione and GDNF concentrations (Naveilhan et al., 1996) which has been shown to attenuate ischaemic brain disease in rodents (Wang et al., 2000). In animal and in vitro models of cerebral ischaemia, vitamin D inhibits antigen cell maturation (Carthy et al., 1989), and stimulates anti-flammatory cytokine production (Timms et al., 2002).

Vitamin D insufficiency (level of 25-50 nmol/l) has been associated with large vessel infarcts and white matter hyperintensity volume and severity (Buell et al., 2010). In addition, vitamin D deficiency has been associated with stroke (Chowdury et al., 2012) as well as atrial fibrillation (Demir et al., 2012). The potential role in cerebrovascular protection could be mediated through its possible affect on blood

pressure (Forman et al., 2005), diabetes (Mathieu et al., 2005), and endothelial dysfunction (Liu et al., 2013).

3.6 Vitamin D / Secondary Hyperparathyroidism & Cognition

Vitamin D deficiency is a known cause of secondary hyperparathyroidism and an inverse relationship exists between serum 25(OH)D and PTH levels. Receptors for PTH have been found in numerous organs including the brain and it is widely distributed in the central nervous system (Weaver et al., 1995; Usdin et al., 1996). PTH appears to pass the blood brain barrier and could therefore affect cerebral function (Joborn et al., 1991). PTH increases intracellular calcium concentration in nerve cells which could have potentially harmful affects like inhibited mitochondrial oxidation and ATP production (Smogorzewski et al., 1991; Massry et al., 1995;) Incubation with PTH in one study caused increased intracellular calcium concentration and cell deterioration in rat hippocampal slices (Hirasawa et al., 2000) It is plausible that PTH and calcium levels may be part of a complex relationship between vitamin D and cognitive function. However, serum PTH is also affected by several other factors including calcium intake, serum phosphate levels and renal function. There is also the potential for the effects of serum calcium on cognition. Higher serum calcium has a been associated with greater cognitive decline in older adults but not in adolescents (Tolppanen et al., 2011).

Primary hyperparathyroidism is associated with cognitive impairment. In elderly patients with primary hyperparathyroidism, dementia is frequently found and often alleviated after parathyroidectomy (Ohrvall et al., 1994). Impairments on tests of cognitive function in patients with primary hyperparathyroidism includes verbal memory, nonverbal abstraction (Walker et al., 2009) and spatial learning (Roman et al, 2005) some of which may improve post parathyroidectomy (Walker et al. 2009).

Clinical studies have also found a correlation between serum PTH and cognitive performance. High PTH levels were associated with low performance in 10 out of 13 tests of cognition in the 5th Tromso Study (Jorde et al., 2005). PTH was associated with impaired cognition at baseline (as measured by MMSE and CDR) and with a five-year cognitive decline in a general aged population independent of calcium and renal function (Bjorkman et al., 2010).

3.7 VDR Polymorphisms and Cognition

Further evidence for the role of vitamin D in cognition comes indirectly from studies of Vitamin D receptor polymorphisms, some of which as discussed earlier are associated with Alzheimer's disease. A positive association between cognitive decline and carriers of the BsmI and TaqI polymorphisms of the VDR gene has been reported. These associations could not be explained by differences in calcium levels or survival suggesting that genetic variance in the VDR gene influences the susceptibility to age related cognitive changes (Kuningas et al., 2009).

3.8 Clinical Studies of Vitamin D & Cognition

A relatively small number of studies have explored the relationship between serum 25-hydroxyvitamin D and adult cognition and include cross sectional, longitudinal and a few intervention trials. Some have been large but used only brief cognitive measures. A few studies have also examined the association between vitamin D intake and cognition.

Whilst most studies show a positive association between serum 25(OH)D and some tests of global or specific cognitive function, results are inconsistent and the relationship remains controversial. It also remains unclear as which cognitive domains are most affected by vitamin D. Annweiler et al. have suggested that hypovitaminosis D is associated with dysfunction of the frontal- subcortical neuronal circuits, particularly the dorsolateral circuit (Annweiler et al., 2012). This is based on the finding of an inverse relationship between vitamin D and executive function in some studies but results are conflicting.

Studies of vitamin D and Cognition - Pitfalls

Inconsistent study findings may be partially explained by a number of factors including choice and sensitivity of cognitive tests used, heterogeneity of the study populations, lack of control for multiple potential confounders and differences in study methodology. Some studies were small and may not have had sufficient statistical power to detect the effect of small differences due to vitamin D.

Cognitive Testing

Vitamin D deficiency / insufficiency has the potential to affect multiple cognitive domains including those specific to Alzheimer's disease and vascular cognitive impairment. Studies that have used a narrow range of cognitive tests or brief global cognitive measures may not be targeting the potential areas that may be affected by vitamin D. In addition, short neuropsychological assessments are likely to lack sensitivity and specificity.

Study Population

Differences in study population characteristics have the potential to affect results, especially were known confounders of either cognition or vitamin D have not be controlled for. Some studies have been small in size and may not be representative of the wider population. Others have included subjects with dementia as opposed to mild cognitive impairment increasing the chance of a positive finding being explained by 'reverse causality'. Most have included community dwelling subjects but some have included institutionalised elderly. In addition, the exclusion criteria of several studies has the potential to bias results. Predisposing genetic factors in different study populations may alter vitamin D response, such as the possible impact of normal human VDR polymorphisms on cognition.

Confounding Factors

Multiple factors influence vitamin D status (as previously discussed) and when not adjusted for could account for false positive results. Cognitive impairment and vitamin D deficiency are common in older age though cognitive impairment may contribute to low vitamin D levels by reducing outdoor activity and hence sunlight exposure. In addition, cognitive impairment could lead to poor dietary habits and a reduction in dietary vitamin D intake.

Tests of cognition are affected by age, gender, education, and psychiatric morbidity, visual and motor impairment. Correlates of cognitive impairment include current smoking status, alcohol consumption, diabetes, cardiovascular disease, stroke and hypertension. Vitamin D deficiency is also associated with hypertension, diabetes, stroke and metabolic syndrome. The absence or incomplete inclusion of potential confounders may account for mixed results from previous studies. Studies have also

varied in their analysis of the relationship, with some exploring associations with cognition as a continuous outcome variable as opposed to abnormal cognitive performance.

Studies of Dietary Vitamin D Intake and Cognition

Since about 90% of vitamin D is obtained from cutaneous synthesis, the effect of diet on vitamin status is small apart from supplements that may be consumed. However, at least three studies to date have specifically looked at vitamin D intake and cognitive status and have found a significant inverse relationship.

Higher vitamin D intake was associated with a lower risk of developing Alzheimer's dementia (AD) within 7 years in a cohort of 5596 community dwelling women (mean age 79.8 years) who were free of vitamin D supplements after adjustment for several confounds (Annweiler et al., 2012). The mean difference in the weekly intake of those who developed AD versus no dementia was 348 IU. The risk reduction for those in the highest versus the lowest quintile of intake of vitamin D was 77%.

Inadequate dietary intake of vitamin D (less than 1400 IU per week) was also associated with cognitive impairment as measured by the SPSMQ score in a study of 5596 community dwelling older females (mean age 80.4 years) after adjustment for several potential confounders (Anweiller et al., 2010). Subjects in the study were not taking vitamin D supplements and hence the difference in vitamin D intake between those with and without cognitive impairment is likely to be small.

An inverse correlation between dietary vitamin D intake (mean 316 IU per day) over a 3 day period and MMSE scores was identified in 59 community dwelling subjects (aged 70-89 years) but no adjustment was made for confounds (Rondanelli et al. 2007).

Cross Sectional Studies

To date, at least 15 out of 22 cross sectional studies which have looked at serum 25(OH)D and cognition have reported a positive association (See table 3.81). Participants have ranged from young to old. Some studies have only included females. Three meta-analyses have been performed. In one of eight case control studies where

participants where dichotomised as having a 25(OH)D of less or more than 50nmol/l, found a higher MMSE in those with the greater vitamin D level though there was significant heterogeneity between studies (Balion et al., 2012). A meta-analysis of five cross sectional and two longitudinal studies involving 7,688 participants also found an increased risk of cognitive impairment in those with lower vitamin D status (OR 2.39, 95% CI 1.91-3.00), (Etgen et al., 2012). In another recent meta-analysis which looked at 11 cross sectional and 3 longitudinal studies lower 25(OH)D prediceted executive function (Annweiller et al., 2013). A recent systematic review found that a worse outcome on one or more cognitive tests or a higher frequency of dementia in 18 out of 25 cross sectional studies which looked at serum vitamin D or vitamin D intake (van der Schaft et al., 2013).

Ten cross sectional studies involved only a small a number of subjects (Kipen et al., 1995; Jorde et al., 2006; Wilkins et al., 2006; Przyelski et al., 2007; Aung et al., 2007; Oudshorn et al., 2008); Dhesi et al., 2002; Hansen et al., 2011; Lasaite et al., 2011; Skalsa et al.,2012) and used only brief measures of cognitive function or had subjects with dementia or adjusted for few or no confounding factors.

Of seven large cross sectional studies (Mc Grath et al., 2007; Lewellyn et al., 2009; Lee et al., 2009; Annweiler et al., 2010; Tolpanenn et al., 2011; Annweiler et al., 2012) only one used a more extensive battery of cognitive tests (Buell et al., 2009).

The largest study included 6257 community dwelling females (mean age 76.6 years) and found an increased risk of global cognitive impairment (-1.5 SD below mean study MMSE) in those who were vitamin D deficient (<25nmol/l) versus replete (≥ 75 nmol/l).. However, no association was found with executive function as assessed by the Trails B test. (Slinin et al., 2012).

In an analysis using data from the third NHANES study, no inverse association was found though cognitive tests were brief (Mc Grath et al., 2007). In a further large study of 4929 adolescents, no association between serum 25(OH)D and specific cognitive tests were found after adjusting for age and ethnicity (Tolppanen et al., 2011). Likewise, no relationship was identified in 4310 older adults aged 60 –90 years

after multivariate adjustment though only a brief test of recall was used (Tolppanen et al., 2011).

A smaller study involving 387 subjects found a positive association when using a comprehensive cognitive test, though no significant difference was found in males when comparing the lowest and highest tertiales of 25(OH)D (Seamans et al., 2010).

Serum 25(OH)D was also significantly associated with cognitive impairment as assessed by the MMSE and CDR in 159 community dwelling older adults (mean age 85 years) after adjustment for age, gender, and education (Peterson et al., 2012). However, vitamin D was taken within 6 months of assessment and no adjustment was made for season.

One study to date has looked at vitamin D status in subjects with mild cognitive impairment and found a significant inverse relationship after multivariate adjustment (Anweiller et al., 2012).

Longitudinal Studies

In a recent review, in four out of six longitudinal studies, subjects with lower baseline vitamin D status had a decline in cognition at 4-7 year follow up (van der Schaft et al., 2013). Studies point to a 25(OH)D level of below 25-50nmol/l as being predictive of future cognitive decline in older adults (See Table 3.82).

The largest study to date found an increased risk of decline in the modified MMSE in 6257 older females (mean age 75.0 years) in those who were severely deficient (< 25nmol/l) or with a level between 25.0-49.9 nmol/l at baseline versus 75 nmol/l or above. However, no effect was found for executive function as assessed with the trail making part B test (Slinin et al., 2012). Likewise, serum 25(OH)D below 25 nmol/l and 50 nmol/l has been associated with cognitive decline as measured by the standard MMSE over a 4-6 year period in a studies after adjustment for several covariates (Llewellyn et al. 2010). Conversely, no significant decline in MMSE scores or performance on Trails B test was identified in a study of 1604 males with 25(OH)D below 19.9 nmol/l at mean follow up of 4.6 years (Slinin et al., 2010).

In a study of 45 highly functioning females (mean age 78.4 years) subjects with 25(OH)D below 25 nmol/l had an increased risk of developing non-Alzheimer's dementia at 7 year follow up though this did not hold true for subjects who developed Alzheimer's, though the study number was small (Anweiller et al., 2011).

In 1639 German subjects aged over 65 or older, poorer performance on the COGTEL phone assessment at 5 years follow up was found in those in the lowest quintile of 25(OH)D at baseline (Breitlin et al., 2012).

In a study of 473 Caucasian women in Perth, Australia, women with vitamin D deficiency during pregnancy (≤ 46nmol/l at 18 weeks term) were two times more likely to have children with a language difficulty at 5 and 10 yrs follow up compared to women with a 25(OH)D > 70 nmol/l (Whitehouse et al., 2012).

Vitamin D –Intervention Trials

There have only been six intervention studies involving vitamin D and measures of cognition, with only three finding a positive result. Of these, four were randomised controlled trials including those with dementia and healthy subjects. Apart from one large study, they were small, of short duration and lacked a comprehensive assessment of cognition (see Table 3.83)

In a post hoc analysis of a large randomised controlled trial (the Women's Health Initiative), no significant effect of vitamin D on cognition was noted during a mean follow up of 7.8 years. In this study which included 2034 females aged over 65 and without probable dementia at baseline, treatment with vitamin D (400 IU) and calcium (1000 mg) daily did not result in any difference in the rate of incident dementia, mild cognitive impairment or global or domain specific cognitive function compared to placebo (Rossom et al., 2012). However, the vitamin D dose used was small and subjects in the placebo group were also able to take over the counter supplements which may have contained vitamin D. In addition, it is unclear as to what effect supplemental calcium may have had.

Supplementation of 16 subjects who had moderately advanced Alzheimer's dementia with high dose vitamin D (7000 IU D2 per day) for 8 weeks was not associated with any improvement in the ADAS-cog or WMS logical memory versus placebo. Study subjects were initially treated for a run in period off 8 weeks with 1000 IU D2 per day before randomisation to high dose vitamin D or placebo with nasal insulin. Findings were negative despite a rise in 25(OH)D from 49 -187 nmol/l in the treatment group. (Stein et al., 2011).

Treatment of 63 healthy young adults (mean age 21-22) in Queensland with 5000 IU D3 per day for 6 weeks was not associated with any change in tests of working memory and executive function (Dean et al., 2011). However, baseline serum 25(OH)D was high (median of 75.0 nmol/l) and subjects were healthy which suggests an inappropriate target group for supplementation.

In a double blind pilot randomised controlled trial of 43 elderly oupatients (mean age 84.7 ± 6.3 yrs) with a new diagnosis of AD, treatment with vitamin D and memantine for six months increased MMSE scores compared to subjects taking memantine or vitamin D alone (Anweiller et al., 2012). The result remained significant after adjustment for covariates, age, gender, pre-treatment score and duration of treatment, however only eight were in the combined vitamin D/memantine group. The results suggest a potential synergistic affect of combined treatment.

An improvement in attention and reaction times was found in 139 ambulatory subjects who were supplemented with vitamin D for six months (Dhesi et al., 2004). Another study was non-blinded and involved treatment of only 25 participants, had a short duration of 28 days and used only two limited tests of cognition (Przybeiski et al., 2008). Whilst it found that an improvement in the Clock Drawing Test was associated with an increase in serum 25(OH)D, there was no significant between-group difference.

Table 3.81: Cross Sectional Studies of 25(OH)D and Cognition

Cross Sectional Studies of 25(OH)D and Cognition					
Author	**No.**	**Age**	**Test / Measure**	**Association**	**Comment**
Mc Grath et al., 2007	9556	20-59 60-90	Adult group: DDST. SDLT Elderly: Short story recall	No	Brief test in elderly
Slinin et al., 2012	6257	76.6	Modified MMSE (3MS), TMB	Yes	Both tests when 25(OH)D < 25 nmol/l versus ≥ 75 nmol/l.
Tolpannen et al., 2011	4310 4290	60-90 20-59	Older adults: story recall Younger adults: DSST, SDLT	No	
Llewellyn et al., 2010	3325	73.7	WAIS digit span, limited MMSE, EBMT(story recall), GCS, MCS	Yes	Only attention, orietantion when 25(OH)D 25 –50 nmol/l, no confounds
Lee et al., 2009	3133	60.0	DSST, ROCT, CTRM,	Yes	Only with DSST
Llewellyn et al., 2009	1766	78.2	AMT	Yes	Only in males in full model, some subject institutionalised
Buell et al., 2009	1080	75.0	Trails A & B, DSST, WMS logical memory and subtests	Yes	Executive tests only and when 25(OH)D< 25nmol/l
Chan et al., 2011	939	> 65	CSI-D	No	In males when comparing 25(OH)D < 63 nmol/l vs. >92 nmol/l
Anweiller et al. 2010	752	80.7	SPMSQ	Yes	Below 25 nmol/l increased risk

DSST - Digit Symbol Substitution Test, AMT - Abbreviated Mental Test, CDR – Clinical Dementia Rating Scale , MMSE – Mini Mental State Examination, SPMSQ – Short Portable Mental State Questionnaire, WAIS –Weschler Adult Intelligience Test., GCS- Global ComposIte Score, MCS- Memory Composite Score, EBMT –Eastern Boston Memory Test., - Weschler Memory Scale. DSMT- Digit Symbol Matching Test, TMB –TrAll making B. CSI Cognitive Style Index, [Ages quoted as range or mean].

Table 3.81: Cross Sectional Studies of 25(OH)D and Cognition

Cross Sectional Studies of 25(OH)D and Cognition					
Author	**No.**	**Age**	**Test / Measure**	**Association**	**Comment**
MeNant et al., 2012	463	78.0	MMSE, DSMT TMT A&B, TMT diff, Block Design Score	Yes	Below 50mol/l reduced score in TMT B & BDS
Seamans et al., 2010	188	76.2	CANTAB	Yes	Only in females
Oudshoorn et al., 2008	225	77.7	MMSE	Yes	
Peterson et al., 2012	159	85	CDR, MMSE	No	No adjustment for season
Jorde et al., 2006	148	62.1	Trails A & B, memory, language & executive tests	No	May have been underpowered
Skalksa et al., 2012	138	79.6	AMT	Yes	Higher risk when 25(OH)D between (23.26-47.8 nmol/l), no confounds
Lasaite et al., 2011	130	18-26	DST, TMA &B	No	No adjustment for confounds
Brouwer-Bolsma et al., 2013	127	65+	Executive function	Yes	
Anweiller et al. 2010	95	71.1	Mild Cognitive Impairment (Winblad et al. criteria)	Yes	
Wilkins et al., 2006	80	74.8	CDR, SBT, MMSE, Language, Memory, Executive tests	Yes	Only with SBT &CDR, Some subjects with dementia
Aung et al., 2007	44	≥ 65	MMSE, CDT	No	No adjustment for confounds
Przyelski et al., 2007	32	79.5	MMSE	Yes	No adjustment for confounds
Hansen et al.. 2011	25	34.6	N-Back test, accuracy & reaction time	Yes	No adjustment for confounds
DSST - Digit Symbol Substitution Test, DSMT- Digit Symbol Matching Test, AMT - Abbreviated Mental Test, CDR – Clinical Dementia Rating Scale , CANTAB – Cambridge Neuropsychological Testing Automated Battery, MMSE – Mini Mental State Examination, SBT – Short Blessed Test, TM A&B –Tr\ail making A & B. [Ages quoted as range or mean]					

Table 3.82: Longitudinal Studies of 25(OH)D and Cognition

Longitudinal Studies of 25(OH)D and Cognition						
Author	**No.**	**Age**	**Length (yrs)**	**Cognitive Outcome**	**Inverse Association**	**Comment**
Slinin et al. , 2012	6257	75.0	4	Modified MMSE , Trails B	Yes	Only with modified MMSE when 25(OH)D <25 or 25-49.9 nmol/l versus ≥ 75 nmol/l)
Breitlin et al., 2012	1639	≥ 65	5	COGTEL	Yes	Yes
Slinin et al., 2010	1604	74.6	4.6	Modified MMSE, Trails B	No	No
Lewellyn et al., 2011	858	>75	3-6	MMSE, Trails A & B	Yes	Both tests, significant when comparing (<25 versus >75 nmol/l)
Whitehouse et al., 2012	473	-	5	Peabody Picture Vocabulary Test	Yes	Children of pregnant women at increased risk when 25(OH)D < 46 nmol/l vs. > 70 nmol/l at 16 weeks gestation
Anweiller et al., 2011	49	76.4	7	Diagnosis of Dementia Alzheiemer's (AD) or Non -Alzhelmer's (NAD)	Yes	Increased risk only with NAD when 25(OH)D< 25 nmol/l Small no. of cases of incident dementia
MMSE – Mini Mental State Examination, COGTEL [Age quoted as mean]						

Table 3.83: Randomised Controlled Trials of Vitamin D and Cognition.

Randomised Controlled Trials of Vitamin D and Cognition							
Author	**No.**	**Population**	**Length**	**Age (yrs)**	**Intervention**	**Cognitive Outcome**	**Result / Comment**
Rossom et al., 2012	4143	Community dwelling females	7.8 years	> 65	400 IU D3 /day + Calcium Placebo	Incident dementia & mild cognitive impairment	No effect Low vitamin D dose Poor compliance
Lewellyn et al., 2011	120	Moderate dementia (MMSE 10-20) Not on dementia drugs	43 weeks	84.7	100,000 IU D3/ month + memantine Placebo/ month + memantine	MMSE, FAB, Trail making A&B, ADAS Cog	Positive effect
Dean et al., 2011	128	Healthy adults in Queensland	6 weeks	21-22	5000 IU D3 /day	Visuospatial working memory (N-Black test) Mental flexibility	No effect High baseline 25(OH)D
Stein et al. 2011	16	Community dwelling Mild /moderate AD (MMSE 12-24)	16 weeks	> 60	1000 IU D3/ day throughout and after 8 wks randomised to either: 1.7000 IU D3 + nasal insulin/day 2. Placebo + nasal insulin /day	ADAS-cog WMS logical memory Disability Assessment in Dementia	No effect
MMSE –Mini Mental State Examination, FAB –Frontal Assessment Battery, ADAS Cog -Alzheimer's Disease Assessment Scale, WMS – Weschler Memory Scale, AD –Alzheimer's Disease							

Conclusion

Relatively few studies to date have examined the relationship between serum vitamin D and cognition. The relationship in cross sectional and longitudinal studies tends to be confined to those who are vitamin D deficient (< 50 nmol/l). It is unclear as to which cognitive domains are most affected by vitamin D, though it has been associated with both measures of executive function as well as global cognitive tests scores. As cognitive impairment is correlated with frailty there is the potential for reversal causality in studies whereby lower vitamin D status is simply explained by inadequate sun exposure in those who are cognitively impaired. Whilst vitamin D may have dynamic and immediate effects on neurotransmitters that play a role in cognition including acetylcholine, proving causality may be difficult if the effects are more long term through modulation of multiple risk factors. This highlights the need for well designed randomised controlled trials of adequate duration and vitamin D dosage.

CHAPTER 4: VITAMIN D AND BLOOD PRESSURE

4.1 Introduction

Increasing evidence has emerged that supports a role for vitamin D in the pathophysiology of hypertension. Disorders of calcium, parathyroid hormone (PTH) and vitamin D metabolism were proposed to be related to the development of hypertension more than twenty years ago (McCarron et al. 1980). Early studies also demonstrated a relationship between vitamin D and blood pressure in rodent models of vitamin D deficiency (Weishaar et al., 1987). Further supportive evidence to date has come from several studies that have looked at the:

1. Affect of loss of VDR function and vitamin D deficiency on blood pressure in animal models.
2. Affect of vitamin D on physiological determinants of blood pressure.
3. Association between vitamin D intake or serum vitamin D and blood pressure.
4. Affect of vitamin D supplementation on blood pressure.

The presence of VDR and 1ά-hydroxylase in the myocardium, vascular smooth muscle cells and endothelium, and the affect of vitamin D on the renin angiotensin aldosterone (RAAS) system supports a plausible biological role for 25(OH)D in the modulation of blood pressure. In addition, numerous studies report an association between serum vitamin D levels and hypertension or higher systolic and diastolic blood pressure. Furthermore, supplementation with vitamin D has been found to have a favourable affect on blood pressure in some intervention trials.

Potential Mechanisms of Action

There are several mechanisms via which vitamin D may affect blood pressure. It is postulated that the vitamin D may modulate blood pressure through it's affect on the:

1. Renin Angiotensin Aldosterone (RAAS) system
2. Parathyroid Hormone levels (PTH)
3. Calcium metabolism
4. Vascular endothelial / smooth muscle function
5. Reno-protection

It may also improve insulin sensitivity and blunt cardiomyocyte hypertrophy which may prevent against hypertension (Cosenso-Martin et al., 2011).

4.2 Vitamin D and Regulation of Renin Angiotensin Aldosterone System

The renin angiotensin aldosterone system (RAAS) plays a critical role in the regulation of blood pressure, electrolyte and plasma volume homeostasis. It's activation leads to increased production of renin and angiotensin which contributes to the development of hypertension (MacKenzie al., 2006). Vitamin D appears to play an important role as a negative regulator of the RAAS. Vitamin D deficiency and supplementation are associated respectively with both up and down regulation of the RAAS, suggesting a beneficial role for vitamin D in control of blood pressure. There may also be an interaction between dietary sodium intake, the RAAS and vitamin D (Vaidya A et al., 2011). However, there has been some inconsistent results. In a small study, treatment of subjects with 25(OH)D or $1,25(OH)_2D$ did not change RAAS parameters (Bernini et al., 2013).

4.21 Vitamin D Deficiency and Up Regulation of RAAS

VDR knockout mice have increased expression of renin and angiotensin II leading to hypertension, cardiac hypertrophy and increased water intake, an effect that was found to be independent of serum calcium and PTH levels (Li et al., 2002). Three fold higher levels of renin mRNA and 2.5 fold higher levels of angiotensin II have been identified in such models, and treatment with an ACE inhibitor or AT1 receptor blocker can prevent the above abnormalities. Renin activity has been inversely associated with $1,25(OH)_2D$ levels in hypertensive patients (Burgess et al., 1990). In normotensive human subjects, serum angiotensin II and intrinsic RAAS activity were found to be higher in those who were vitamin D deficient (< 30 nmol/l) and insufficient (37.5–75 nmol/l) compared to those who were vitamin D sufficient (> 75 nmol/l) (Forman et al., 2010). A trend for higher plasma renin activity was also found in 50 subjects (mean age 49.5 ± 7.8 years) with lower vitamin D status (Kota et al., 2011). Serum 25(OH)D was also inversed associated with plasma renin activity in 223 hypertensive caucasians (Vaidya et al., 2011).

4.22 Vitamin D and Down Regulation of RAAS

Vitamin D is a potent suppressor of renin synthesis, an effect which appears to be independent of calcium and PTH levels (Zhao et al., 2008). In cell cultures, $1,25(OH)_2D$ directly suppresses renin gene transcription by a VDR dependent mechanism. In normal mice vitamin D deficiency stimulates rennin expression, whereas injection of 1,25(OH)D reduces renin synthesis. In transgenic mouse models that over express the VDR receptor, suppression of renin synthesis by $1,25(OH)_2D$ in vivo was also found to be independent of PTH and calcium (Kong et al., 2008). Correction of vitamin D levels has been found to blunt systemic RAAS activity. In vitamin D deficient mice, with increased systolic and diastolic blood pressure, high plasma renin levels were reversed in six weeks by a vitamin D sufficient diet (Weng et al., 2013).

In a small study involving 15 drug free patients with essential hypertension and low vitamin D levels, treatment with oral vitamin D (25,000 IU once a week) significantly reduced plasma renin and aldosterone levels, with a trend also for a reduction in angitotensin II levels (Carrara et al., 2013).

4.23 VDR Polymorphisms and RAAS

Genetic variation at the FokI polymorphism of the VDR gene, in combination with higher vitamin D levels were associated with lower plasma renin activity in 375 hypertensives subjects (Vaidya et al., 2011). However, 25(OH)D was not associated with renin activity in normotensives in the same study. In addition, the Fok1 polymorphism has also associated with an increased risk of hypertension in a case control study involving 480 patients (Swapna et al., 2011).

4.3 Vitamin D, PTH and Hypertension

Vitamin D deficiency is associated with elevated PTH levels and an inverse relationship exists between vitamin D and PTH (Thomas et al., 1998). It has been proposed that the possible affect of vitamin D on blood pressure may be in part mediated by PTH.

Several observational studies report a high prevalence of hypertension in states of primary or secondary hyperparathyroidism which often normalise post

parathyroidectomy. In addition, several cross sectional studies report a significant positive correlation between PTH levels and blood pressure. The association may be more marked in those with low calcium intake and/or elevated PTH (Jorde et al., 2000). While evidence points to a direct or indirect physiological role of PTH in blood pressure homeostasis, the nature of the relationship is unclear.

Potential Mechanisms of PTH on Blood Pressure

The underlying relationship between PTH and blood pressure may be complex. Chronic PTH infusion is known to cause increased blood pressure in healthy subjects though this may be due to high calcium levels (Hulter et al., 1986). Conversely, a hypotensive and vasodepressor effect has been reported in animal species (Saglikes et al., 1985).

Some studies point to a potential role of PTH in vascular smooth muscle function, it's association with a PTH hypertensive factor, a possible affect on renin secretion and vascular inflammation and atherosclerosis.

PTH and Vascular Smooth Muscle

Receptors for PTH and PTH-related peptide have been located in vascular smooth muscle cells and in the arterial endothelium (Usdin et al., 1996). PTH has a pro-sclerotic effect on vascular smooth muscle cells which may contribute to vessel wall thickening and consequently to higher blood pressure (Perkovic et al., 2003). A positive correlation between PTH and intracellular calcium suggests that PTH may act as an ionophore for calcium entry into cells which could lead to hypertension (Fardella et al., 1995).

PTH and 1,25 $(OH)_2D$ levels

High levels of $1,25(OH)_2D$ which can be due to elevated PTH have been associated with hypertension in humans in many studies (Rensick et al, 1986; Sowers et al, 1988; Brickmann et al. 1990; Morimoto et al, 1991; St. John et al., 1994), but not in all (Young et al., 1990; Lind et al., 1995; Duprez et al.,1994). $1,25(OH)_2D$ has been shown to increase intracellular calcium concentration in isolated vascular smooth muscle cells (Kawashima et al., 1988). Increased levels of $1,25(OH)_2D$ has also been reported in spontaneous hypertensive rats (Kurtz et al., 1986).

$1,25(OH)_2D$ reportedly increases the uptake of calcium into vascular smooth muscle cells (Bukoski et al., 1987) and has been shown to increase calcium adenosine triphosphate (Ca-ATPase) activity in a vascular smooth muscle cell line (Kawashima et al., 1988). It is postulated that increased $1,25(OH)_2D$ levels in response to elevated PTH may lead to increased calcium influx into vascular smooth muscle cells resulting in contraction and increased peripheral resistance, increasing blood pressure (Zemel et al., 2001).

PTH and PTH Hypertensive Factor

The existence of the above factor was first proposed in 1989 (Pang et al., 1989). The co-release of a PTH hypertensive factor along with PTH has been reported in spontaneously hypertensive rats (SHR) which when injected into normotensive rats produced a delayed hypertensive effect (Pang et al., 1989). Parathyroid hypertensive factor-like activity levels have also been found to be elevated in hypertensive individuals (Resnick et al., 1993).

PTH and the RAAS

A dose dependant increase in plasma renin activity was found following a PTH infusion in 22 normotensive human subjects (Broulik et al, 1986) though this has not been replicated by other studies (Hulter et al. 1986). High plasma renin activity and aldosterone levels in hypertensive patients with primary hyperparathyroidism were found to normalise along with blood pressure in most patients after parathyroidectomy in one case series (Gennari et al., 1995) suggesting the possible direct effect of PTH on renin secretion. However, increased levels of $1,25(OH)_2D$ which is a potent suppressor of RAAS is known to occur in association with high PTH levels. In addition, elevated PTH levels have been found in individuals with low renin hypertension (Resnick et al., 1986).

Hypertension and Primary / Secondary Hyperparathyroidism

Vitamin D deficiency is prevalent in patients with primary hyperparathyroidism. (Grey et al., 2005; Silverberg et al., 2007; Grubbs et al., 2008) and is also a cause of secondary hyperparathyroidism. In addition, patients with primary hyperparathyroidism have been found to have increased radial artery stiffness (Rubin

et al., 2005) and both increased stiffness and intimal thickness of the carotid artery (Walker et al., 2009).

Several case reports and series describe improvement in blood pressure post parathyroidectomy in patients with hypertension and hyperparathyroidism (Stefenelli et al.,1993; Gennari et al., 1995; Puepet et al., 2008), though some have reported no difference (Bollerslev et al., 2009) and others an increase in blood pressure (Rydeberg et al., 2010).

Clinical Studies of PTH and Blood Pressure

While several cross sectional studies have found a positive association between PTH levels and blood pressure (Gennari et al., 1986; Rensick et al, 1986; 1987; Hvarfner 1987; Brickmann et al. 1990; Young et al.,1990; St John et al., 1994; Morfis et al., 1997; Jorde et al., 2000; Jorde et al., 2005; Snijder et al., 2007; Zhao et al., 2010), (He et al., 2011; Chan et al., 2012; Mateus-Hamdan et al., 2012) others do not show any significant relationship (Kristal – Bonch et al., 1997; Kim et al., 2010),

In at least two studies where PTH was associated with blood pressure, no corresponding association was identified with vitamin D (Snidjer MB et al., 2007), (Chan et al., 2012) but this may have been due to a high vitamin D status of study subjects. In a recent large population study (N=7561), most of the association between 25(OH)D and blood pressure was attenuated by PTH (He et al., 2011) suggesting that it was an explanatory variable.

One longitudinal cohort study found PTH to be a significant predictor of rise in systolic blood pressure over a seven year period in men but not in women (Jorde et al., 2005). In addition, in one randomised controlled trial of vitamin D and calcium supplementation, a significant positive correlation between the change in PTH and the change in systolic blood pressure was found (Pfeiffer et al. 2001, $r = 0.9$).

Confounding Factors for PTH

PTH levels can be influenced by factors other than vitamin D including the intake of calcium, phosphate and sodium (Bucher et al., 1996; Calvo et al 1996; Altun et al. 2006). Low serum calcium was reported as the commonest cause of elevated PTH in

the 5th Tromso study. No significant difference in serum 25(OH)D were found between a cohort with normal and high PTH levels in one study despite finding a positive correlation between PTH and blood pressure (Jorde et al., 2000). Sodium intake and calcium excretion are tightly related (Zemel et al., 2001) and several studies report that increased dietary sodium intake can increase urinary calcium excretion (MacGregor et al., 1993) which could result in an increase in PTH and also $1,25(OH)_2D$. Some studies also report increased renal calcium excretion in hypertensive subjects (Gennari et al., 1986) which has been proposed as cause for elevated PTH levels in patients with essential hypertension (McCarron et al., 1980; St. John et al., 1994).

4.4 Vitamin D, Calcium and Blood Pressure

Vitamin D is critical for calcium absorption and homeostasis. Dietary calcium may lower the activity of the renin angiotensin system (Resnick et al., 1983) and inhibit vascular smooth muscle contraction (Bohr et al., 1963) and improve sodium/ potassium balance (Resnick et al., 1999). It is possible that the relationship between vitamin D and blood pressure could be mediated by its affect on calcium homeostasis. Several studies have identified a relationship between calcium intake or serum calcium levels and blood pressure. In addition, numerous randomised control trials of calcium supplementation have reported statistically significant reductions in systolic and / or diastolic blood pressure though results are inconsistent and the effect is small.

Calcium Intake and Blood Pressure

A relationship between calcium intake and / or supplementation and blood pressure has been identified in several meta-analyses (Griffith et al., 1999; Allender et al., 1996; Bucher et al., 1996; Birkett et al., 1998;Dickinson et al., 2006), In a systematic review by Chung et al., calcium supplementation in trials of hypertensive adults was associated with lower systolic but not diastolic blood pressure (Chung et al., 2009).

Serum Calcium and Blood Pressure

A significant inverse relationship between plasma ionized calcium and blood pressure has been found in a few studies (Hvarfer et al., 1986; Lind et al., 1987; Brickmann et al., 1990; Orwoll et al., 1990).

4.5 Vitamin D and Endothelial Dysfunction

Several studies including randomised controlled trials have provided evidence which suggests that vitamin D has a positive effect on endothelial function. Results however, have been inconsistent. Despite this, there is a strong biological plausibility underpinning vitamin D's potential effect.

The VDR and 1-α hydroxlase are found in endothelial and vascular smooth muscle cells (Somjen et al., 2005). Vitamin D regulates expression of several proteins in the arterial wall including vascular endothelial growth factor, matrix metalloproteinase type 9, myosin, elastin, type I collagen, and gamma carboxyglutamic acid, a protein that protects against arterial calcification (Norman et al., 2005).

It is believed that 1,25 dihydroxyvitamin D may exert a vasculo-protective effect through it's anti-inflammatory properties and down regulation of endothelial adhesion molecules as well as increasing the activity of endothelial nitric oxide synthetase (Talmor et al., 2008), (Molinari et al., 2011). It has been shown to increase prostacyclin production which may have an affect on vasodilation and atherosclerosis (Wakasugi et al., 1991). It has been suggested that 1,25(OH)D could increase vascular resistance by increasing the sensitivity to vasoconstrictors (Bukoski et al.,1990).

Chronic treatment with vitamin D reduces endothelial contractions and lowers arterial blood pressure in the aorta of the spontaneously hypertensive rat (Wong et al., 2010). Vascular smooth muscle tone was also doubled and nitric oxide evoked vasodilatation halved in young vitamin D deficient mice who also had markedly elevated blood pressure and heat rates (Tare et al., 2011).

Cross Sectional Studies

Most cross sectional studies in humans have shown an inverse association between vitamin D and endothelial dysfunction using different measures (Liu et al., 2013). Some studies were relatively large (N=554) (Yu et al., 2011) but many have included much smaller sample sizes (Duprez et al., 1993; Ertek et al., 2012; Jablonski et al., 2011).

In addition, to endothelial dysfunction studies have also found vitamin D to independently associated with increased carotid intimal/media thickness (Targher et al., 2006) decreased arterial compliance (London et al., 2007) and arterial calcification (Watson et al., 1997).

Randomised Controlled Trials

At least 12 randomised controlled trials of vitamin D and endothelial function have been performed (Liu et al., 2013). Results have varied with only six reporting a positive effect of supplementation (Schleithoff et al., 2006), (Sugden et al., 2008), (Tarcin et al., 2009; Dong et al., 2010; Harris et al., 2011; Witham et al., 2012). Different findings may in part be due to differences in study methodologies, vitamin D dose used, outcomes measures and participant characteristics. Study sizes were small and included those with varied conditions including stroke, diabetes, heart failure and peripheral arterial disease.

4.6 Vitamin D and Renoprotection

Vitamin D may also have reno-protective properties which might be mediated by it's affect on podocyte hypertrophy (Kuhlmann et al., 2004) and suppression of mesangial cell proliferation in the kidney (Hariharin et al., 1991). Vitamin D deficiency has been associated with an increased risk of proteinuria in the US adult population (De Boer et al., 2007). Vitamin D could protect against this, as reduction of angiotensin II through RAAS blockade may have an antiproteinuric and antifibrotic effects (Shroff et al., 2012). These reno-protective properties could explain how vitamin D might positively influence blood pressure status.

4.7 Studies Supporting a Role For Vitamin D in Hypertension

UVB Exposure, Vitamin D and Hypertension

Some cross sectional studies reporting geographical differences in blood pressure support an association between vitamin D and hypertension. This is based on the fact that UVB exposure is a surrogate marker of vitamin D synthesis in the skin which declines with increasing distance form the equator and is lower in the Winter than in the Summer.

Systolic and diastolic blood pressure were significantly and positively associated with distance from the equator in the INTERSALT study which examined more than 10,000 participants form around the world (Rostand et al., 1997). Geographic differences in blood pressure have also been observed in individuals of African origin, with those residing in northern regions having higher blood pressure than those living closer to the equator (Cooper et al., 1994).

Several studies have shown seasonal variations in blood pressure with it peaking in the Winter and falling in the Summer (Kristal-Boneh et al.,1996; Minami et al., 1996; Rosenthal et al.,2004)..

It has been postulated that UVB sun exposure might have the potential to lower blood pressure by inducing the cutaneous production of nitric oxide (Juzeniene et al., 2012) an effect which would be independent of vitamin D. In addition, a seasonal change in both systolic and diastolic blood pressure was found independent of vitamin D dose in a trial of 305 healthy postmenopausal woman aged 60-70 supplemented with vitamin D (Wood et al., 2012).

UVB Treatment and Blood Pressure

At least one study that investigated the effect of UVB treatment on blood pressure. In 18 individuals with mild hypertension aged 26 - 66 years who were randomised to receive UV-B or UV-A exposure three times weekly for six weeks, a 162% rise in serum 25(OH)D in the UV-B cohort was associated with a 6 mmHg drop in both systolic and diastolic blood pressure. No change in blood pressure was observed with UV-A exposure. Most subjects in the study had serum 25(OH)D levels well below 50 nmol/l suggesting that lower levels of vitamin D are associated with hypertension (Krause et al., 1998).

Vitamin D and Pre-eclampsia

Studies suggest that vitamin D may play a role in pathogenesis of pre-eclampsia, a condition that causes hypertension. There appears to be a racial disparity in pre-eclampsia with black females being more likely both to develop severe preeclampsia and suffer greater morbidity with it than white women (Eskenazi et al., 1991). The incidence of pre-eclampsia has been found to vary with season being the highest in

the Winter (Wellington et al., 2012) also supporting the hypothesis that vitamin D may play a role. In one study, an early pregnancy serum 25(OH)D of less than 37.5 and 50 nmol/l was associated with a respective fivefold and twofold increased risk after confounding for several factors (Bodnar et al., 2007). $1,25(OH)_2D$ regulation of vascular endothelial growth factor (VEGF) (Cardus et al., 2009) which has been reported as a predictive marker for pre-eclampsia (Bills et al., 2009) may be a possible mechanism to account for this. Vitamin D therefore may have a role in the pathogenesis of hypertension via decreased VEGF production (Ullah et al., 2010),

4.8 Clinical Studies of Vitamin D and Blood Pressure

Clinical studies to date that have explored the relationship between vitamin D and blood pressure have included cross sectional and longitudinal studies of vitamin D intake or serum 25(OH)D, as well as randomised controlled trials. Many studies were not primarily designed to look specifically at the relationship with blood pressure.

The majority of studies have found an inverse relationship, though there have been inconsistent findings. This may reflect a number of different factors such as differences in study population characteristics or inclusion criteria, methods of measuring blood pressure or defining hypertension, evaluation of 25(OH)D levels, treatment interventions and adjustment for potential confounding factors for blood pressure and vitamin D.

4.81 Vitamin D and Blood Pressure Studies -Pitfalls

Study Population

As many studies looked at blood pressure as a secondary outcome, many participants had other medical conditions including obesity, metabolic syndrome and cardiac disease and were therefore were not representative of the general population. It is also not known whether genetic factors in specific populations may account for a differential effect of vitamin D on blood pressure.

Confounding Factors

Several factors are known to affect blood pressure including sodium, calcium and alcohol intake, BMI, physical activity, use of medications including anti-

hypertensives, smoking, exercise and family history. Some factors that can affect vitamin D (as have been previously discussed) are also associated with blood pressure. Residual confounding may occur in studies where there has been inadequate adjustment for these covariates.

Factors related to the effect of vitamin D on Blood Pressure

It is not clear what level of vitamin D deficiency will activate the RAAS or affect blood pressure and hence the supplementation dose and duration of treatment that might be required to achieve a beneficial outcome. It is also unclear whether the affect of supplementation may be greater in those with higher blood pressure or a diagnosis of hypertension and whether it is age dependent. It has been suggested that in subjects taking medications which antagonise the RAAS system, the effect of vitamin D might be minimal as this is one of the potential mechanisms via which it exerts it's anti-hypertensive effect. Several trials of vitamin D supplementation did not measure baseline or post treatment serum 25(OH)D levels making it difficult to interpret results, especially in studies with poor compliance.

Blood Pressure Measurement and Definition

Methods of blood pressure measurement have varied between studies with many only taking one or two readings and only a few using 24 hour blood pressure monitors. Other studies have relied on 'self-reported' hypertension which may not be as reliable. Given the phenomen of 'white coat hypertension' it is possible that some studies may have included subjects as hypertensive who may have been normotensive giving false results. Studies have used different definitions of hypertension and some have looked only at the relationship with blood pressure readings. In addition, others have included only normotensive subjects.

4.82 Studies of Vitamin D intake and Blood Pressure

Studies that have looked at the relationship between vitamin D intake and blood pressure have given conflicting results but this may be due to the relatively small amounts of vitamin D consumed in the diet. A higher intake of cod-liver oil (which is a rich source of vitamin D) has also been inversely associated with systolic blood pressure in a study of 236 Icelandic adults aged 65-91 (Arnarson et al., 2012).

Negative Studies

The Nurses Health Study I (77,436 women aged 30 - 55 years with follow up at 18 years), the Nurses Health Study II (93,803 women aged 40 – 75 years with follow up at 8 years) and the Health Professional Study (51,529 men aged 40 -75 years with follow up at 16 years) found no significant association between self reported vitamin D intake and incident hypertension in multivariate analysis (Forman et al., 2005). This was despite some subjects consuming more than 1600 IU of vitamin D per day.

A cross sectional study of 15,596 community dwelling adults in Norway aged between 25 to 69 years, found no significant association between vitamin D intake and blood pressure after adjusting for age, BMI, alcohol and coffee consumption, cigarette smoking and physical activity (Jorde and Bonaa et al., 2000). The estimated mean daily intake for men and women was 240 IU and 360 IU which may not have been enough to detect a correlation.

Positive Studies

Normotensive subjects in the Women's Health Study (N= 8,886) aged 45 or older who had increased intake of dietary but not supplemental vitamin D had a reduced risk of self reported incident hypertension at ten year follow up after adjusting for several confounding factors (Wang et al., 2008). The discrepancy between the effect of supplemental and dietary vitamin D suggests other possible confounding factors.

Vitamin D intake but not supplementation was also associated with diastolic and systolic blood pressure in 615 males of Japanese descent in the Honolulu Heart Program though the correlation was small (Joffres et al., 1987).

Participants in the Women's Health Study (N=10,066) were found to have a reduced incidence of hypertension across increasing quartiles of dietary and supplementary vitamin D intake, though the only covariate adjusted for was age and blood pressure was self reported (Liu et al., 2005)

A study of normotensive females (N=86) found a significantly higher systolic blood pressure (+ 6mmHg) in those aged 20 to 35 years who consumed less than 400 IU of daily vitamin D after adjusting for age, BMI, calcium and alcohol intake. The same

comparison in older women aged 55 to 80 years (n = 222) was also associated with a higher systolic blood pressure (+ 4mmHg), (Sowers et al.,1985).

4.83 Clinical studies of serum vitamin D and blood pressure

Cross Sectional

Most cross sectional studies have found a inverse relationship between 25(OH)D and either systolic or diastolic blood pressure or a diagnosis of pre-hypertension/hypertension. Study methodology has varied widely and has included children, adolescents, middle age and older adults. Only few have included subjects not taking anti-hypertensives. Study sample sizes varied from very small to up to 27,153 and have included children, adolescents and older adults. Most studies show an association across all ages, though some adjusted for only few potential confounders. Many used the mean of two or three blood pressure readings in diagnosing hypertension but a few used 24 hour blood pressure monitors. The relationship appears to be more consistent in those who are vitamin D deficient (<50 nmol/l) with the potential effect on blood pressure reduction being greater for systolic than diastolic blood pressure.

In a meta-analyses of 18 studies, 14 cross sectional and 4 prospective, serum 25(OH)D was inversely associated with hypertension (OR 0.73, 95% CI 0.63-0.84). with a 16% reduction in the risk (OR 0.84, 95% CI 0.78-0.90) for an every 40 nmol/l increase in 25(OH)D (Burgaz et al., 2011).

In the largest study to date (using data from NHANES), increased systolic blood pressure in community dwelling US adults aged over 20 years was associated with reducing categories of vitamin D status (moving from 75-90 nmol/l down to less than 25 nmol/l) after adjustment for age, gender, ethnicity, body mass index, physical activity, smoking, co morbidities and blood pressure treatment (Scragg et al., 2010). Other large studies using data from the Nutritional Health and Examination Survey's (NHANES) in the US have found an inverse association (Martins et al., 2007; Scragg et al., 2007; Judd et al., 2008; Kumar et al., 2009; Nam et al., 2009; Sabanayagam et al., 2011; Gupta et al., 2011; Williams et al., 2011; Zhao et al., 2012).

An increased risk (OR 1.48) of pre-hypertension (SBP 120-139 or DBP 80-89 mmHg) was also found comparing the lowest versus the highest quartile of 25(OH)D in 9,215 participants free of hypertension in the 3rd NHANES study independent of BMI, GFR, and other factors (Sabanayagam et al., 2011).

In 3060 participants of the European Male Ageing study (males age 40-79 years), systolic blood pressure was associated with higher quintiles of vitamin D (<37.5 to 85.9nmol/l) (Lee et al., 2009).

In the Shangai Health Study which included 1460 subjects aged 40-74 years, 25(OH)D was inversely associated with both systolic diastolic blood pressure as well as hypertension, though only in males. Conversely, Chan et al., found no association in Chinese age over 65 years though mean 25(OH)D was high (Chan et al., 2012).

A serum 25(OH)D below 37.5 nmol/l versus 50-74.9 nmol/l was associated with an increased risk of hypertension (OR 3.2, 95% CI 1.0-11.1) in 833 males (mean age 71.1 ± 0.6 years) in a study that assessed blood pressure with a 24 hour blood pressure monitor (Burgaz et al., 2011). Other studies have shown a decrease in both diastolic and systolic blood pressure going from 25(OH)D levels below 25 –30.6 nmol/l up to and above 75 nmol/l (Farouhi et al., 2009; Pilz et al., 2009). Almirall et al also found an inverse relationship between systolic and diastolic blood pressure and 25(OH)D in those aged between 64-93 years of age (Almirall et al., 2009).

At least two out of three studies which included subjects not taking anti-hypertensives found a inverse association (Zhao et al., 2010; Jungert et al., 2012; Li et al., 2012)

Some studies have looked at the association between vitamin D and pulse pressure (the difference between systolic and diastolic blood pressure). A higher pulse pressure (as assessed by 24 hour blood pressure monitor) was associated with lower vitamin D status in a study of 171 subjects with stage 4/5 chronic kidney disease (Garcia-Canton et al., 2010). Conversely, no association between pulse pressure and 25(OH)D was found in a similarly small study of 156 adults though all were normotensive (Ertek et al., 2011).

In one small case control study using a 24 BP monitor (N=80), vitamin D was found to be lower in hypertensive dipper patients (32 nmol/l versus 54.8) versus non-dipper hypertensives despite similar daytime systolic and diastolic blood pressure as (Demir et al., 2012).

Negative Studies

Some negative studies have included subjects with relatively high mean serum 25(OH)D, (Lind et al., 1995; Scragg et al., 1995; Peters et al., 2009; Chan et al., 2011). Others have not adjusted for season and a had a small number who were hypertensive (Michos et al., 2009) or may not have been of sufficient statistical power and only recorded one blood pressure reading (Snidjer et al., 2007). No association was found by Frost et al. even when comparing those who were vitamin D deficient versus sufficient, however the study population was young and more likely to be normotensive (Frost et al., 2010). The study by Schmitz et al. was negative but only after adjustment for BMI (Schmitz et al., 2009). Rachel et al found no difference in diastolic blood pressure in males in a small case control study but did not measure systolic blood pressure (Reichel et al., 1992). Two studies reported an unexpected positive correlation between serum 25(OH)D and blood pressure but both were small and one involved dialysis patients (Brickmann et al., 1990; Argiles et al., 2002).

Cross Sectional Studies of 1,25 (OH)D and blood pressure.

There has been inconsistent findings in studies that looked at $1,25(OH)_2D$ and blood pressure with some finding positive, negative or no associations. However, 1,25 $(OH)_2D$ levels are affected by renal function and also depend on serum calcium levels. In addition, low, normal or high levels can be found in vitamin D deficiency or insufficiency (Pilz et al., 2009).

A weak but significant positive correlation between $1,25(OH)_2D$ and mean blood pressure was reported in 583 elderly not receiving any anti-hypertensive treatment (St John et al., 1994). However, other positive studies have been small (Sowers et al., 1988; Brickman et al., 1990; Al- Saleh et al., 2013).

Table 4.83: Cross sectional studies of serum 25(OH)D and Blood Pressure

Cross Sectional Studies of 25(OH)D and Blood Pressure				
Author	**Number**	**BP**	**HTN**	**Comment**
Scragg et al., 2010	27,153	Yes	-	
Martins et al., 2007	15,088	-	Yes	
Scragg et al., 2007	12,644	Yes	-	SBP and pulse pressure
Judd et al., 2008	7699	Yes	--	
He et al. 2011	7561	No	-	Insignificant in full model
Hypponen et., 2008	6810	-	Yes	
Kumar et al., 2009	6275	Yes	-	Younger adults, SBP
Zhao et al., 2010	5414	Yes	Yes	
Williams et al., 2011	4807	Yes	-	Adolescents
Hintzpeter et al., 2008	4030	-	Yes	
Sabanaygam et al., 2012	3712	Yes	-	
Reis et al., 2009	3577	Yes	Yes	Adolescents
Lee et al., 2009	3069	Yes	-	SBP
Gupta et al., 2011	1711	-	Yes	Pre-hypertension
Nam et al., 2012	1504	No	-	Aged 12-18 yrs
Dorijgochoo et al., 2012	1460	Yes	Yes	Only in males
Schimtz et al., 2009	1334	No	-	
Snijder et al., 2007	1205		No	
Chan et al., 2012	939	No	-	High mean 25(OH)D
Pasco et al., 2009	861	Yes	Yes	
Burgaz et al., 2011	833	Yes	-	24 BP monitor
Frost et al., 2010	783	No	-	Young adults
Michos et al., 2009	650	No	No	
Pilz et al., 2009	648	Yes	-	
Forouhi et al., 2008	524	Yes	-	SBP & DBP
Seiki et al., 2012	495	Yes		Severe kidney disease, DBP
Gannage-Yared et al., 2009	381	Yes	-	Young adults, SBP
Garcia et al., 2013	332	No	No	PTH assoc with SBP/DBP
BP – Systolic or Diastolic Blood Pressure, HTN - Hypertension				

Table 4.83: Cross Sectional Studies of 25(OH)D and Blood Pressure

Cross Sectional Studies of 25(OH)D and Blood Pressure				
Author	**Number**	**BP**	**HTN**	**Comment**
Scragg et al., 1992	295	-	No	
Kruger et al., 2013	291	Yes	-	SBP &DBP, middle aged
Thomas et al., 1998	290	-	Yes	
Almirall et al., 2009	237	Yes	-	SBP &DBP
Caro et al., 2012	219	No	-	Most were overweight,
Smotkin-Tangorra et al., 2007	217	Yes	-	SBP
Kokot et al., 1981	212	No	Yes	
Peters et al., 2009	205	No	-	Adolescents
Reichel et al., 1992	157	No	-	
Ulu et al., 2013	152	Yes	-	
Zhou et al., 2011	140	Yes	-	Obese children
Jungert et al., 2012	132	Yes	-	Only in males
Kulah et al., 2007	118	Yes	-	24 BP monitor
Kristal-Boneth et al., 1997	100	No	-	

4.84 Longitudinal Studies of serum 25(OH)D and Blood Pressure

Several prospective studies show an inverse relationship between lower vitamin D status and incident hypertension (Forman et. al 2007; Forman et al., 2008; Griffin et al., 2010; Kim et al., 2010). However, there has been inconsistent findings with some reporting no association (Forouhi et al., 2008; Jorde et al., 2010; Bolland et al., 2010; Margolis et al., 2012; Skaaby et al., 2012; Ke et al., 2013). Studies that have a found an increased risk of hypertension have usually included subjects who are vitamin D deficient (< 50 nmol/l).

In a recent meta-anaylsis of seven prospective studies, a reduced risk of incident hypertension was found when comparing the highest versus the lowest tertiale of serum 25(OH)D (RR. 0.70, 95% CI 0.58-0.86). A 12% reduction in incident hypertension was identified with every 25 nmol/l rise in 25(OH)D from baseline (Kunutsor et al., 2013). Meta analysis of four other studies by the same authors found

no overall change in the risk of future hypertension when comparing oral intake of vitamin D (RR 1.0, 95% CI, 0.95-1.05), (Kunutsor et al., 2013).

An earlier meta-analysis of three studies also found an increased risk of incident hypertension after 7-8 years (RR 1.76, 95% CI 1.3-2.4) when comparing subjects in the lowest concentration category (< 37 – 51 nmol/l) with those in the highest (> 75 to 81 nmol/l) (Pittas et al., 2010).

Positive Studies

In 613 men in the Health Professional Follow Up Study (HPFS) and 1198 women in the Nurses Health Study (NHS) there was an increased risk of incident hypertension (OR 3.18) in those with 25(OH)D below 37.4 nmol/l versus 75 nmol/l or greater (Forman et al., 2007). A further analysis which included 38,383 males in the HPFS and 77,531 females in the NHS but used predicted 25(OH)D concentrations, found an increased risk of hypertension in both cohorts (OR 2.31, 95% CI 20.3-2.63) and (OR 1.57, 95% CI1.44-1.72) comparing the lowest with the highest decile of vitamin D (Forman et al., 2007).

A nested case control study of 1484 in the NHS also found an increased risk of hypertension when comparing the lowest with the highest quartile of vitamin D (OR 1.66, 95% CI 1.11-2.48). An 8% increased risk was found for every 12.5 nmol/l decrease in 25(OH)D (Forman et al., 2008). Both studies above by Forman et al. did not measure blood pressure but relied on a self reported diagnosis of hypertension, however, participants were nurse's or healthcare workers.

In 559 females aged between 22 - 44 years, 25(OHD)D below 80 nmol/l was associated with an increased risk of systolic hypertension (OR 3.0, 95% CI 1.01 –8.7) at 14 year follow up, but not rate of change of blood pressure (Griffin et al., 2011).

A 25(OH)D below 46.8 nmol/l at baseline was associated with an increased risk of hypertension at 4 year follow up (OR 2.74, 95% CI 1.40 - 5.34) in 324 Korean subjects aged over 40 (Kim et al., 2010).

Negative Studies

In an analysis of 4863 participants of the WHI, no significant difference in the adjusted mean change in systolic or diastolic blood pressure or incident hypertension was found over a 7 year follow up. There was no difference in incident hypertension comparing those with 25(OH)D below 25 nmol/l versus above 50 nmol/l after adjusting for covariates. A slightly higher risk of incident hypertension (OR 0.67, 95% CI 0.46 - 0.96) was however, identified in those with 25(OH)D between 47.7 – 64.6 nmo/l versus less than 34.4 nmol/l but may have been due to chance (Margolis et al., 2012). However, diagnosis of hypertension was self reported and no follow up 25(OH)D levels were taken.

In a study of 2385 subjects (mean age 56.2 ± 9.3 years) no association was found between baseline vitamin D status and change in blood pressure at 14 year follow up. In addition, the lowest 5th and 10th percentile of vitamin D (<29.6 nmol/l and <34 nmol/l) did not predict future hypertension. This was despite finding a significant inverse relationship with baseline 25(OH)D (Jorde et al., 2010).

No increased risk of risk of blood pressure was found in a study of 4,330 subjects aged 30 – 60 years, after a follow up of five years. The median 25(OH)D at baseline was 48.0 nmol/l (Skaaby et al., 2012).

Likewise, in 1,957 middle aged male Finnish smokers, no association was found between 25(OH)D and blood pressure at 4 year follow despite a significant inverse relationship with systolic blood pressure at baseline. A large proportion (63%) of subjects had hypertension at study entry (Ke et al., 2013).

In 1,471 community dwelling females (mean age 74 years), those with a seasonally adjusted 25(OH)D of less than 50 nmmol/l did not have an increased risk of higher systolic or diastolic blood pressure at approximately 5 year follow up. However, subjects were participants of a trial of calcium supplementation and were largely free of medical disease (Bolland et al., 2010).

Finally, in the Ely study baseline 25(OH)D levels in 524 subjects aged 40-69 years were not associated with new onset hypertension or changes in blood pressure at 10 year follow up despite an association with blood pressure readings at baseline (Forouhi et al., 2008). However, age adjusted mean 25(OH)D was relatively high being 64.5nmol/l and 57.2 nmol/l in males and females respectively (Forouhi et al., 2008).

Table 4.84: Longitudinal Studies of 25(OH)D and Blood Pressure or Hypertension

Author	No.	Age	Duration	HTN	BP	Comments
Margolis et al. 2012	4863	66.0	7 yrs	No	No	Overall, no difference across quartiles of 25(OH)D
Skaaby et al., 2012	4330	30-60	5 yrs	-	No	High baseline 25(OH)D
Jorde et al., 2010	2385	56.2	15 yrs	-	No	No difference comparing 5^{th} versus 95^{th} percentile of 25(OH)D
Ke et al., 2013	1957	-	4 yrs	-	No	Association with SBP at baseline
Foreman et al., 2008	1484	43.0*	8 yrs	Yes	Yes	OR 1.47 when 25(OH)D < 50 nmol/l
Bolland et al., 2010	1471	74.0	5 yrs	-	No	No increased risk when comparing <50nmol/l vs. ≥ 50nmol/l
Kim et al., 2010	1337	65.8*	4 yrs	Yes	-	Risk in normotensives when 25(OH)D < 46.8 nmol/l
Foreman et al., 2007	1198	57.0	8 yrs	Yes	-	OR 1.70 when 25(OH)D <37.5nmol/
Foreman et al., 2007	613	65.0	8 yrs	Yes	-	OR 3.53 when 25(OH)D <37.5nmol/l,
Griffin et al., 2010	559	24 - 44	14 yrs	-	Yes	OR 3.0 when 25(OH)D < 80 nmol/l
Forouhi et al., 2008	524	40-69	10 yrs	-	No	No difference, high baseline vitamin D
HTN – Hypertension, BP – Blood Pressure. [Age quoted as range, mean or median*]						

4.85 Intervention trials of vitamin D and blood pressure.

There has been a lack of well designed randomised controlled trials to examine the effect of vitamin D on blood pressure and many have been small in size. The vast majority of studies were not primarily designed to investigate blood pressure as a primary outcome and have included a heterogeneous groups of patients including pregnant women, subjects with obesity, chronic renal disease and heart failure. Some have used high dose vitamin D in deficient older adults and found no effect. Other trials have used activated vitamin D [$1,25(OH)_2D$] and found an improvement in blood pressure. Overall, results are conflicting but suggest a possible small effect on blood pressure.

Meta-analyses of RCT'S of vitamin D and Blood pressure

Four meta-analyses of randomised controlled trials involving vitamin D and blood pressure have been performed (Witham et al., 2009; Pittas et al., 2010; Wu et al., 2010; Elamin et al., 2011). Three showed a positive effect of vitamin D on either diastolic or systolic blood pressure.

The largest of these included ten randomised controlled trials (N=32,181) and found a non-significant reduction in systolic blood pressure (weighted mean difference -1.9 mmHg) and no effect on diastolic blood pressure (Pittas et al., 2010). Two of the trials in the analysis used vitamin D in combination with calcium, one used vitamin D2 and another examined the affect of UVA exposure. The dose range of vitamin D varied from 400 to 8571 IU. Follow up ranged from 5 to 52 weeks in most studies and was 7 years in the Women's Health Initiative trial. No difference in the change in systolic and diastolic blood pressure was found between trials that used vitamin D alone or in combination in with calcium. The change in systolic blood pressure did not differ between trials that used higher ($\geq$ 1000 IU / day) versus lower (< 1000 IU / day) vitamin D doses. However, there was a significant difference on the affect on diastolic blood pressure in trials that used a higher dose of vitamin D (-1.5 mmHg, P = 0.039), (Pittas et al., 2010).

A separate meta-analysis of eight randomised trials (N=5500) showed a non-significant reduction in systolic blood pressure but a small and statistically significant

reduction in diastolic blood pressure (-3.1 mmHg). This analysis included four trials that used activated vitamin D, though subgroup analysis suggested that the inactivated form produced a greater fall in systolic blood pressure (Witham et al., 2010). In addition, no effect was seen in studies where subjects where normotensive at baseline.

A meta-analysis of 14 randomised controlled trials involving 1518 subjects found no significant effect of vitamin D supplementation. However, there was a trend for a reduction, though there was significant heterogeneity between studies (Elamin et al., 2011).

Finally, the smallest meta-analysis which included four randomised controlled trials involving 429 participants found that vitamin D supplementation resulted in a significant reduction in systolic blood pressure by 2.44 mmHg but not diastolic blood pressure compared with placebo. Subgroup analysis suggested that the change in blood pressure did not vary much across the dose of vitamin D used or study length (Wu et al., 2010).

RCT's using non activated vitamin D [25(OH)D]

Positive Studies

Seven intervention studies to date using non-activated vitamin D [25(OH)D] have found a favourable effect of vitamin D on blood pressure. Positive trials have been small and of short duration (between eight to twenty weeks) and generally included those with low serum 25(OH)D (Pfeiffer et al., 2001; Sugden et al., 2008; Larsen et al., 2012; Goel et al., 2011; Wood et al., 2012; Asemi et al., 2013; Forman et al., 2013), Most positive studies report a reduction in systolic but not diastolic blood pressure.

In 250 black subjects (median age 51 years), treatment with vitamin D3, 1000, 2000 or 4000 IU daily resulted in a respective reduction in SBP of -0.66, -3.4 and -4.0 mmHg at 3 months versus placebo (Forman et al., 2013). For every 2.49 nmol/l increase in 25(OH)D there was a 0.2 mmHg reduction in SBP. No effect of vitamin D on DBP was identified.

In 148 normotensive community dwelling females aged seventy or over and who had serum 25(OH)D levels of less than 50 nmol/l, supplementation with 800 IU of vitamin D3 plus 1200 mg of calcium for eight weeks significantly reduced systolic blood pressure versus treatment with calcium alone. Whilst there was a significant net difference in systolic blood pressure of - 7.4 mmHg between groups (P=0.02) no difference in diastolic blood pressure was found. Serum 25(OH)D increased by 20.48 nmol/l in the combined treatment versus the calcium only cohort (Pfieffer et al., 2001).

In a study of 112 patients (mean age 61 years) with 25(OH)D levels below 80 nmol/l, treatment with vitamin D3 resulted in a significant reduction in SBP and DBP as assessed by 24 BP monitor (Larsen et al., 2012).

In another RCT involving 34 patients with stable diabetes and serum 25(OH) D levels of less than 50 nmol/l, those assigned a one time treatment with 100,000 IU of vitamin D2 were found to have a significant decrease in systolic blood pressure (- 7.3 mmHg) versus the placebo group (+ 6.6 mmHg) at eight weeks (P = 0.001) Whilst there was a reduction in diastolic blood pressure (net difference -4.5 mmHg) it did not reach significance (P = 0.08). Serum 25(OH)D increased by 15.3 nmol/l relative to placebo (Sugden et al., 2008).

The addition of vitamin D supplement (33,000 IU D3 every 2 weeks for 3 months) to 100 hypertensive patients (aged over 35 years) already assigned to receive conventional treatment was found to reduce systolic blood pressure compared to controls on blood pressure therapy only (Goel et al., 2011).

Finally, in one small RCT, treatment of 24 pregnant women with 400 IU of vitamin D3 daily for nine weeks resulted in a significant reduction in systolic and diastolic blood pressure compared to placebo (Asemi et al., 2013).

Negative Studies

In the largest (N=36,282) and longest trial the Women's Health Initiative, supplementation with 1000mg of calcium and 400 IU of vitamin D3 daily versus placebo in a random double blind fashion in postmenopausal women aged between 50

to 79 years did not show any significant decrease in the incidence of hypertension after a median follow up of 7 years, even when comparing those in the lowest versus highest quartile of 25(OH)D at baseline (Margoli et al., 2008). However, a number of factors might account for this result. The level of compliance was only modest at 60%, blood pressure criteria were not strict and were probably not blinded to staff and participants, and incident hypertension was self reported. The dose of vitamin D used was relatively low and post treatment 25(OH)D were not measured. On the basis of doses and adherence, the effect of supplementation was estimated to increase 25(OH)D by only 5nmol/l which is unlikely to have had an affect.

In an RCT of 330 obese subjects aged 21 to 75 with diabetes, daily treatment with calcium (500 mg) and vitamin D (20,000 IU or 40,000 IU per week) for one year did not result in a change in blood pressure versus placebo. This was despite an increase in serum 25(OH) D by 43 and 92 nmol/l in the two respective groups of vitamin D supplementation. Conversely, there was a small but significant increase in systolic blood pressure in those treated with 20,000 IU per week versus the placebo group (Jorde et al., 2010).

Similarly, in a trial of 165 subjects with a BMI greater than 27 kgm^{-2} and aged between 18 to 70, no significant difference in systolic or diastolic blood pressure was found between those randomised to receive daily vitamin D3 (3332 IU) or placebo. Mean 25(OH)D at baseline was 30 nmol/l and increased by 43.2 nmol/l after supplementation (Zittermann et al., 2009). However, the primary goal was to measure weight loss and fat loss. In addition, blood pressure was only measured once at the beginning and end of the study. Patients were also overweight which may have affected the response to vitamin D.

Another RCT of 159 older adults (mean age 77 years) with isolated hypertension, treatment with 100,000 IU of vitamin D3 versus placebo every three months for one year did not result in any reduction in office readings in SBP (Witham et al., 2013). In addition, no effect on 24 hour blood pressure was found despite a rise in mean serum 25(OH)D from 44.8 nmol/l to 69.8nmol/l. Whilst this was the largest RCT primarily designed to investigate the effect of vitamin D on blood pressure the number was still relatively small. However, there was a statistically non-significant between group

difference in SBP of –0.7 mmHg suggesting a potential small effect of vitamin D on blood pressure.

In a trial involving 189 females aged 63 -76 (mean age 70) with a mean 25(OH)D of 33nmol/l, no change in blood pressure was found at five weeks after supplementation with a single dose of 100,000 IU D3 during the Winter months. This was despite a net rise in 25(OH)D of 18.9 nmol/l. There was however a trend towards reduction in both systolic and diastolic blood pressure (Scragg et al., 1995).

In a trial of 63 subjects (mean age 41-43) with a body mass index between 27-40, blood pressure less than 160/95 and daily calcium intake of less than 800 mg per day, no significant difference in systolic or diastolic blood pressure was found between groups randomised to receive daily vitamin D3 (400 IU) and calcium (1200 mg) or placebo over a 15 week period. No serum 25(OH)D levels were checked and the dose of vitamin D was relatively small (Major et al., 2007).

One negative trial included treatment of 90 subjects who were vitamin D deficient (<50 nmol/l) with high dose vitamin D for three months (Sokoi et al., 2013). Similarly, another involved 52 subjects who were also vitamin D deficient (<50 nmoll) and treated with high dose vitamin D (7000 IU daily) for 26 weeks but were obese (Wamberg et al., 2013). In addition, treatment of 75 patients (mean age 66 years) with 100,000 IU of vitamin D3 at baseline, two and four months did not result in any significant change in SBP or DBP (Witham et al., 2013). Similarly, in an RCT of 46 subjects, treatment with 50,000 IU of vitamin D / week for 12 weeks followed by 50,000 IU every alternate week for 40 weeks did not result in any significant change in blood pressure compared to placebo. However, study participants had stage two-three chronic kidney disease (Alvarez et al., 2012).

Another trial involved treatment of 265 post-menopausal woman aged 60-70 years for one year with either 400 IU or 1000 IU of vitamin D3 but found no change in blood pressure versus placebo (Wood et al., 2012).

Most other negative interventions have involved subjects with either higher 25(OH)D levels or younger age or lower baseline blood pressure (Schleithoff et al., 2006;

Myrup et al., 1992; Orwoll et al., 1990; Zittermann et al., 2009; Nagpal et al., 2009; Jorde et al., 2010; Witham et al., 2010). Some trials that were negative used a relatively low dose of vitamin D (Margolis et al., 2008; Major et al., 2007).

Intervention studies involving supplementation with alphacalcidiol [1,25(OH)$_2$D]

Of six trials of oral alphacalcidiol supplementation, four found a blood pressure lowering effect (Lind et al., 1987; Lind et al., 1988; Lind et al., 1988; Lind et al., 1989; Myrup et al., 1992: Judd et al., 2010). None of these studies measured serum vitamin D, the supplementation dose used was low and duration was short. Apart from one study, those that were positive included subjects who hypertensive or older adults. Two trials that were negative involved subjects who were either normotensive or younger.

Intravenous 1,25(OH)D$_2$ has also been shown to reduce systolic and diastolic blood pressure but the effect is inconsistent (Mak et al., 1992; Jespersen et al., 1998). Conversely, administration of paracacitriol (a vitamin D analogue) was found to have no effect on 24 hour ambulatory systolic of diastolic blood pressure (Alborzi et al., 2008).

Positive Studies

In a non randomised non-placebo study, treatment of 3 subjects (mean age 46.5 years) with 0.5 mcg of alphacalcidiol twice daily for one week resulted in a 9% decrease in mean SBP as measured by a 24 hour blood pressure monitor with blood pressure returning to pre-treatment levels following discontinuation (Judd et al., 2010).

In a trial involving 65 males aged 61 to 65 with impaired glucose tolerance, daily supplementation with 0.75 mcg of alphacalcidiol versus placebo for twelve weeks was associated with a significant net reduction in systolic blood pressure by 4.0 mmHg and DBP by 1.6 mmHg but only in those with pre-treatment hypertension. (Lind et al., 1988).

In two other trials (Lind et al., 1987:Lind et al., 1988) involving hypertensive subjects with a mean age of 65 and 63 respectively, treatment with 1.0 mcg alphacalcidiol for six months resulted in a significant net reduction in diastolic blood pressure. A

reduction in systolic blood pressure was also found in one of these two trials (Lind et al., 1987) though subjects in both studies included those with primary hyperparathyroidism and intermittent hypercalcaemia.

Negative Studies

In a randomised controlled trial of 42 subjects with untreated mild to moderate hypertension and with a mean age of 51 years, daily supplementation with 1μg of alphacalcidiol for 18 weeks did not result in any change in systolic blood pressure. (Lind et al., 1989)

Finally, treatment with alphacalcidiol in normotensive women aged 70 years for twelve months resulted in no significant change in blood pressure. Patients were randomised to any one of four treatment arms which included ether placebo alone or in combination with alphacaldiol (0.5mcg or 1.0 mg /day) or (oestrogen/ norethindrone). No change in blood pressure within or between groups was found. (Myrup et al., 1992).

Table 4.85: Intervetion Trials of Vitamin D and Blood Pressure

Intervention Trials of Vitamin D and Blood Pressure					
Author	**No.**	**Duration**	**Intervention**	**Effect**	**Comment**
Margoli et al., 2008	36,282	7 yrs	400 IU D3 + 1000 mg Ca^{2+}	No	Low dose of vitamin D, poor compliance
Jorde et al., 2010	330	1 yr	40,000 IU D3 + 500 mg Ca^{2+}	No	Diabetic
Wood et al, 2012	265	I yr	400 or 1000 IU daily vs placebo	No	Healthy post menopausal aged 60-70
Forman et al.,2013	250	3 months	1000, 2000, or 4000 IU D3 daily vs placebo	Yes	Reduction in SBP
Goel et al., 2011	200	3 months	33,000 IU D3 or placebo/ 2 weeks + BP tablet	Yes	Reduction in SBP
Scragg et al., 1995	189	5 wks	100,000 IU D3 stat	No	Trend for reduction in DBP &SBP, mean 25(OH)D 33 nmol/l
Zittermann et al., 2009	165	1 yr	3332 IU D3 / day	No	Overweight, mean 25(OH)D 33 nmol/l
Witham et al.,2013	159	1 yr	100,000 IU D3 every 3 months vs. placebo	No	Baseline 25(OH)D was 44.8 nmol/l, hypertensive
Pfeiffer et al., 2001	145	8 wks	800 IU D3 / day	Yes	Subjects 25(OH)D < 50 nmol/l
Larsen et al.,2012	112	20 wks	3000 IU D3 day vs placebo	Yes	Baseline 25(OH)D 57.2nmol/l, Reduction in SBP
Schleithoff et al., 2006	93	35 wks	2000 IU D3 / day	No	Subjects with heart failure, mean 25(OH)D 37 nmol/l
Sokol et al., 2012	90	12 wks	50,000 D2 weekly vs placebo	No	Subjects with 25(OH)D < 50 nmol/l
Witham et al.,2013	75	6 months	100.000 IU D3 at, 2, 4 ,6 months vs. placebo	No	
Myrup et al., 1992	74	1 yr	0.5 mcg 1α D / day	No	Normotensive
Nagpal et al., 2009	71	6 wks	120,000 IU D3 every 2 wks	No	Mean baseline 25(OH)D –63 nmol/l and BP 124/78
Lind et al., 1988	65	12 wks	0.75 mcg 1α D / day	Yes	Reduction in SBP &DBP, only in those with hypertension

Table 4.85: Intervention Trials of Vitamin D and Blood Pressure

Intervention Trials of Vitamin D and Blood Pressure					
Author	**Number**	Duration	**Intervention**	**Effect**	**Comment**
Orwoll et al., 1990	65	3 yrs	1000 IU D3 + 1000 mg Ca^{2+} /day	No	Normotensive
Major et al., 2007	63	15 wks	400 IU D3 + 1200 mg Ca^{2+}/day	No	Overweight, young (aged 27-40).
Witham et al., 2010	61	8 wks	100,000 or 200,000 IU D3 stat	Yes	Diabetic subjects, SBP reduction
Witham et al., 2010	58	8 wks	100,000 IU D2 vs placebo	No	Mean 25(OH)D 38 nmol/l and BP 128/72
Wamberg et al., 2013	52	26 wks	7000 IU D3 daily vs placebo	No	Subject BMI >30. Baseline 25(OH)D < 50 nmol/l
Asemi et al., 2013	48	9 wks	400 IU D3 daily vs placebo	Yes	Pregnant women, reduction in SBP & DBP
Alvarez et al., 2012	46	1 yr	50,000 IU/wk x 12 wks vs placebo	No	Subjects with chronic kidney disease (stage 2-3)
Lind et al., 1989	42	18 wks	1.0 mcg 1α D / day	No	Hypertensive
Sugden et al., 2008	34	8 wks	100,000 IU D2 / day	Yes	25(OH)D < 50 nmol/l, reduction in SBP
Lind et al., 1988	33	6 months	1.0 mcg 1α D / day	Yes	Hypertensive, reduction in DBP
Lind et al., 1987	29	6 months	1.0 mcg 1α D / day	Yes	Hypertesnsive, reduction in SBP & DBP
Judd et al., 2010	3	I week	0.5mcg 1α D /day	Yes	24 BP monitor, non randomised, non placebo

4.9 Conclusion.

Most studies show an inverse relationship between serum 25(OH) and blood pressure. Results from longitudinal studies are however inconsistent. In addition, there is a distinct lack of a high quality large randomised controlled trials and insufficient evidence to date to prove causality or recommend it as a treatment. Despite this, there is a good biological plausibility underpinning the potential mechanisms through which vitamin D could lower blood pressure. These include regulation of the renin angiotensin aldosterone system, endothelial function, serum calcium and PTH levels and potential renoprotective effects. Most large population studies suggest that vitamin D may have a greater effect on systolic compared to diastolic blood pressure. Lack of adjustment for confounders, inadequate blood pressure measurements and non-exclusion of study participants taking anti-hypertensive medications may account for some studies being negative or falsely positive. It is possible that long-term vitamin D deficiency may give rise to a 'hypertensive disease state' that may not be amenable to treatment. The relationship appears more consistent in those who are hypertensive and vitamin D deficient (< 50 mmol/l) and this target group may be more appropriate for future intervention trials.

CHAPTER 5: VITAMIN D AND FALLS

5.1 Introduction

Falls are common in older adults with one in three people aged 65 or older and up to one in two aged 80 or older experiencing one or more falls each year (Tinetti et al., 1988) (Annweiler et al., 2010). Whilst multiple factors contribute to falls, it is known that gradual loss of muscle strength below a certain threshold results in functional impairment and increased risk of falls and fractures (Wolfson et al., 1995).

Vitamin D deficiency appears to be related to falls as it is associated with proximal muscle weakness (Schott et al., 1976). Furthermore, there is strong biological evidence supporting a role for vitamin D in muscle function. The vitamin D receptor (VDR) and the metabolic pathway for it's activation is found in human muscle cells where it is known to affect metabolism and calcium uptake (Celgia et al., 2008).

Several studies have shown that vitamin D status is positively associated with muscle strength and physical performance and inversely associated with falls. Vitamin D supplementation has also been shown to improve tests of muscle function and balance and positively impact on muscle fibre composition and morphology in vitamin D deficient older adults (Ceglia et al., 2008). Finally, numerous randomised controlled trials have found that vitamin D reduces either the rate of falls or falls risk. Despite this, the effect of vitamin D on falls appears to depend on several factors and it remains unclear who benefits most. The absolute threshold level of 25(OH)D needed to prevent falls in an elderly population is not known and selecting patients at risk for falls and defining the appropriate dose remain as areas in need of further research (Rosen et al., 2012). It may be that vitamin D supplementation with adequate calcium may be required for falls prevention and that the effect may depend on baseline 25(OH)D status, age and mobility (Ringe et al., 2012).

5.2 Vitamin D and Myopathy

The first associations between vitamin D and muscle function were made from clinical observations of muscle weakness in patients with osteomalacia due to vitamin D deficiency (Prineas et al., 1965). This generally presented in adults with proximal

muscle weakness and is known to develop independently of metabolic abnormalities such as hypocalcaemia, hypophosphataemia and hyperparathyroidism (Boland et al., 1986). A similar myopathy has been described in patients with end stage renal failure or malabsorption syndromes, both of which are associated with vitamin D deficiency (Ekbom et al., 1964; Smith et al., 1967).

Vitamin D and Muscle Morphology

Vitamin D deficiency has been associated with predominantly type II muscle fibre atrophy in adults. The selective affect on type II muscle fibres (which are fast-twitch and are first to be recruited to prevent a fall) may help to explain the falling tendency of vitamin D deficient elderly individuals (Snidjer et al., 2006).. Muscle biopsy histology in vitamin D deficient adults also reveals fat infiltration, fibrosis, glycogen granules and enlarged interfibrillar spaces (Yoshikawa et al., 1979)..

Two studies have reported a positive affect of vitamin D supplementation on muscle fibre composition. Sorenson et al., showed that treatment with $1,25(OH)_2D$ and calcium for 3 to 6 months resulted in an increase in the relative composition and fibre area of type IIa muscle fibres (Sorenson et al., 1979). Treatment of elderly stroke survivors with 1000 IU of vitamin D2 over a two year period significantly increased the percentage and mean diameter of type II muscle fibres (Sato et al., 2005). In the same study, serum 25(OH)D levels were also correlated with type II muscle fibre diameter at baseline and at two year follow up.

5.21 Mechanism of Action of Vitamin D in Muscle

Vitamin D acts on muscle through VDR dependent and non-dependent mechanisms. VDR is expressed in cloned human skeletal muscle cells (Costa et al., 1986) and has been detected in situ in human skeletal muscle tissue (Bischoff et al., 2001) where its expression decreases significantly with increasing age (Bischoff-Ferrarri et al., 2004).

Regulation of Calcium Uptake

$1,25(OH)_2D$ regulates muscle calcium uptake in chick skeletal muscle cells by modulating the activity of muscle calcium pumps in the sarcoplasma reticulum and sarcolemma. It also regulates calcium influx via voltage –sensitive calcium channels, thereby modulating intracellular calcium (Boland et al., 1986) which affects muscle

function (Ebashi et al., 1985). Treatment of cultured myoblasts with $1,25(OH)_2D$ also results in increased calcium uptake (de Boland AR et al, 1985; Walters et al., 1987). Activation of the vitamin D receptor increases the synthesis of calmodulin, a calcium binding protein that plays a role in muscle contraction (Zanello et al., 1995).
$1,25(OH)_2D$ also appears to play a role in phosphate metabolism stimulating accelerated phosphate uptake and accumulation in cells, an affect thought to mediated via a VDR dependent affect on de novo protein synthesis (Boland et al., 1995).

Muscle Cell Differentiation

$1,25(OH)_2D$ appears to play role in muscle cell differentiation and proliferation. It has been shown to increase both cell density and fusion in cultured chicken embryo myoblasts (Giuliani et al., 1984) and have a mitogenic affect in proliferating myoblasts (Drittranti et al., 1989) affecting expression of cell cycle genes such as c-myc, and c-fos and other skeletal muscle cell proteins (Drittanti et al., 1989).

Rapid Non Genomic affects

$1,25(OH)_2D$ has been shown to have a rapid affect on calcium uptake in skeletal muscle cells in vitamin D deficient chicks (de Boland and Boland, 1987) believed to be mediated by a non-VDR dependent mechanism. The non-genomic response to $1,25(OH)_2D$ may depend upon activation of MAPK pathways which are known to regulate several cells processes. $1,25(OH)_2D$ activates ERK-1/2, phospholipase C and c–myc, components of the intracellular signal pathway (Morelli et al., 2001).

5.22 Vitamin D, PTH and Muscle

The potential effect of vitamin D on falls may be in part mediated by PTH which may have direct affects on skeletal muscle. Indeed, higher PTH levels in nursing home residents has been associated with falling independent of 25(OH)D (Stein et al., 1999). Likewise, serum PTH was found to be an independent predictor of time to first falls in a study of frail elderly (Sambrook et al., 2004). In patients with hyperparathyroidism, symptoms of muscle fatigue and weakness are found (Kristoffersson et al., 1992) and muscle biopsies reveal atrophy of type II muscle fibres as in vitamin D deficiency (Patten et al., 1974). Secondary hyperparathyroidism

has also been associated with impaired muscle strength (Mallette et al., 1975; Hedman et al., 1984). Studies in rat models have shown that PTH induces muscle catabolism (Garber et al., 1983) and reduces calcium transport and metabolism in skeletal muscle (Smogorzewski et al., 1988). Hypophosphataemia resulting from high PTH levels could also result in muscle weakness (Hoogendoorn et al., 2003).

5.3 Vitamin D and Physical performance

There are conflicting results from studies looking at the relationship between vitamin D and physical performance. Many have included older people though vitamin D status has also been positively associated with improved physical performance in young adults (Foo et al., 2009; Ward et al., 2009). Vitamin D deficiency (<50nmol/l) has been associated with several physical measures including increased risk of body sway (Pfeifer et al., 2001; Okuno et al., 2009), and poorer performance in balance, gait speed, physical activity level (Gerdhem et al., 2005) and timed up and go (Dukas et al., 2005).

A systematic review in 2009 examining the effects of low serum 25(OH)D and vitamin D supplementation on muscle, balance and gait performance among people aged 65 and older found inconsistent results (Annweiler et al., 2009). In a more recent meta-analysis of adults aged 60 years or older, supplemental vitamin D (800 –1000 IU daily) was found to reduce postural sway, improve timed up and go performance and increased lower extremity strength (Muir et al., 2011). Differences in response to treatment may be due to significant heterogeneity in baseline vitamin D levels and physical status.

Cross sectional Studies

The association between 25(OH)D and physical performance tends to be in those with vitamin D deficiency (<50 nmol/l). The relationship in the 'oldest old' may be more complex and indeed no association was found between 25(OH)D and physical performance in 367 octagenarians in the Befrail study after adjustment for multiple confounds (Mathei et al., 2013). Despite this, serum 25(OH)D was associated with appendicular lean mass and physical performance in a smaller study of frail elderly (Tieland et al., 2013). In addition, vitamin D was associated with walking speed, and

muscle strength in 1659 community dwelling elderly Autralian males (Hirani et al., 2013).

Levels below 25 and 50 nmol/l were associated with deterioration in physical performance and strength in 2641 subjects aged 71-80 years over a four year period (Houston et al., 2012). Likewise, in 1234 community dwelling elderly (mean age 75.3 years) in the longitudinal aging study of Amsterdam (LASA), subjects with 25(OH)D below 25 nmol/l scored lower on three tests of physical performance including the chair and tandem stand and walk test (Wicherts et al., 2007). In a further analysis of participants in the LASA and B vitamins for prevention of osteoporotic fractures study, a 25(OH)D level below 50 nmol/l was also associated with lower physical performance though did not predict physical decline (Sohl et al., 2013). In addition, in elderly females those with 25(OH)D below 25nmol/l or between 25-50 nmol/l had a higher rate of functional decline. A potential threshold level of about 60 nmol/l for physical performance was identified in one study of 802 subjects aged 40-80 years (Janssen et al., 2013).

However, there has been some inconsistencies. In a study of 4200 subjects aged over 60 years, higher serum 25(OH)D levels up to 94nmol/l were associated with faster walking speed (8-foot walk test) and faster times in getting out of a chair (sit to stand test), (Bischoff-Ferrari et al, 2004). Despite this, most improvement in both measures was noted at 25(OH)D level between 25 - 40 nmol/l. In addition, a higher 25(OH)D level of up to 115 nmol/l was associated with less prospective decline in function in older adults (Dam et al., 2009). Futhermore, in a recent small study of 35 older adults with 25(OH)D levels above 75 nmol/l, those with higher vitamin D status (up to 135 nmol/l) had better performance on one balance test (Anweiller et al., 2013).

Effect of Vitamin D Supplementation on Physical Performance

In a meta-analysis of 13 RCT'S, vitamin D supplementation reduced body sway, decreased TUG times and improved lower extremity strength A beneficial effect on muscle strength was found in all studies that used 800 IU or more of vitamin D and no effect on gait was identified (Muir et al., 2011). In a meta-analysis of 17 randomised controlled trials involving 5072 adults supplemented with vitamin D, improvements

in muscle strength only occurred in two studies in those with a 25(OH)D level of less than 25 nmol/l (Stockton et al., 2011).

However, some studies suggest an effect at higher vitamin D levels. In a randomized control trial of 242 ambulatory community dwelling subjects (mean age 77 years), a rise in mean 25(OH)D from 55.4 to 84 nmol/l following treatment with 800 IU D3 and 1000 mg of calcium for 20 months resulted in a 28%, 11% and 39% reduction in body sway, Timed Up and Go and falls respectively (Pfeifer et al., 2009). In two other randomised controlled trials involving 363 older adults, a rise in 25(OH)D from 25.7-66.0 nmol/l (Lips et al., 2010) and 34.75- 65.5 nmol/l was also associated with a reduction in body sway (Pfeifer et al., 2000).

5.4 VDR Polymorphisms and Falls

Several studies have shown an association between VDR polymorphisms and muscle strength or falls. In particular carriers of the bb allele appear to have greater muscle strength and less falls. The bb allele has been associated with a 7% higher grip strength and a 23% higher quadriceps strength in non obese women aged 70 or older compared to the BB genotype (Geusens et al., 1997). The bb allele was also associated with a reduced rate of falls in a study of 259 older adults aged 80 years or older (Onder et al., 2008). Conversely, subjects who were carriers of the B allele were found to have an increased risk of falls in participants of the Aberdeen Prospective Osteoporosis Screening Study (Barr et al., 2010).

In addition to the above, the C allele has also been associated with reduced fat free mass and quadriceps strength in healthy elderly men (Roth et al., 2004). Furthermore, young healthy women with the bb allele in combination with the C allele were found to have lower fat free mass and hamstring (but not quadriceps) strength compared to those with the BB allele (Grundberg et al., 2004).

5.5 Clinical Studies of Vitamin D and Falls

Numerous studies including cross sectional, longitudinal and randomised controlled trials have looked at the relationship between vitamin D and falls. Several have also looked at the association between vitamin D and measures of muscle strength and physical performance.

Study - Pitfalls

Studies have varied in their definition of what a fall is and how they have categorised falls. Falls history may not be reliable particularly when it is dependent on 'self report' which could result in non-capture of falls weakening any potential relationship. As many factors contribute to falls risk, the relative importance of vitamin D may vary in different subjects groups. For example, the relationship may be more pronounced in those who are older and frailer and vitamin D deficient. However, the presence of medical morbidities like stroke and orthostatic hypotension could also obscure any relationship. In observational studies, there may be residual confounding due to lack of adjustment for factors such as visual and cognitive impairment and medication use. Finally, in randomised controlled trials there has been significant heterogeneity across studies in terms study participant characteristics, vitamin D dose and treatment duration.

5.51 Cross Sectional Studies

Analysis in a recent Agency for Healthcare Research and Quality (AHRQ) systematic review identified a significant inconsistency in the relationship between vitamin D and falls in both cross sectional studies and longitudinal studies (Chung et al., 2009). The relationship between vitamin D and falls in the elderly suggests that those with vitamin D deficiency (<50 nmol/l) are likely at increased risk, though there is also considerable heterogeneity among subjects, calcium intake and also vitamin D assays used (Rosen et al., 2012).

Positive Studies

In a study of 2957 community dwelling elderly Japanese aged 65 to 92 years and with a mean 25(OH)D of less than 30 nmol/l, the reported rate of falls in the previous year was significantly higher in women in the lowest quartile of 25(OH)D and in those with 25(OH)D insufficiency (Suzuki et al., 2008). This association was only found in females who accounted for 58% of the total cohort. 25(OH)D was also positively correlated with performance on three tests of physical function including hand grip strength, stork standing time and normal walking speed.

In a study of 349 elderly patients (246 hospitalised and 103 living at home), 25(OH)D was positively associated with the absence of falls and also hand grip strength, ability to climb stairs and outdoor activity (Mowe et al., 1999).

In a small study of community dwelling older adults (mean age: 85 years) fallers had significantly lower serum vitamin D than non-fallers (81.9 versus 97.6 nmol/l) after adjusting for age, health status and supplement use though levels were still in the sufficient range (Peterson et al., 2012).

Negative Studies

In a cross sectional study of 495 post menopausal females (mean age 74) in the 8th Hawaii Osteoporosis study, no association was found between serum 25(OH)D and falls after adjusting for age, weight and height (Pamyothin et al., 2009). The mean 25(OH)D level was relatively high at 79.5 nmol/l and only 8.3% had a level below 50 nmol/l which may account for the negative result. There was however, a correlation found between quadriceps strength and serum 25(OH)D.

No significant association was found between self reported falls in the previous year and serum 25(OH)D in 54 healthy volunteers aged between 65-91 years despite finding an association with some physical performance measures including the Berg Balance Score. Whilst adjusting for some confounding factors, this study was small and 93% of subjects had 25(OH)D levels greater than or equal to 50 nmol/l. (Shahar et al., 2009).

In a study of 627 ambulatory subjects who were institutionalised, an inverse association between 25(OH)D and falls became non-significant after adjustment for multiple confounds (Sambrook et al., 2004). Similarly, in a cross sectional retrospective analysis of 83 ambulant nursing home and hostel residents, those who fell were found to have lower serum 1,25(OH) but not 25(OH)D than other residents (Stein et al., 1999).

Serum vitamin D was not associated with falls in the previous year in a study of 342 older adults, though postural body sway was higher in those with deficiency (Kruase et al.,, 2013).

5.52 Longitudinal Studies

A few studies have examined the effect of vitamin D future falls risk. At least two have found a significant inverse relationship though one was negative.

Severe vitamin D deficiency (< 25 nmol/l) was associated with an increased risk of repeated "self reported" falls (OR 1.78, 95% CI 1.06-2.99) in 1231 community dwelling adults aged over 65 years who were participants of the Longitudinal Aging Study Amsterdam (Snidjer et al., 2006). The association was stronger in those under 75 years of age and remained significant after adjusting for several confounding factors. In addition, lower vitamin D status also predicted falls at three year follow up though the association was weaker.

In a prospective study of 1261 elderly females in residential care, 25(OH)D was associated with first time to fall at a follow up of between 145 – 168 days. This was after adjusting for their weight, cognition, psychotropic drug use, wandering behaviour and the presence of a previous Colles fracture. (Flicker et al., 2003). 45% in low level care and 22% in high level care had a 25(OH)D less than 25nmol/l.

No increase in the risk of falls was found at 5 year follow up in a study of 1471 healthy community dwelling females (mean age of 74 years) participating in a randomised control trial of calcium supplementation (Bolland et al., 2010). The seasonally adjusted serum 25(OH)D was less than 50 nmol/l in 50% of subjects. 25(OH)D status did not predict other measures at five years including grip strength or fractures. However, study participants were healthy which may account for the negative finding.

5.53 Randomised Controlled Trials of Vitamin D and Falls

Numerous randomised controlled trials of vitamin D supplementation and falls have been performed. Most have found that vitamin D reduced either falls risk of rate of falling. There were however, some inconsistencies with some trials reporting no benefit or an increased risk with similar or higher doses. In two studies, the use of 500,000 IU and 300,000 IU administered annually was reported to both increase and have no affect on the risk of falls (Smith et al., 2007: Sanders et al., 2011). Mean

baseline 25(OH)D levels were relatively high suggesting an inappropriate target group for supplementation. The evidence supporting a reduction in falls with supplementation among individuals with very low levels of 25(OH)D has become more robust and relatively consistent in systematic reviews, including the recent report from the U.S. Public Health Services Task Force (Rosen et al., 2012).

Several meta-analysis of double blind randomised controlled trials have been performed (See table). Results have been varied with some showing no effect or an effect only on rate of falls as opposed to falls risk. However, analyses have differed in their entry criteria for participants which have included community dwelling, institutionalised and hospitalised patients. In addition, there were differences in vitamin D dose used, duration of treatment and exclusion or inclusion of those taking calcium supplements. Only one meta-analysis looked specifically at falls in relation to vitamin D status and found that a level of least 60nmol/l was required (Bischoff Ferrari et al., 2009).

It is not necessarily surprising that vitamin D might reduce recurrent falls as opposed to prevent falls in the elderly, particularly those who are institutionalised or who are frail. Indeed, a systematic review found that treatment with vitamin D in nursing home residents is not effective at reducing the number of fallers but is of benefit in preventing fall recurrence (Chua et al., 2011). It has been suggested that larger randomised controlled trials using greater doses of vitamin D are needed to further clarify on the role of vitamin D only supplementation without calcium on falls risk (Scragg et al., 2012).

Meta-analyses of vitamin D and falls

In the most recent Cochrane meta-analysis, treatment of subjects residing in care facilities with vitamin D reduced the rate of falls (RR 0.63, 95% CI 0.46-0.86) but not the risk of falling (RR 0.99, 95% 0.90-1.08) (Cameron et al., 2012). A further meta-analysis of those in care facilities also found similar results with a 28% reduction in falls rate (N = 4512) but no effect on risk of falling (Gillespie et al., 2012). Similarly, in a separate meta-analyses vitamin D was found to reduce falls rate but not risk of falling in residents in care facilities (Cameron et al., 2010). However, in a Cochrane

meta-analysis which included community dwelling subjects, no overall effect of vitamin D on reducing falls risk (N=26,747) or falls rate (N=9324) was identified though it was suggested there may be of benefit in those who were vitamin D deficient (Gillespie et al., 2012).

Bischoff et al. looked at eight randomised control trials (N=2426) of older individuals (mean age 65) and found that doses of 700 -1000 IU / day reduced the risk of falling by 19%. Whilst there was a trend for a reduction in falls with the use of vitamin D3 versus vitamin D2 this was not significant and no difference was found between the uses of active or nonactive forms of vitamin D. A 25(OH)D level of at least 60 nmol/l and /or a supplement dose of more than 700IU per day were required to be effective. The effect of treatment was apparent from 2- 5 months and was sustained for up to 36 months. Similar reduction in falls was seen with vitamin D and calcium versus calcium alone (Bischoff et al., 2009).

In a meta-analysis of 26 trials involving elderly participants (N=45.782), supplementation with vitamin D was associated with a 14% reduction in the risk of falls. The effect was more prominent in those who were vitamin D deficient at baseline and was only found in studies where calcium was co-administered with vitamin D (Murad et al., 2011).

In a meta-analyses of 5 trials (N = 3756) the pooled relative risk (RR) for vitamin D(3) preventing falls was 0.88 (95%CI 0.78-1.00). When a subgroup analysis on post-menopausal females was performed, the pooled RR for vitamin D_3 preventing falls was 0.92 (95%CI 0.75–1.12), compared with no vitamin D_3 (Jackson et al., 2007).

Kalyani et al., examined 10 trials (N = 932) of subjects aged 60 years or older and found that in pooled analysis daily vitamin D therapy (200 -1000 IU) resulted in a 14% reduction in falls versus placebo or calcium only. The subgroup of subjects with significantly fewer falls included those were taking 800 IU D or more daily, were under the age of 80, and had no prior history off falls and who were on treatment for longer than 6 months (Kalayani et al., 2010). Subjects included hospitalised patients, institutionalised and community dwelling.

In a meta-analysis of 14 randomised controlled trials (N=21,268), treatment with vitamin D hormone anologs resulted in lower risk of falling (OR 0.79, 95% CI 0.64-0.96) versus native vitamin D (OR 0.94, 95% CI 0.87-1.01), (Richy et al., 2008).

An earlier meta-analyses of five randomised control trials (N=1237) of subjects with a mean age of 60 years, vitamin D was found to reduce the risk of falls in ambulatory or institutionalised elderly with stable health by 22% (OR 0.78, 95% CI 0.64-0.92). Subgroup analysis suggested that this effect was independent of type of vitamin D, and calcium supplementation but reduced sample sizes made this sub-analysis non-significant (Bischoff Ferrari et a., 2005).

Table 5.53: Meta-analyses of Randomised Controlled Trials of Vitamin D supplementation and Falls

Meta-analyses of Randomised Controlled Trials of Vitamin D and Falls					
Author	**RCT'S**	**No.**	**Participants**	**Outcome (OR)**	**Comment**
Gillepsie et al., 2012	5	4,603	Care Facilities	0.63 (0.46-0.86) ↓ rate of falls	No effect on risk of falling
Gillepsie et al., 2012	7	9,324	Community dwelling	No effect on falls rate	Possible benefit in deficiency
Gillepsie et al., 2012	13	26,747	Community dwelling	No effect on falls risk	Possible benefit in deficiency
Murad et al., 2011	26	45,782	Community dwelling, institutionalised	0.86 (0.77-0.96) ↓ risk of falls	Greater effect in deficiency and with Calcium
Cameron et al., 2012	5	4,603	Care Facilities	0.63 (0.46 to 0.86) ↓ rate of falls	No effect on risk of falling
	6	5,186	Care Facilities	No effect	Subjects had low baseline vitamin D
Cameron et al., 2010		4,512	Care Facilities	0.72 (0.55 –0.95) ↓ rate of falls	No effect on risk of falling
		5,095	Care Facilities	Non effect	
Richy et al., 2008	14	21,268	Active and native vitamin D	0.79 (0.64-0.96) ↓ risk of falls (OR for active vitamin D)	Duration 12 months, greater effect of active vitamin D.
Gillepsie et al., 2009			Community dwelling	0.95 (0.80 -1.14) ↓ risk of falls	Possible benefit when vitamin D lower
Jackson et al., 2007	5	3756	Post menopausal women and older men	0.88 (0.78-1.00) ↓ risk of falls	
Kalyani et al., 2010	10	2932	Community dwelling, Institutionalised/hospitalised, aged ≥ 60	0.86 (0.79-0.93) ↓ rate of falls	Greater effect when vitamin D>800 /day, aged < 80, taking calcium, no falls history
Bischoff et al., 2009	7	2426		0.81 (0.71-0.92) ↓ risk of falls	High dose vitamin D or serum level ≥ 60 nmol/l
Bischoff et al., 2004	5	1237	Community dwelling	0.78 (0.64-0.92) ↓ risk of falls	-

5.6 Conclusion

Several meta-analyses of randomised controlled trials support a role for vitamin D supplementation in either reducing falls risk or rate of falls. Vitamin D is known to act directly on skeletal muscle and may mediate it's effect on falls by improving muscle strength, balance and body sway. Optimisation of parathyroid hormone and serum calcium may also play a role. The effect size varies hugely across different studies, reflective of different responses in different subject groups. It is unclear as to which patient's benefit most from supplementation, whether the effect is altered by supplemental calcium and the optimal level of 25(OH)D required. Studies suggest the benefit is greater in those who are vitamin D deficient and have a history of falls, and that a level of at least 60 nmol/l should be achieved. Evidence also suggests that increasing levels above this may be of benefit in both improving physical performance and reducing falls.

CHAPTER 6 : VITAMIN D AND MOOD

6.1 Introduction

It has been proposed that vitamin D may play a role in the pathogenesis of depression and affective disorders (Parker et al., 2011). The presence of the vitamin D receptor and metabolic pathways for vitamin D in several brain areas implicated in depression, coupled with a seasonal variation in mood when levels are low supports this theory. Several studies point to the role of vitamin D in brain function and development. Supportive evidence has also come from studies that have explored the relationship between vitamin D and depression, other psychiatric illness and affective disorders including Seasonal Affective disorder (SAD) and Premenstrual Syndrome (PMS).

Seasonal Variation in Depression

Depression is known to have a seasonal pattern with US data suggesting Summer and Winter peaks (Wehr et al., 1989). In addition, depression in the Winter but not in the Summer has been shown to be responsive to light therapy (Rosenthal et al., 1988). Seasonal change in the photoperiod is integral to most hypotheses that explain the seasonality of mood (Eagles et al., 2003).

Bone Mineral Density and Depression

Low bone mineral density (which is associated with lower vitamin D status) is disproportionately common in those with depression (Coelho et al., 1999: Mitchelson et al., 1996: Jacka et al., 2005: Fazeli et al., 2013). Robbins et al. also found a significant association between bone mineral density and depressive symptoms after adjustment for osteoporotic risk factors and postulated that an unmeasured third factor such as an endogenous steroid might be responsible (Robbins et al., 2001).

6.2 Vitamin D and Affective Disorders

Mental Illness

Some studies have found an association between vitamin D and psychiatric illness including schizophrenia and bipolar affective disorder (Eyles et al., 2013) but most have been small and have included subjects who were medical inpatients. The finding

of an inverse relationship is not surprising and may be as a consequence of factors associated with the very illness itself including less time spent outdoors and poor dietary intake of vitamin D.

Berk et al., found that mean serum 25(OH)D was 29% lower in a sample of 53 inpatients in a private a psychiatric clinic versus 691 controls without psychiatric illness (46.4 nmol/l versus 65.3 nmol/l) after adjusting for season and age (Berk et al., 2008). A higher prevalence of vitamin D deficiency has also been found in young adolescents with severe mental illness where levels were also associated with the presence of psychosis independent of race (Graciuos et al., 2012). Likewise, in elderly residing in nursing homes in Belgium, a consistent tendency for more depressive symptoms and use of antidepressants was found in those with greater vitamin D deficiency (Verheoven et al., 2012).

Vitamin D has also been associated with schizophrenia which is more common in individuals born during the Winter and Spring when maternal vitamin D levels are low (Harms et al., 2008) and for which vitamin D supplementation in early life may reduce the risk (Mc Grath et al., 2004). A high prevalence (74%) of vitamin D deficiency (<50 nmol/l) was found in a study of 102 subjects who were psychiatric inpatients, with those with schizophrenia having markedly lower levels than those with mania or depression (Menkes et al. 2012). Similarly, in a another study of 133 patients, those with schizophrenia and depression had lower vitamin D status compared to healthy controls though no association was found with disease activity (Itzhaky et al., 2012.)

It has been hypothesised that vitamin D deficiency could be linked to suicide (Tariq et al., 2011). Lower 25(OH)D (< 38.6 nmol/l) was associated with an increased risk of suicide in a nested case control study (N=495 in each group) involving US military personnel (Umhau et al., 2013).

Premenstrual Syndrome (PMS)

Premenstrual Syndrome (PMS) is a recognised mood disorder (DSM-IV) characterised by moderate to severe physical and emotional symptoms in the luteal phase of the menstrual cycle that can substantially interfere with normal life activities

and interpersonal relationships (Mortola, 1992). It has been suggested that women who experience symptoms consistent with Premenstrual Syndrome (PMS) may have vitamin D deficiency, calcium dysregulation and hyperparathyroidism. However, only a few studies have explored this relationship and results are inconsistent.

Lower levels of vitamin D and higher PTH levels are associated with Premenstrual SyndromeThys-Jacobs et al., 2000). Changes in 1,25(OH)D levels were noted in 68 women with Premenstrual Dysphoric Disorder (PMDD) versus 47 healthy female controls during the menstrual cycle (Thys-Jacobs et al., 2007).

Higher intake of vitamin D (median intake of 706 IU/day versus 112 IU/day) has been associated with a reduced risk (OR 0.59, 95% CI 0.50-0.97) of PMS in a longitudinal study of females who were aged between 27-44 years (Bertone-Johnson et al., 2005). Likewise, a food intake of only 100 IU or more of vitamin D per day in a study of 186 females (aged 18-30 years) was associated with a reduced risk (OR, 0.32, 95% CI 01.0-0.98) of PMS (Bertone-Johnson et al., 2010). However, premenstrual symptoms were not related to vitamin D, PTH calcium or dairy product consumption in a cross sectional study of 177 young females who had primary dysmenorrhea (Obeidat et al., 2012).

Postpartum Depression

Vitamin D has been implicated as factor in postpartum depression for which a possible psycho-neuro-immunological mechanism has been proposed (Ellsworth et al., 2012). A significant relationship over time was found between low 25(OH)D and postpartum depression (as measured by the Edinburgh Postpartum Depression Scale) in 97 women attending an outpatient clinic over a seven month period (Murphy et al., 2010). There is little evidence overall though to support a role for vitamin D in postpartum depression.

Seasonal Affective Disorder

A role for vitamin D in the aetiology of Seasonal Affective Disorder (SAD) was first proposed by Stumpf and Privette in 1989 (Stumpf et al., 1989). SAD is a disorder characterised by symptoms of depression, anxiety, irritability, appetite changes,

hypersomnia, and fatigue that occurs during the winter months (Lurie et al., 2006). However, few studies have explored this relationship and the results of intervention trials of vitamin D supplementation in this group have been inconsistent. Some have reported no improvement in those with SAD symptoms (Harris et al., 1993; Partonen et al., 1996; Dumville et al., 2006) whilst others found a beneficial effect (Landsdowne et al., 1998: Gloth et al., 1999).

6.3 Vitamin D and Depression– Potential Mechanisms

VDR and the Brain

VDR has been identified in multiple areas of the brain including the prefrontal cortex, cingulated gryus, thalamus and hypothalamus, many of which have been implicated in the pathophysiology of depression (Drevets et al., 2008). High concentration of VDR are found in the hypothalamus (Eyles et al., 2003) where the suprachiasmatic nucleus is located and believed to be involved in affective disorders (Kalsbeek et al., 1998). VDR and hydroxylases have also been found in the amygdala, which plays a central role in the limbic system and in the regulation of behaviour and emotion (Walbert et al., 2001). Vitamin D metabolites including $1,25(OH)_2D$ and 25(OH)D can cross the blood brain barrier (McCann et al., 2008) and metabolic pathways for it's activation exist in the brain (as discussed previously). Vitamin D is a cofactor in the synthesis of the brains main free radical scavenger glutathione, deficiency of which has been implicated in many psychiatric disorders including depression (Berk et al., 2008). Vitamin D receptor knockout mice have increased anxiety like behaviour though this may be mediated by loss of vitamin D function (Kaleuff et al., 2006).

Vitamin D and Monoamines

To date, some evidence links vitamin D to monoamines implicated in depression. Summer sunlight has been found to increase brain serotonin levels at least twice as much as winter sunlight (Lambert et al., 2002) suggesting a potential role for vitamin D. More importantly, preclinical findings show that vitamin D can regulate catecholamine levels (Eyles et al., 2013). $1,25(OH)_2D$ appears to increase expression of genes encoding tyrosine hydroxylase (the rate limiting enzyme for catecholamine synthesis) in adrenal medullary cells by threefold (Puchaaz et al., 1996). In addition,

Vitamin D Responsive Elements have been identified in silico promoter regions in serotonin receptors and tryptophan hydroxylase, two genes associated with depression (Wang et al., 2005). Vitamin D has also been found to protect against serotonin depleting effects of neurotoxic doses of amphetamine in rodents (Cass et al., 2006).

Vitamin D and Hypothalamic Adrenal Axis (HPA)

One theory underlying depression is the activation of the hypothalamic pituitary adrenal (HPA) axis. It is possible vitamin D may act on the suprachiasmatic nucleus in the hypothalamus, which is known to have an inhibitory action on the HPA axis (Kalsbeek et al., 1998). There is also some evidence to suggest a relationship between vitamin D, stress and cortisol. It has been proposed that vitamin D might be involved in hippocampal neurogenesis in response to stress, as in vitro studies suggest cross talk between VDR and glucorticoid receptors in the hippocampus (Obradovic et al., 2006). Recently, an increased expression of hydroyxlase enzymes, VDR and vitamin D target gene mRNA (calbindin and neurotrophin-3) as well as higher $1,25(OH)_2D$ was found in the hippocampus of stress induced depressed rats. This suggests that local vitamin D signalling may be a compensatory mechanism to protect against the affect of stress in the brain (Jiang et al., 2013)..

Vitamin D, Parathyroid Hormone and Depression

A possible mechanism by which vitamin D might cause depression is through elevated parathyroid hormone (PTH) levels. As previously discussed, PTH receptors are found in the central nervous system and it can cross the blood brain barrier. High PTH levels which are often a consequence of low serum vitamin D have been associated with low mood and depression in some cross sectional studies (Hoogendik et al., 2008). In addition, low mood is commonly found in patients with hyperparathyroidism, which when corrected can lead to improvement (Chiba et al., 2007). However, studies have given conflicting results and some have found no independent relationship (Lee et al., 2011).

Inflammation

Evidence suggests that increased levels of pro-inflammatory cytokines in the brain may be associated with depression (Song et al., 2011). Vitamin D has been shown to

down regulate a number of inflammatory mediators including nuclear KB (McCann et al., 2008) which has been implicated in depression (Miller et .2009).

Cerebrovascular Disease

The 'vascular depression hypothesis' sites that cerebrovascular disease contributes to the development of depression in later life (Paulson et al., 2013). Vitamin D through modulation of vascular risk factors (as previously discussed) could in theory reduce cerebrovascular disease burden and hence depression.

6.4 VDR Polymorphisms and Depression

In a study of 563 subjects, carriers of the ApaI VDR polymorphism were more likely to have less depressive symptoms (Kuningas et al., 2009). The ff VDR genotype was found to be more common in patients with bipolar disorders in case control study and was also associated with lower expression of the dopamine D1 receptor gene (Ahmadi et al., 2012). Results suggest that the effect of vitamin D on mood is modulated by differences in the VDR. However, other polymorphisms of the VDR (BsmI-β, TaqI-β) were not associated with depression or response to intervention (Yalamanchili et al., 2012).

6.5 Clinical Studies

Results of clinical studies looking at the relationship between vitamin D and mood have given inconsistent results. An outline of cross sectional and longitudinal studies of serum 25(OH)D and depression as well as randomised controlled trials is discussed. A small number of studies that have looked at vitamin D intake and depression are also included.

Studies of Vitamin D and Mood - Pitfalls

Several factors related to vitamin D status have the potential to effect study results, some of which have been previously outlined. In particular, depression may lead to lower vitamin D levels via reduced sun exposure and altered dietary habits resulting in reduced vitamin D intake. Most studies have not taken account of a prior history of depression or the presence of family history of depression. Assessments of depression have varied widely with most using validated scales and some making a formal clinical diagnosis. Differences in the sensitivity and specificity of scales used could

account for false negative or positive outcomes. Some scales only reflect mood in the preceding week which has the potential to weaken the relationship. In longitudinal studies the frequency of mood assessment has varied and in some the follow up has been short. It is possible that clinically detectable mood disorders may take many years to develop and hence be missed in shorter studies. Finally, as in other randomised controlled trials of vitamin D, a lack of a positive outcome may relate to inappropriate treatment dose / duration or target subject group.

6.51 Vitamin D Intake and Depression

In 81,189 participants of the Women's Health Initiative aged 50-79 years, a total daily vitamin D intake of 800 IU or more versus less than 100 IU was associated with a reduced risk of depression (OR 0.79, CI 0.71 – 0.89). In an analysis including those without evidence of depression, a daily intake of more than 400 IU D3 versus less than 100 IU was also associated with a 20% reduction in the risk of depression at three years (Bertone-Johnston et al., 2011).

Women who consumed less than 400 IU of daily vitamin D in the Iowa Women's Health study had significantly lower mental health related Quality of Life after controlling for several confounding factors including physical activity (Motsinger et al., 2012).

6.52 Cross Sectional Studies of 25(OH)D and Mood

Of 24 cross sectional studies, 15 found an inverse association between serum 25(OH)D and measures of depression/anxiety. Some have been large population studies whiles others have included small numbers in case control models. Most large studies have been positive, though two were negative but only after further adjustment in multivariate analysis (See Table 6.5). 25(OH)D levels above 75 nmol/l appear to be associated with lower risk of depression/depressive symptoms in several studies that have found an inverse relationship. A recent meta-analysis of ten cross sectional and one case control study found that lower vitamin D levels were associated with an increased risk of depression (OR 1.31, 95% CI 1.0-1.71), (Anglin et al., 2013).

Positive Studies

In the largest study to date involving 12,954 community dwelling adults (mean age 51.7 ± 11.0 years) who were participants of the Cooper Center Longitudinal Study, higher 25(OH)D levels were associated with a reduced risk of depression at baseline (Hoang et al., 2011). However, the finding only remained significant in those with prior history of depression.

In the 6th Tromso study, a higher risk of depressive symptoms was found in 10,086 subjects after adjusting for multiple confounds (Kjaergaard et al., 2011). The analysis was performed separately in smokers and non-smokers where the respective odds ratio were (OR 0.59, 95% CI 0.39-0.89) and (OR 0.74, 95% CI 0.58-0.95).

In 7970 participants of the 3rd NHANES study who were aged 15-39 years, a higher risk of depression (OR 1.85, 95% CI 0.90-3.81) was identified in multivariate analysis in those with vitamin D deficiency (<50 nmol/l) versus those with a 25(OH)D of 75nmol/l or above (Ganji et al. 2010).

In a study of 4101 pregnant women, vitamin D deficiency (≤ 29.9 nmol/l) and insufficiency (30-49.9 nmol/l) at a median of 13 weeks gestation were associated with higher level of depressive symptoms (OR 1.48, 95% CI 1.13-1.95) and (OR 1.44, 95% CI 1.12-1.85) respectively after adjustment for several covariates (Brandebarg et al., 2012). In linear regression model, there was mean 5% increased risk found for every 10 nmol/l drop in 25(OH)D.

In a national representative sample of 4002 Jordanian's aged 25 or older, an increased risk of depression was identified in those in the lowest two versus the highest quartile of vitamin D (OR 1.21 –1.39) after adjusting for several confounds (Jaddou et al., 2012). A relatively high 25(OH)D of 105 nmol/l was identified as the level when the decreased risk of depression was significant.

An increased risk of depression was found in 3669 European males (mean age 60 ± 11 years) across decreasing quartiles of vitamin D (>78.4 nmol/l to < 39.0 nmol/l) in multivariate analysis (Lee et al., 2011). Likewise, lower 25(OH)D status was

associated with both depression and depression severity in 1282 participants aged 65-95 years in the Longitudinal Ageing Study Amsterdam after multivariate adjustment (Hoogendik et al., 2008).

Depressive symptoms were associated with vitamin D deficiency (<25nmol/l) in 2070 participants aged 65 older in the 2005 Health Survey England after adjustment for several confounds (OR 1.46, 95% CI 1.02-2.08), (Stewart et al., 2010).

An increased risk of depression was found in 939 Chinese males in Hong Kong after multivariate adjustment when comparing those in the highest quartile (> 92 nmol/l) versus the lowest quartile (< 63nmol/l) of 25(OH)D (Chan et al., 2011).

In a study of 178 African American pregnant women, a significant inverse relationship was found between 25(OH)D and antenatal depression (CES-D≥16), with a 46% reduction in the risk of depression for every 6.8 nmol/l increase in vitamin D status (Cassidy Bushrow et al., 2012). The mean 25(OH)D was 33.3 ± 20.1 nmol/l suggesting that those who are deficient were more likely to be depressed.

Remaining positive studies were small and included an heterogenous group of subjects including hospitalised patients, subjects with hyperparathyroidism, Alzheimer's dementia, fibromyalgia (Sneider et al., 2000; Jorde et al., 2006; Wilkins et al., 2006; Eskandari et al., 2007; Armstrong et al., 2007).

Negative Studies

Two negative studies involved large numbers of subjects, used validated tests and adjusted for several confounders. A lower prevalence of depressive symptoms was identified in the highest tertiale of 25(OH)D (mean 65.1 ± 16.0 nmol/l) in a study of 3262 community dwelling Chinese aged 50-70 years. However, after adjustment for geographic location the find became insignificant (Pan et al., 2009).

Similarly, while an association between vitamin D and depression was found in 3916 participants of the 2005-6 NHANES study (aged ≥ 20 years), it became insignificant after adjustment for multiple confounds (Zhao et al., 2010).

In 527 Japanese subjects aged 21-67 years no significant association was identified between vitamin D and depressive symptoms in multivariate analysis (Nanri et al., 2009). Other negative studies have been much smaller. Two were case controls (Michelson et al., 1996; Herran et al., 2000) and used no formal measures of mood. In addition, another involved a small number of chronic haemodialysis patients and though finding a correlation between 25(OH)D and Beck Depression Inventory scores this did not remain significant in multivariate analysis (Bossola et al., 2010).

In another small study of 138 females aged between 18-24 years no association was found between 25(OH)D and depression (Kwasky et al., 2012). In a sample of 118 participants aged 70-75 years in the Survey in Europe and Nutrition in the Elderly (SENECA) study, no significant association was between increasing tertiales of 25(OH)D (<34 nmol/l to 52-152 nmol/l) and Geriatric Depression Scale scores after adjustment for several lifestyle factors (Brouwer Brolsma et al., 2013). A negative correlation between vitamin D status and depression was found in 59 patients with multiple sclerosis but this became insignificant when adjusted for disability and fatigue (Knippenberg et al., 2011).

Table 6.52: Cross Sectional Studies of 25(OH)D and Mood

Cross Sectional Studies of 25(OH)D and Mood				
Author	**No**	**Measure**	**Risk**	**Comments**
Hoang et al., 2011	12594	CES-D (short form)	Yes	Not significant in those without prior history of depression
Kjaergaard et al., 2011	10086	SCL-10	Yes	Stronger association in females
Ganji et al., 2010	7970	DIS	Yes	Risk when 25(OH)D < 50 nmol/l
Brandebarg	4101	CES-D	Yes	Pregnant women, risk when 25(OH)D < 74.5nmol/l
Jaddou et al., 2011	4002	DASS21	Yes	Reduced risk significant when 25(OH)D >105 nmol/l
Zhao et al., 2010	3916	PHQ-9	No	Negative only in fully adjusted model
Lee et al., 2010	3369	BDI-II	Yes	Risk reduced with rising 25(OH)D from <39.0 to >78.4 nmol/
Pan et al., 2009	3262	CES-D	No	Non significant only after adjusting for location
Stewart et al, 2010	2070	GDS	Yes	Risk when 25(OH)D <25 nmol/l
Hoogendik et al., 2008	1282	CES-D	Yes	
Chan et al., 2011	939	GDS	Yes	Risk comparing <63 nmol/l versus > 92nmol/l
Nanri et al., 2009	527	CES-D	No	
CES-D Center for Epidemiological Studies Depression scale, SCL-10 –Symptom Checklist Depression Scale -10, DASS-21- Depression, Anxiety Stress Scale -21, BDI- Beck Depression Inventory, GDS – Geriatric Depression Scale, PHQ-9 - Patient Health Questionnaire –9.				

Table 6.52: Cross Sectional Studies of 25(OH)D and Mood

Cross Sectional Studies of 25(OH)D and Mood				
Author	**No**	**Measure**	**Risk**	**Comments**
Cassidy Bushrow, et al., 2012	178	CES-D	Yes	Pregnant women, results suggest that risk when 25(OH)D<50 nmol/l
Eskandari et al, 2007	133	Psychiatric Assessment	Yes	Case control study
Kwasky et al., 2012	139	BDI	No	
Brouwer Brolsma et al., 2012		GDS	No	No relationship when 25(OH)D rising forom <34 nmol/l to 52-152 nmol/l
Bossola et al., 2010	80	BDI II (Italian version)	No	Subjects on dialysis
Wilkins et al., 2006	80	DSI	Yes	Risk when 25(OH)D < 25 or 50 nmol/l
Armstrong et al., 2007	75	HADS	Yes	Subjects with fibromyalgia – outcome was anxiety
Jorde et al., 2006	84	BDI	Yes	Lower BDI when 25(OH)D below 50 nmol/l
Sneider et al., 2000	60	Psychiatric Assessment	Yes	Case control study
Knippenberg et al., 2013	59	HADS	No	Subjects with multiple sclerosis, no association in full mdoel
Michelson et al., 1996	48	Psychiatric Assessment	No	Case control study
Herran et al., 2000	38	Psychiatric Assessment	No	Case control study
CES-D – Center for Epidemiologic Studies Depression scale, BDI-Beck Depression Inventory, GDS – Geriatric Depression Scale, HADS – Hospital Anxiety Depression Scale, DSI – Depression Symptoms Inventory.				

6.53 Longitudinal Studies

Of six longitudinal studies to date that looked at vitamin D and mood, five identified an increased risk of developing depressive symptoms or depression in subjects with lower serum vitamin D status. Study subjects were middle aged or older adults apart from one study which included children and follow up ranged from between one and six years. In a recent meta-analysis of three longitudinal studies an increased risk of depression was found in the highest versus lowest categories of vitamin D (OR 2.21, 95% CI 1.40 – 3.49), (Anglin et al., 2013).

Positive Studies

An increased risk of depression (at up to one year follow up) was found in 7538 subjects aged over 50 years who had lower vitamin D levels and known cardiovascular disease but no known prior history of depression. The relative risk was 2.7 and 2.5 for those with 25(OH)D levels below 37.5 nmol/l and between 40-75 nmol/l respectively compared to those having a level above 125 nmol/l.. However, adjustment was made for few confounds (May et al., 2010).

An inverse relationship between 25(OH)D and depression was also found in 7401 participants (aged 45 years) in the 1958 British Birth Cohort after adjustment for vitamin D related behaviours (OR 0.57, 95% CI0.40-0.81). Furthermore, those with 25(OH)D below 50 and 85 nmol/l respectively had an increased risk of subsequent depression at 50 years of age (Maddock et al., 2013).

Higher vitamin D status was associated a with a reduced risk of depressive symptoms at four years follow up in 2752 children (OR 0.90, 95% CI 0.86-0.95) independent of several confounding factors (Tolpannen et al., 2012).

In an analysis of 2396 participants (aged 18-65 years) of the Netherlands Study of Depression and Anxiety (NESDA), lower vitamin D status was associated with an increased risk of depressive disorders at two year follow up. Vitamin D was also inversely associated with current depression and symptom severity at baseline (Milaneschi et al., 2013).

Negative Studies

Lower vitamin D status was not associated with an increased risk of incident depression in 629 Chinese men (aged over 65 years) at four year follow up (Chan et al., 2011). However, a self-report measure of mood was used and there was a small number of cases of incident depression.

Table 6.53: Longitudinal Studies of 25(OH)D and Depression

Longitudinal Studies of 25(OH)D						
Author	**No.**	**Age***	**Duration**	**Measure**	**Risk**	**Comments**
May et al., 2010	7538	73.1	1 yr	ICD-10	Yes	No adjustment for season
Maddock et al., 2013	7401	45.0	5 yrs	MHI	Yes	Risk when <50 or 85 nmol/l.
Tolapannen et al., 2012	2752	9.8	4 yrs	MFQ	Yes	Outcome measure was only depressive symptoms
Milaneschi et al., 2013	1596	18-65	2 yrs	DSM - IV	Yes	OR 0.90
Milaneschi et al., 2010	954	75.0	3-6 yrs	CES-D ≥ 16, CES-D.	Yes	Risk when < 50 nmol/l for both outcomes except in males at 6 yrs,
Chan et al., 2011	629	> 65	4 yrs	GDS > 8	No	Small number of cases of incident depression

MHI – Mental Health Inventory, MFQ – Mood & Feelings Questionnaire, CES-D – Center for EpidemiologIcal Studies Depression Scale, GDS – Geriatric Depression Scale, ICD-10 – International Classification of Diseases -10, DSM IV – Diagnostic Statistical Manual IV. [*Age quoted as mean, range or median]

6.54 Intervention Trials

Of 16 intervention studies involving vitamin D and mood eight were positive. Two large randomised controlled trials have examined the effect of vitamin D on mood but only one was primarily designed to do this (Sanders et al., 2011; Bertone-Johnson et al., 2012). Others studies have been small and/or of short duration. One used active vitamin D but found no improvement (Yalamanchili et al., 2012).

Negative

The largest trial to date involving 2263 females aged 50-79 years in the Women's Health Initiative (WHI) found that 400 IU D3 with 1000mg calcium daily resulted in a increase in depressive symptoms after two years. However, the dose of vitamin D was small and there is also the potential for a negative affect from the calcium supplementation (Bertone-Johnson et al., 2012). In addition, compliance with treatment was only 60-63 % and use of antidepressants was used as a proxy for depressive symptoms for some patients.

Another large study of over 2000 community dwelling subjects aged 70 or more who received a large annual dose of vitamin D (500,000 IU) found no improvement in mental health scores at 3-5 year follow up (Sanders et al., 2011). This however, may have been confounded by an increase in falls and fractures in the treatment group versus placebo. In addition, of a subset of participants who had serum 25(OH)D measured, only a small proportion had vitamin D deficiency (less than 25 nmol/l) and more than half had levels above 50 nmol/l. This suggests that it may have been an inappropriate target group for supplementation.

In a double blind placebo controlled trial of 489 post menopausal women (aged 65-77 years), treatment with calcitriol [$1,25(OH)_2D$] or calitriol combined with hormone therapy for three years had no effect on depression as assessed by the Geriatric Depression Scale (Yalamanchili et al., 2012).

Of other negative trials, the dose of vitamin D was low in one (Harris et al., 2009). In another, treatment involved bright light only for a short period and though resulting in an improvement in the Beck Depression Inventory was not associated with any

change in serum vitamin D (Partonen et al., 1996). However, in four other trials higher doses of vitamin D did not result in any improvement in different mental health outcomes. In a study of 921 women aged 70 years or more, treatment with 800 IU vitamin D and calcium versus placebo resulted in no significant change in the Mental Component Score (MCS) at six months (Dumville et al., 2006). Supplementation of 65 healthy community dwelling men (aged 65-87 years) with 1000 IU OF vitamin D per day also did not improve health perception (Kenny et al., 2003).

Finally, treatment with vitamin D (5000 IU/day) for a mean of 8.1 days did not result in any benefit in mood (as assessed by the Profile of Mood States) compared with vitamin C. This might be explained by the fact that 25(OH)D was still in a sub-normal range post treatment (Wang et al., 2013).

Positive Studies

A significant decrease in the Beck Depression Inventory score in 441 subjects aged 21 –70 years was found post treatment with once weekly 40,000 IU D2 for one year, though the drop out rate was high at 25% and subjects only had depressive traits as opposed to a diagnosis of depression (Jorde et al., 2008).

In a double blind randomised placebo controlled trial of eight weeks duration involving 40 subjects with depression, treatment with combined vitamin D3 (1500 IU per day) and fluoxetine was found to be better than fluoxetine alone in reducing, depressive severity. The effect was noticed from the fourth week of treatment onwards (Khoraminya et al., 2013).

Treatment of 48 depressed Swedish adults with vitamin D deficiency (<50 nmol/l), for three months with vitamin D3 led to a significant reduction in depression on the MFQ-S and also an improvement in eight out of nine items in a vitamin D deficiency scale (Hogberg et al., 2012).

Likewise, treatment of 40 vitamin D deficient subjects suffering with depression (BDI $\geq$ 16) with a once off intramuscular injection of 300,000 IU D3 resulted in a significant improvement in BDI scores compared to 40 similar subjects who received

no intervention. Mean post treatment 25(OH)D was 60.2 nmol/l. (Mozaffari-Khosravi et al., 2013).

Administration of 100,000 IU of vitamin D once off to eight patients with Seasonal Affective Disorder also resulted in an improvement in several measures of mood (Gloth et al., 1999). In 82 outpatients with a 25(OH)D level of less than 61 nmol/l, supplementation with either 4000 IU or 600 IU per day of vitamin D3 for three months over two consecutive winters resulted in an improvement in wellbeing score. (Vieth et al., 2004).

In a random double blind study of 44 healthy subjects, treatment with vitamin D (400IU or 800IU) daily for 5 days in late winter was associated with an improvement in self reported affect with some evidence for a reduction in negative affect (Landsdowne et al., 1998). In a study of six females with a 25(OH)D level below 100 nmol/l, supplementation with D3 was associated with a decline in scores on the Beck Depression Inventory (BDI). Mean increase in 25(OH)D was 67.5 nmol/l (Shipowick et al., 2009).

Table 6.54: Studies of vitamin D supplementation and Depression

Intervention Trials involving serum 25(OH)D and Mood						
Author	**No.**	**Duration**	**Treatment**	**Measure**	**Effect**	
Bertone-Jonhston et al., 2012	2263	2 years	400 IU D3 +1000 mg Ca /day*	Burnam Scale / Antidepressant use	No	Low dose of vitamin D
Sanders et al., 2011	2012	3-5 years	500,000 IU D3 stat /year	GHQ, SFHS, others	No	Few had 25(OH)D < 25 nmol/l
Drumville et al., 2006	912	6 months	800 IU D3 + 1000 mg Ca / day	MCS	No	
Yalamanchilli et al.,2012	489	3 years	$1,25(OH)_2D3$ + HRT	GDS	No	
Jorde et al., 2008	441	1 year	20,000 or 40,000 IU D2 / wk	BDI	Yes	Drop out rate 25%, small decrease in BDI
Harris et al., 1993	250	12 month	400 IU D2 + 337 mg Ca / day	PMQ	No	
Dean et al., 2011	128	6 weeks	5000 IU D3 /day	BDI, STAI	No	High baseline vitamin D
Vieth et al., 2004	82	6 months	4000 IU or 600 IU D3 / day	Well being score	Yes	No difference with higher dose
Mozaffari-Khosravi et al., 2013	80	3 months	300,000 /100,000 IU D3 stat	BDI-II	Yes	Higher dose more effective
Kenny et al., 2003	65	6 months	1000 IU D3 + Ca / day	Health perception	No	
Hogberg et al., 2012	48	3 months		WHO wellbeing scale, MFQ-S	Yes	Improvement in several domains
Landsdowne et al.,1998	44	5 days	400 IU or 800 IU D3 / day	Self Report Measure of Affect	Yes	
Khoraminya et al., 2013	40	8 weeks	800 IU D3 + Fluoxetine or placebo	HDRS, BDI	Yes	Vitamin D with SSRI better
Partonen et al., 1996	29	2 weeks	Bright light	Depressive Symptoms	No	
Gloth et al., 1999	15	1 month	100,000 IU D3 stat	HDS	Yes	
Shipowick et al., 2009	6	8 weeks	5000 IU D3 daily	BDI -II	Yes	

GHQ – General Health Questionnaire, SHFS- Short Form Health Survey, PGIIS –Patient Global Impression Improvement Scale, STAI – Strait Anxiety Inventory, HDRS – Hamilton Depression Rating Scale ,WHO –World Health Organisation.

Conclusion

The evidence to date supports a potential role for vitamin D in the aetiopathology of depression. Vitamin D is known to cross the blood brain barrier and metabolic pathways exist for it's activation in the brain. The vitamin D receptor (VDR) is found in multiple brain areas implicated in depression including the prefrontal cortex, amygadala and hypothalamus. Vitamin D may play a role in mood through it's possible effect on the hypothalamic pituitary adrenal (HPA) axis, brain serotonin and serum parathyroid hormone levels. Vitamin D is also known to have anti-inflammatory, neuro-protective and vasculo-protective affects in the brain, which might protect against depression.

Both cross sectional and longitudinal studies exploring the relationship vitamin D and depression have given conflicting results, though most support an inverse association, A recent meta-analysis of 10 cross sectional and three cohort studies (N=31,424) found that lower vitamin D status was associated with a higher rate of depression than controls. In addition, lower vitamin D was also associated with an increased risk of incident depression (Anglin RE et al., 2013).

Despite this, randomised controlled trials have given inconsistent results, though some have been of short duration, have included only small sample sizes, used too low a dose of vitamin D or treated those who weren't vitamin D deficient at baseline. Other intervention studies have not been placebo controlled.

As with other studies involving vitamin D, there is the potential for residual confounding whereby other factors explain the lower vitamin D status. In particular, those who are depressed may have lower vitamin D levels on account of spending less time outside and have poor oral intake of vitamin D. Well designed randomised controlled trials in the future will be needed to establish the potential role of vitamin D in depression.

SECTION 3: STUDY POPULATION

CHAPTER 7: STUDY DESIGN

7.1 Study Population and Design

The study population comprised participants of the TUDA (Trinity, University of Ulster, Dept. of Agriculture) study. This is a large collaborative, cross sectional study designed to create a phenotype/genotype database for three cohorts of community dwelling subjects aged over sixty years. It aims to explore the effect and possible interaction between nutritional and genetic factors on age related diseases.

Two of these cohorts, each with either cognitive impairment or osteopaenia/osteoporosis were recruited from outpatient services at the Department of Medicine for the Elderly at St. James's Hospital, Dublin. Patients attending general geriatric clinics and a day hospital service were recruited into the cognitive cohort. Those attending specialist bone health clinics and the department's DXA service comprised subjects in the bone cohort. The diagnosis of ostoeporosis/osteopaenia was based on DXA results using standard WHO criteria. The remaining cohort included subjects with hypertension (physician diagnosed) and were recruited from GP practises in the catchment areas of Altnagelvin and Causeway Hospital's in Northern Ireland.

Subjects aged over 60 years, who were able to provide consent and scored 16 or more on the Mini Mental State Examination (MMSE) were eligible for entry into the study. Recruitment started in Jan 2009 and was completed by May 2012. Trained researchers and doctors did study assessments. Subjects who were eligible for recruitment were assessed on the day of their outpatient attendance or were retrospectively selected and asked to come back on another occasion. All subjects were contacted by telephone in advance and sent study information by post. All participants provided written consent. Ethical approval was obtained from both Hospital and GP ethics committees.

7.2 Overview of Assessments

All participants underwent a single assessment lasting about 70 minutes which included structured interview recording self reported information on demographic factors, education, medical history, current medications, diet, smoking and alcohol drinking status, physical activity, sun exposure, falls, and psychosocial history.

Validated questionnaires that screen for depression and measure functional status were administered in addition to neuropsychological testing. Biophysical measurements and blood tests were also taken.

Study Scales

Physical function was measured using self-report instruments in the domains of Instrumental Activities of Daily Living (IADL) and Physical Self-Maintenance Scale (PSMS) (Lawton and Brody, 1969). These scales include questions measuring ability to prepare food and to go shopping. The Centre for Epidemiological Studies-Depression Scale (CES-D) was chosen as a screening test for depressive symptoms (Radloff, 1977).

Lifestyle Factors

Subjects were asked to report on the current use of any supplements including cod liver oil or whether they consumed oily fish, eggs or margarine (foods known to contain vitamin D). They were also questioned on alcohol use and smoking history and asked to report on sun holiday travel in the six months prior to their assessment, as well their preference for spending time in the shade or sun when outside.

Medical History / Medications

All medications that subjects were taking were recorded. Details of medical history were also noted including a diagnosis of hypertension, diabetes mellitus, myocardial infarction, stroke and peripheral arterial disease. Subjects also self reported on a physican diagnosis of depression and whether or not they had a fall in the last year. A fall was defined as an event which resulted in a person coming to rest inadvertently on the ground or floor or other lower level. They were also asked if they suffered with dizziness on standing up.

Neuropsychological Assessment

Cognitive status was assessed in a variety of domains using standardised and validated instruments. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), (Randolph et al, 1998) was used to assess immediate and delayed memory, attention, visuoconstruction and language domains. It is a relatively brief but comprehensive test of cognition that takes approximately 30

minutes to administer. Global cognitive performance in the RBANS was represented by a composite Total Scale. Executive function was measured specifically with the Frontal Assessment Battery (FAB) (Dubois et al., 2000) and a brief global assessment of cognitive function was also measured with the Mini Mental State Examination (MMSE) (Folstein et al., 1975).

Biophysical Measurements

Height, hip and waist circumference were measured with standard tape measure accurate to 1 mm. Weight was measured with a calibrated scale accurate to 0.1 kg and with the subject wearing indoor clothes and shoes. Body mass index (weight/ height^2) were calculated according to the standard definition. Subjects were also evaluated with the Timed Up and Go test (TUG). This is a measure of the time it takes to get out of a chair, walk three metres, turn around and walk back to return to their original seated position (Podsiadlo et al., 1991). Blood pressure was measured with a clinically validated automated blood pressure recording device (Omron 705CP-II) with subjects in a seated position (El Assaad et al., 2003). Readings were taken until both systolic and diastolic blood pressure were within 5 mmHg or otherwise until a total of five readings were made.

Blood Sampling

Non-fasting blood samples were taken either on the day of assessment or within the following week. These included full blood count, renal, bone and liver profile, random lipids and glucose, glyscosylated haemoglobin (Hba1c), C reactive protein (CRP), red cell folate and serum vitamin B12.

Bloods were processed on the day of collection and centrifuged within one hour. All of the above bloods in the cognitive and bone cohort were analysed at St. James's Hospital, Dublin and those in the hypertensive cohort at Altnagelvin and Causeway Hospitals in Northern Ireland.

In addtition, samples were also taken for 25-hydroxyvitamin D and parathyroid hormone. All vitamin D and PTH samples were stored at –70°C and batched for later analysis at the biochemistry laboratory at St James's Hospital, Dublin which is DEQAS accredited. 25-hydroxyvitamin D levels were measured by liquid

chromatography mass spectroscopy (LC-MS) using a standardised assay (MassChrom®). Blood samples for parathyroid hormone were stored on ice until delivered to the lab and were measured by electrochemiluminesce (Roche Assay).

CHAPTER 8: STUDY POPULATION CHARACTERISTICS

Study data was available for 1800, 1704 and 1404 participants in the hypertensive, cognitive and bone cohorts respectively. 533 subjects were excluded after application of study inclusion criteria (MMSE ≥ 24). The above MMSE cut-off was chosen so as to avoid including subjects with dementia in our study. A further 278 were excluded due to missing or incomplete data, leaving the respective number in each of the above cohorts 1568, 1330 and 1199.

Statistical Analysis

Descriptive analysis and comparison of cohort characteristics were made using the one way anova, unpaired t-test, wilcoxon rank and chi squared tests as appropriate. All statistical analysis was performed using JMP Edition 10.1 (SAS Institute, 2010).

The demographic, clinical, psychosocial and lifestyle characteristics, as well as serum vitamin D status for each cohort are summarised in Table 8.1. Significant differences between cohorts existed for nearly all of the above characteristics and are discussed in detail below.

Cognitive Cohort

Subjects in the cognitive cohort were older (mean age 80.4 ± 6.8 years), had a lower education (median of 10.0 years) and were more likely to suffer with depression (13.1%) and live alone (47.0%). They also had poorer functional (median score for IADL 21 and PSMS 23) and cognitive (mean MMSE 27.1 ± 1.7) status and the highest prevalence of falls (51.4%) and stroke (16.4%). They also had higher prevalence of hypertension compared to bone cohort participants (80.9% versus 65.0%). Mean Timed Up and Go was approximately twice that of subjects in the other cohorts (mean 21.1 ± 11.0 seconds).

Hypertensive Cohort

Subjects in the hypertensive cohort were more than 10 years younger than cognitive cohort participants (mean age 69.5 ± 6.1 years) though were only marginally younger than those in the bone cohort (mean difference 1.7 years, P <0.001). They were least likely to live alone (24.0%) and had the lowest prevalence of falls (22.3%).

Conversely, they had the highest body mass index (mean 29.9 ± 5.0 kgm^{-2}) and prevalence of type II diabetes (17.2%) and of vitamin D deficiency (59.5%). However, they also had the smallest proportion taking vitamin D supplements (25.6%).

Bone Cohort

Bone cohort participants were more likely to be female (85.2%) and had the lowest body mass index (26.3 ± 5.1 kgm^{-2}) and prevalence of type II diabetes (5.3%), hypertension (65.0%) and cardiovascular disease (MI 4.5%, stroke 2.1%, PAD 2.2%). They also had the highest proportion taking vitamin D supplements (73.2%).

As the bone and hypertensive cohort were similarly aged, a separate analysis comparing characteristics specifically between both was performed. In addition to the findings above, those in the hypertensive cohort were found to have a higher prevalence of stroke ($P = 0.03$) and myocardial infarction ($P < 0.001$), were more likely to score lower on the Frontal Assessment Battery (FAB) ($P < 0.001$) and have a current or a past history of depression ($P = 0.001$). However, there was no difference in physical frailty as measured by the Timed Up and Go ($P = 0.27$) or function assessed by the Physical Self Maintenance Scale ($P = 0.09$) or in performance on the MMSE ($P = 0.34$).

Table 8.1: Study Cohort Characteristics

Cohort Characteristics				
	Hypertensive (N = 1568)	**Bone (N = 1199)**	**Cognitive (N = 1330)**	**P-Value**
Demographics				
Age (yrs)	69.5 ± 6.1	71.2 ± 7.4	80.4 ± 6.8	<0.001[a]
Gender (female, %)	56.0	85.2	66.6	<0.001[b]
Education (median)	11.0	12.0	10.0	<0.001[c]
Living alone (%)	24.0	33.2	47.0	<0.001[b]
Psychosocial Factors				
Alcohol (past or current)	75.4	79.8	72.9	<0.001[b]
Smoking (past or current)	43.8	52.8	54.8	0.01[b]
Exercise (in past 2 weeks)	80.0	88.0	66.3	<0.001[b]
History of Depression	25.2	19.4	25.6	0.001[b]
Current Depression (CESD-≥16)	9.5	6.6	13.1	<0.001[b]
Serum vitamin D				
25(OH)D ($nmoll^{-1}$)	49.0 ± 25.1	77.5 ± 30.2	56.6 ± 31.8	<0.001[a]
Severe deficiency (<25$nmoll^{-1}$)	15.5	3.4	17.8	<0.001[b]
Deficiency (25-49.9 $nmoll^{-1}$)	44.0	15.3	28.6	<0.001[b]
Insufficiency (50-74.9 $nmoll^{-1}$)	25.4	27.4	23.5	<0.001[b]
Cognitive Scales				
MMSE (mean)	27.9 ± 1.4	28.0 ± 1.6	27.1 ± 1.7	<0.001[a]
FAB (median)	16	17	15	<0.001[c]
Functional Scales				
IADL (median)	27	27	21	<0.001[c]
PSMS (median)	24	24	23	<0.001[c]
Medical Disease				
Myocardial infarction (MI) (%)	12.1	4.5	12.1	<0.001[b]
Peripheral Arterial Disease (PAD) (%)	2.9	2.2	6.5	<0.001[b]
Diabetes (%)	17.2	5.3	13.2	<0.001[b]
Hypertension* (%)	100	65.0	80.9	<0.001[b]
Stroke (%)	3.7	2.1	16.4	<0.001[b]
Falls (in last year) (%)	22.3	35.5	51.4	<0.001[b]
Physical Measures				
Timed up and Go (TUG) (sec)	10.1 ± 3.8[d]	10.0 ± 5.8[e]	21.1 ± 11.0[f]	<0.001[a]
Body Mass Index (kgm^{-2})	29.9 ± 5.0	26.3 ± 5.1	27.1 ± 5.5	<0.001[a]
Vitamin D Supplements				<0.001[b]
Prescribed vitamin D + Calcium (%)	5.9	64.5	42.3	
Cod liver oil/fish oil (%)	16.3	4.4	5.4	
Active vitamin D (%)	0.5	0.3	1.5	
Non prescribed vitamin D (%)	2.9	4.0	3.2	
[a] Anova test, [b] Chi Squared Test, [c] Wilcoxon rank test, [d] N= 1536, [e] N= 1181, [f] N= 1181, Mean ± SD				
* Hypertension - self reported and/or systolic BP≥ 140 or diastolic BP ≥ 90 mmHg.				

CHAPTER 9: DISCUSSION

Participants in TUDA have a higher prevalence of ill health across a number of self-reported and objective outcomes than would be expected in an unselected, general population study (TILDA Study, Barrett et al., 2011). This is not surprising given that the cohorts were 'disease' defined and therefore also likely to have subjects with other co-morbidities.

In particular, the cognitive cohorts represent a group of 'older' old frail adults with significant physical, functional and cognitive impairment. Their gender (female, 67%) and age (80.4 ± 6.8 years) is reflective of patients attending a general geriatric outpatient service. Timed up and Go scores (mean 21.1± 11.0 sec) were significantly higher than what is considered normal for older adults, indicative of a high level of physical frailty (Bischoff et al., 2003; Herman et al., 2011). It compares to a proposed normal cut-off time of 12.7 seconds identified in a meta-analysis (N=1102) of older adults aged 80-99 years of age (Bohannon, 2006). Not surprisingly this cohort had the highest rate of having a fall in the previous year (50.1%) with a similar prevalence to that found in other studies of 'older' old. It also had the highest prevalence of depression (13.1%). This may be explained by the fact that it had the highest proportion of participants living alone and with cognitive and functional impairment, which are risk factors for depression (Cervilla et al., 1997; Prince et al., 1997). In addition, the prevalence of stroke was highest in this cohort which may directly account for physical frailty and cognitive impairment. Overall, several interconnected factors (physical, cognitive and medical) are likely to contribute to the frail status of subjects in this cohort

Participants in the bone and hypertensive cohort were significantly younger (approximately 10 years), but also varied across a variety of characteristics reflective of other co-morbidities associated with their respective cohort diagnoses. In particular, the prevalence of diabetes (17.2%) was greatest in the hypertensive cohort and was high by comparison to population data for Northern Ireland (9.4% prevalence in those aged between 65-74 years, Slan Report, 2007). Those who are diabetic may be more likely to have their blood pressure checked and been known 'hypertensives' introducing bias selection as a potential cause. These subjects also had the highest

body mass index which may also be related to the high level of diabetes. Given the high prevalence of diabetes combined with their diagnosis of hypertension, it is not surprising that cardiovascular disease (stroke, MI) was more common than in similarly aged participants in the bone cohort. Indeed, the prevalence of MI was identical to that of the much older cognitive cohort participants. Interestingly, performance on the Frontal Assessment Battery (FAB) which measures executive function was lower than in bone cohort subjects ($P < 0.001$). A higher rate of stroke and possibly silent cerebrovascular disease (given the greater prevalence of vascular risk factors) may account for this.

As expected subjects in the bone cohort were predominantly female and had the highest proportion taking prescribed vitamin D supplements. This is not surprising given that osteopaenia / osteoporosis is more common in females who would have been prescribed vitamin D routinely following diagnosis. The significant proportion not taking supplements can be explained by the fact that approximately 300 subjects were recruited prospectively on the day of DXA diagnosis before any vitamin D treatment may have been instituted. Falls prevalence was greater in the bone than the hypertensive cohort, which may simply reflect bias selection, as those who fall are more likely to fracture or be sent for DXA assessment. Despite the higher rate of falls, there was no significant difference in physical frailty as assessed with the TUG ($P = 0.26$) suggesting a role for other factors.

Vitamin D Status

There was a significant difference in serum 25(OH)D across all cohorts, with the lowest level identified in the hypertensive (49.0 ± 25.1 nmol/l) and cognitive (56.6 ± 31.8 nmol/l) cohorts and the highest level in the bone cohort (77.5 ± 30.2 nmol/l), ($P < 0.001$). Whilst many factors are likely to contribute to this finding, the cohort differences in supplement use may be the most important. As the majority of bone cohort participants were taking supplements, it is not surprising that mean vitamin D was much higher than in hypertensive cohort subjects. Likewise, the higher vitamin D level (+ 7.6 nmol/l) observed in the cognitive versus the hypertensive cohort is also likely to be due to their greater supplement use. The difference was however small, and may have been attenuated by other factors, such as their high level of physical

frailty which has the potential to lower vitamin D status via reduced physical activity and sun exposure. This is also likely to account for the significantly lower vitamin D (-20.9 nmol/l) in the cognitive versus the bone cohort.

It is also important to note that the different geographical location of hypertensive cohort participants (55^{o} versus 53^{o} N latitude) may help to explain their lower vitamin D status. For a more detailed analysis and discussion see Chapter 9.

Conclusions

TUDA study participants have a higher level of disability and ill health than in the general population, reflective of multiple co-morbidities associated with each 'disease' defined cohort. Significant differences in participant characteristics were found between all cohorts. Cognitive cohort subjects were older (approximately ten years), frailer and had more falls, physical, cognitive and functional impairment. Those in the hypertensive cohort were youngest, but had the highest prevalence of diabetes and for their age a relatively high proportion had cardiovascular disease. Finally, bone cohort subjects were more likely to be female, had the least prevalence of cardiovascular disease and depression and had better cognitive status.

SECTION 4: CORRELATES OF VITAMIN D IN OLDER IRISH ADULTS

CHAPTER 10: INTRODUCTION

Several factors are associated with serum 25-hydroxyvitamin D. As approximately 90% of all vitamin D is synthesised by our skin, many behavioural practises which determine sunlight exposure play an important role. Geographical location and geophysical factors also significantly influence ultraviolet B irradiation and thereby vitamin D production. In addition, lifestyles factors including dietary and supplement intake also contribute to vitamin D levels.

Vitamin D deficiency is prevalent, but varies widely and at population level may be important for many non-bone health outcomes including cancer, depression, cardiovascular, neuro-cognitive and autoimmune disease. The relative importance of different factors in determining vitamin D status may vary by location and between different population groups. In particular, in locations such as Ireland, production is nonexistent for about six months of the year in what has been called the 'Vitamin D Winter'. An increased prevalence of deficiency is found in older adults who have reduced capacity for cutaneous synthesis and may spend less time outdoors, thereby requiring a greater dietary intake to meet their vitamin D requirements.

The purpose of this study is to determine the correlates of vitamin D in a well defined group of community dwelling older Irish adults living at 53^{o} and 55^{o} North Latitude by exploring the relationship with multiple factors (geophysical, biophysical, psychosocial and lifestyle). It also aims to identify the prevalence of vitamin D insufficiency/deficiency and to explore the extent to which this is affected by season and supplementation.

CHAPTER 11: METHODOLOGY

Study Population and Design

The study population comprised participants of the TUDA (Trinity, University of Ulster, Dept. of Agriculture) study which been previously described. This is a large collaborative, cross sectional study designed to create a phenotype/genotype database for three cohorts of community dwelling subjects aged over sixty years. Study data was available for 1800, 1704 and 1404 participants in the hypertensive, cognitive and bone cohort respectively.

Methodology

Study methods are as previously outlined (see chapter 8). Participants with MMSE scores below 24 were excluded as we aimed to look for correlates of vitamin D in non-dementia subjects. Serum 25-hydroxyvitamin D was measured in non-fasting blood samples taken either on the day of assessment (bone and cognitive cohort) or within the following week (hypertensive cohort). Samples were centrifuged within one hour and stored at $-70^{o}C$ until further batched for analysis. 25(OH)D was measured by liquid chromatography mass spectroscopy (LCMS) at the biochemistry laboratory at St James's Hospital, Dublin which is accredited by the Vitamin D External Quality Assessment Scheme (DEQAS).

Statistical Analysis

All parameters were inspected for normality and if significantly skewed were appropriately transformed. All statistical analysis was performed using JMP Edition 10.0.2. (SAS Institute, 2010).

Study Covariates

The total monthly global solar radiation (GSR) which represents the total amount of solar irradiation received per unit area per month ($KJcm^{-2}$) was used as a surrogate marker of UVB exposure. GSR data was obtained by request from the Irish Meteorological Service. Data from weather stations nearest to study recruitment sites (Dublin Airport) and (Malin Head, Donegal) were used respectively for analysis in the Cognitive / Bone and Hypertensive cohort.

Season was defined according to the standard meteorological definition used in Ireland (Irish Meteorological Service) with the following months comprising Winter (Dec, Jan, Feb), Spring (Mar, Apr, May), Summer (Jun, Jul, Aug), and Autumn (Sept, Oct, Nov).

Timed Up and Go (TUG) measurements were divided into quintiles as it was not possible to measure it in a small proportion of subjects with significant physical disability. These subjects were categorised in the lowest quintile of TUG performance.

Statistical Models

Mean 25(OH)D levels were compared between both groups in each cohort, as well as across each season, with the use of the one way Anova, unpaired-t and Chi-squared tests as appropriate. Graphical plots were made to visually explore the seasonal variation in 25(OH)D in both supplemented and non-supplemented subjects in each cohort. Supplements included prescribed and over the counter vitamin D as well as use of cod liver oil.

Associations between serum 25(OH)D and potential lifestyle, geophysical, psychosocial and clinical confounders were explored in a forward regression model in each cohort. Factors suspected of influencing vitamin D status including age, gender, season, GSR, BMI, supplement use, sun holiday travel, preference for sunshine or shade, depression status, education, marital status, smoking and alcohol drinking status, margarine, eggs and oily fish consumption were included in the model. Those that were identified as significant were included in a final model along with well established confounds (age, gender, season and body mass index).

In addition, as participants in the hypertensive cohort were in a different geographical location (+2^{o} North latitude), a comparison was made between the mean seasonal global solar radiation (GSR) across cohorts (during the study recruitment period 2010-2012).

CHAPTER 12: RESULTS

Five hundred and thirty three subjects were excluded after application of study inclusion criteria (MMSE ≥ 24). A further 278 subjects were excluded due to missing or incomplete data, leaving the respective number in the hypertensive, cognitive and bone cohort 1568, 1330 and 1199. For an outline of study cohort participant characteristics see Table 8.1.

Vitamin D Status

The mean serum 25(OH)D of supplemented subjects were 82.8, 72.3 and 62.3 nmol/l in the respective bone, cognitive and hypertensive cohorts. The corresponding values for non-supplemented cohort participants were 63.0, 39.1 and 44.5 nmol/l (See Table 12.1).

Serum Vitamin D and supplementation

The overall mean difference in serum 25(OH)D between supplemented and non-supplemented subjects were 19.8 nmol/l, 33.2 nmol/l and 17.8 nmol/l in the bone, cognitive and hypertensive cohort respectively (See Table 12.1).

The prevalence of vitamin D deficiency (<50 nmol/l) in non-supplemented subjects were 40.2%, 73.6%, 67.4% and in the bone, cognitive and hypertensive cohorts respectively. Approximately one quarter (24.8%) of those in the cognitive, one third (34.1%) in the hypertensive and only 10.8% in the bone cohort remained deficient despite being on supplements (See Table 12.2).

Seasonal Variation in vitamin D

There was a significant seasonal variation in serum 25(OH)D in all three cohorts in both supplemented and non-supplemented subjects ($P < 0.001$). Serum 25(OH)D was lowest in the Spring and highest in the Summer/Autumn ($P < 0.001$) with trough and peak levels in the months of February/March and August respectively. 25(OH)D levels remained statistically higher in supplemented versus non-supplemented subjects in each cohort across all seasons ($P < 0.001$) apart from in the bone cohort during Winter where the difference was non-significant (See Table 12.3).

The trough to peak seasonal difference in vitamin D for supplemented and non-supplemented subjects was (16.2 versus 21.4 nmol/l) in the hypertensive, (9.2 versus 18.0 nmol/l) in the bone and (16.2 versus 14.0 nmol/l) in the cognitive cohort.

Table 12.1: Comparison of serum 25(OH)D across cohorts

Serum 25(OH)D in cohorts (nmol/l)				
	Supplemented	Non-Supplemented	Difference	P- value
Cognitive	72.3 ± 30.2 (N = 700)	39.1 ± 23.0 (N = 630)	33.2	<0.001[a]
Bone	82.8 ± 27.0 (N = 878)	63.0 ± 33.7 (N = 321)	19.8	<0.001[a]
Hypertensive	62.3 ± 23.1 (N = 399)	44.5 ± 26.8 (N = 1169)	17.8	<0.001[a]
[a] unpaired t-test				

Table 12.2: Comparison of serum 25(OH)D across cohorts by category.

Serum 25(OH)D by category in different cohorts (nmol/l)						
	Cognitive (N=1330)		Bone (N=1199)		Hypertensive (N=1568)	
25(OH)D nmol/l	**Y**	**N**	**Y**	**N**	**Y**	**N**
< 25.0 (%)	5.7	31.2	1.6	8.4	4.8	19.2
25.0- 49.9 (%)	19.1	42.4	9.2	31.8	29.3	48.2
50.0- 74.9 (%)	28.8	17.8	26.7	29.6	34.3	22.3
≥ 75 (%)	46.6	8.6	62.5	30.2	29.3	10.3
Y = Supplemented, N= Non-supplemented						

Table 12.3: Seasonal Variation in serum 25(OH)D by cohort

Seasonal Variation in serum 25(OH)D (nmol/l)				
Cognitive				
	Supplemented	**Non-supplemented**	**Difference**	**P- value**
Spring	63.8 ± 28.0 (N=213)	33.3 ± 20.3 (N=173)	30.5	<0.001[a]
Summer	77.5 ± 30.0 (N=177)	41.5 ± 22.7 (N=163)	36.0	<0.001[a]
Autumn	80.0 ± 32.0 (N=164)	47.3 ± 26.0 (N=160)	32.7	<0.001[a]
Winter	69.4 ± 28.3 (N=146)	33.8 ± 21.1 (N=134)	35.6	<0.001[a]
P-value (season)	<0.001[b]	<0.001[b]		
Hypertensive				
Spring	53.9 ± 22.7 (N=93)	35.3 ± 17.9 (N=262)	18.6	<0.001[a]
Summer	70.1 ± 22.7 (N=129)	56.7 ± 24.5 (N=346)	13.4	<0.001[a]
Autumn	62.4 ± 23.9 (N=111)	44.0 ± 20.8 (N=375)	18.4	<0.001[a]
Winter	58.4 ± 28.8 (N=66)	35.4 ± 20.1 (N=186)	23.0	<0.001[a]
P-value (season)	<0.001[b]	<0.001[b]		
Bone				
Spring	77.1± 26.6 (N=224)	53.7 ± 29.7 (N=83)	23.4	<0.001[a]
Summer	85.4 ± 24.6 (N=237)	65.6 ± 23.4 (N=83)	19.8	<0.001[a]
Autumn	86.3 ± 25.8 (N=258)	62.2 ± 26.0 (N=83)	24.1	<0.001[a]
Winter	81.8 ± 31.2 (N=159)	71.7 ± 49.9 (N=72)	10.1	<0.08[a]
P-value (season)	<0.001[b]	<0.001[b]		
[a] Unpaired t-test [b] Anova test				

Table 12.4: Mean Seasonal Global Solar Radiation (GSR) by cohort

Mean Seasonal Globar Solar Radiation (2010-2012) by cohort (Jcm^{-2})				
	Cognitive	**Bone**	**Hypertensive**	**P-value**
Spring	41861	44242	43085	0.09[a]
Autumn	19257	19838	16732	<0.001[a]
Winter	9457	8264	7391	<0.001[a]
Summer	49319	48912	49021	0.08[a]
[a] One way anova test				

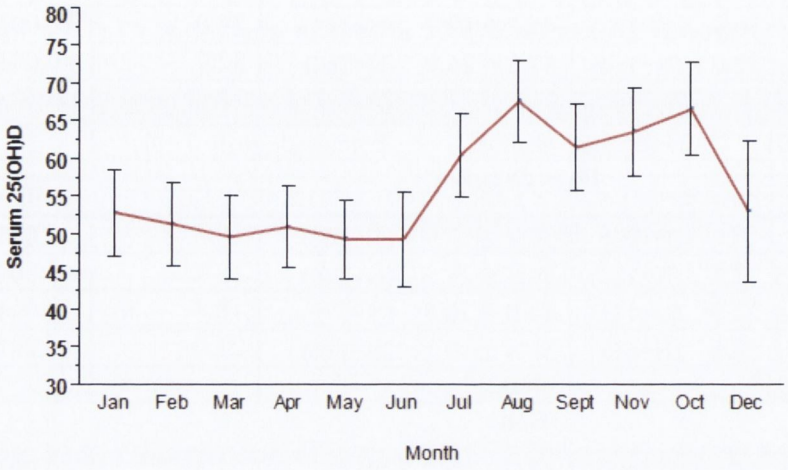
As shown above, mean seasonal global solar radiation was significantly lower in the Autumn (P <0.001) and Winter (P <0.001) in the hypertensive cohort.

Graphs showing Seasonal Variation in serum [25(OH)D] in the Cognitive cohort

Error bars represent one standard error

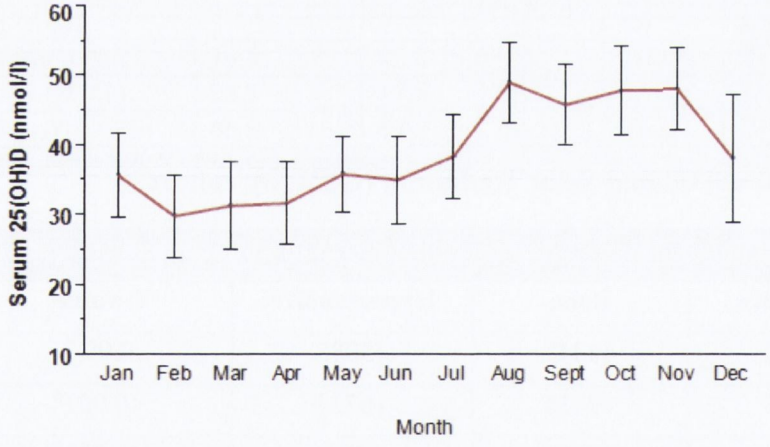
P-value: after adjustment for age, gender, BMI and /or supplement use

Fig 12.1: Total Cohort (N = 1330)



P < 0.001

Fig 12.12: Non-supplemented subjects (N = 630)



P < 0.001

Fig 12.13: Supplemented subjects (N = 700)

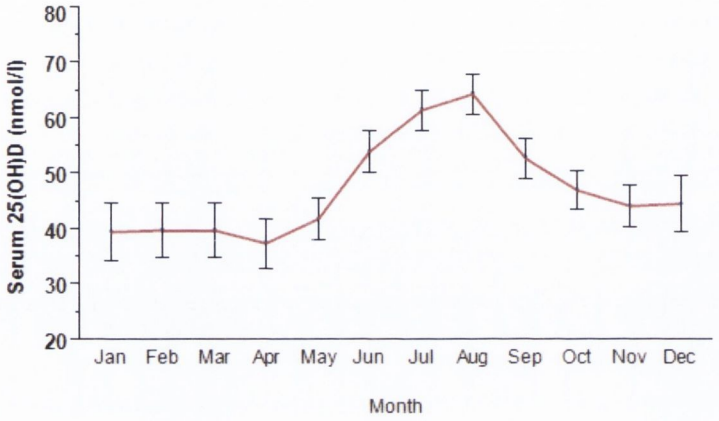
P < 0.001

Graphs showing Seasonal Variation in serum [25(OH)D] in Hypertensive cohort.

Error bars represent one standard error.

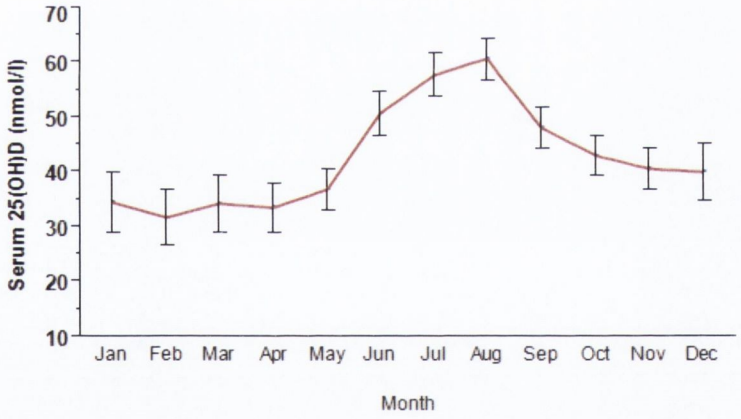
P-value: after adjustment for age, gender, BMI and supplement use (where appropriate)

Fig 12.2: Total Cohort (N = 1568)



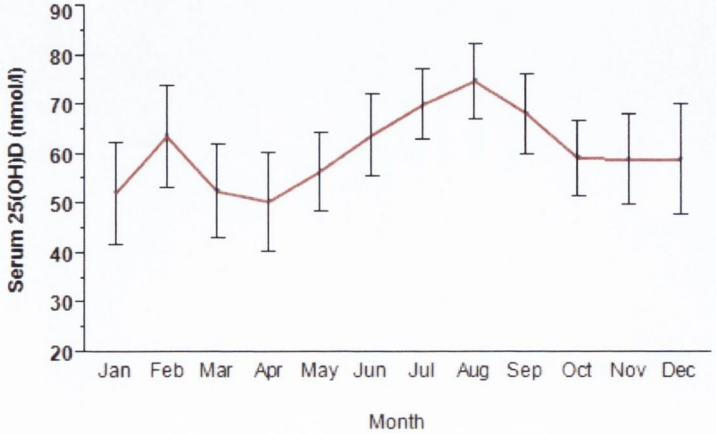
P < 0.001

Fig 12.22: Non-supplemented subjects (N = 1169)



P < 0.001

Fig 12.23: Supplemented subjects (N = 399)



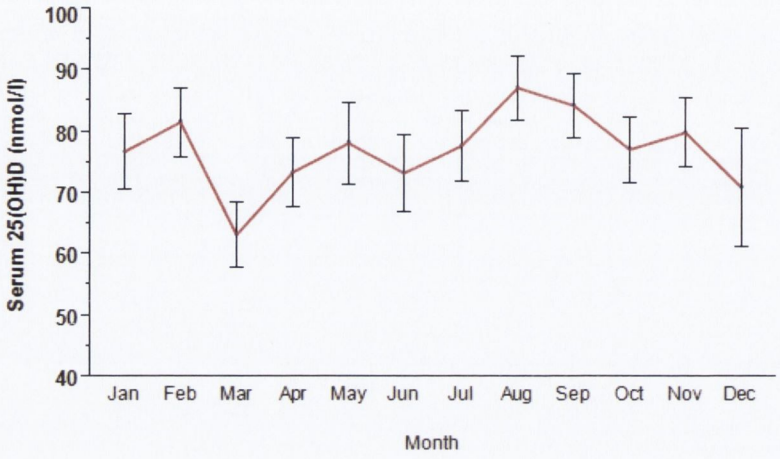
P < 0.001

Graphs showing Seasonal Variation in serum [25(OH)D] in the Bone Cohort

Error bars represent one standard error

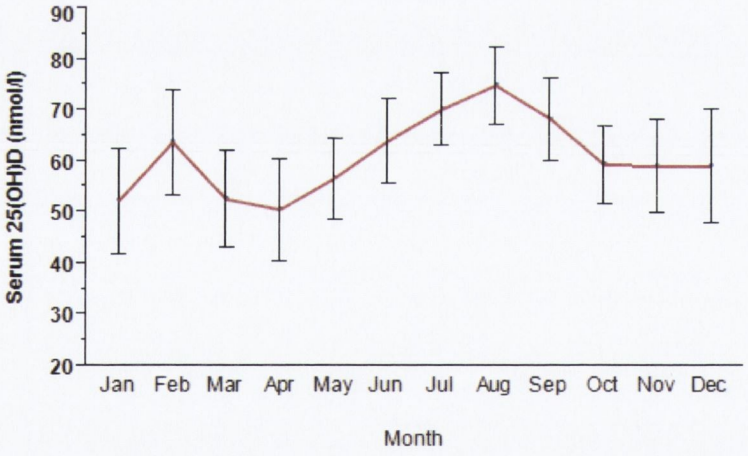
P-value: after adjustment for age, gender, BMI and supplement use (where appropriate).

Fig 12.3: Total Cohort (N = 1199)



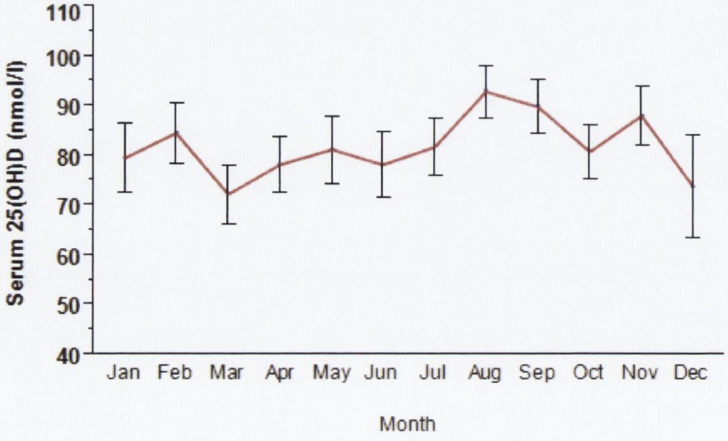
P <0.001

Fig 12.31: Non-supplemented subjects (N = 321)



P < 0.001

Fig 12.32: Supplemented (N = 878)



P < 0.001

Correlates of Vitamin D in Linear Regression Model

Collinearity was identified between Global Solar Radiation (GSR) and season (Cognitive cohort, $r^2 = 0.5$, P <0.001; Hypertensive cohort $r^2 = 0.49$, P <0.001, Bone cohort $r^2 = 0.52$, P <0.001). Due to this season or GSR was entered separately as covariates into the forward regression model used to explore correlates of vitamin D.

Table 12.5: Correlates of serum [25(OH)D] in Cognitive Cohort

Correlates of serum 25(OH)D in Cognitive cohort (N=1330) [$r^2 = 0.33$]		
	β coefficient	**P-value**
Age(yrs)	-0.02	0.83
Gender (female)	2.89	0.0004
Education	0.49	0.06
Autumn	7.08	<0.001
Spring	-6.92	<0.01
Summer	-6.11	0.008
Body mass index	-0.33	0.016
Timed Up and Go	-2.79	<0.001
Preference for sunshine	3.57	0.0018
Supplement use	16.1	<0.001

As only 5.6% (N=75) of all cognitive cohort participants went on a sun holiday in the prior six months it was excluded as a covariate in a the model.

When GSR was substituted for season in the above forward regression model, the significance of other covariates were not changed nor were new correlates identified. In addition, no association was found between serum 25(OH)D and GSR (P = 0.23)

Table 12.6: Correlates of serum [25(OH)D] in Hypertensive Cohort

Correlates of serum 25(OH)D in Hypertensive cohort (N=1568) [$r^2 = 0.32$]		
	β coefficient	**P-value**
Age(yrs)	-0.05	0.61
Gender (female)	-1.52	0.005
Autumn	7.38	<0.0001
Spring	-7.83	<0.0001
Summer	-1.40	0.46
BMI	-0.74	<0.0001
Avoid sunshine	-3.07	<0.0001
Supplement use	8.30	<0.001
Sun holiday travel	6.50	<0.001
Smoking (current)	-3.10	<0.0004
CES-D ≤16	2.13	<0.02
Oily Fish	1.29	0.08
Supplement use	8.24	<0.0001

When GSR was substituted for season in the forward regression model, the significance of other covariates were unchanged. In addition, a positive association was found between serum 25(OH)D and GSR (β = 0.000, P =0.001).

Table 12.7: Correlates of serum [25(OH)D] in Bone Cohort

Correlates of serum 25(OH)D in Bone cohort (N=1199) [$r^2 = 0.19$]		
	β coefficient	**P-value**
Age(yrs)	-0.04	0.69
Gender (female)	-1.60	0.0074
Autumn	7.70	<0.0001
Spring	-6.53	<0.0001
Summer	-1.40	0.46
BMI	-0.33	0.016
Enjoy sunshine	3.55	<0.001
Supplement use	8.32	<0.001
Sun holiday travel	7.11	<0.001
Smoking (current)	-4.69	<0.001
CES-D ≤16	2.03	<0.001
Oily Fish	1.29	0.06

When GSR was substituted for season in the forward regression model, an association was found between serum vitamin D and oily fish consumption though the significance of other covariates were unchanged (P=0.02).

Overall, the models explained up to 33%, 32% and 19% of the variation in serum 25(OH)D in the hypertensive, cognitive bone cohort respectively. Positive predictors of serum 25(OH)D in all three cohorts were season of Autumn and preference for sunshine outdoors, whilst negative predictors were season of Spring/Summer, no supplement use and body mass index.

There were however, some differences between cohorts in other predictors. For example, vitamin D levels were inversely related to physical frailty (as measured by the Timed Up and Go) only in the cognitive cohort. In addition, female gender was associated with higher and conversely lower vitamin D status in the cognitive and bone/hypertensive cohorts respectively. In the hypertensive and bone cohort other predictors of higher vitamin D status were sun holiday travel in the last 6 months and absence of depression, whilst negative correlates were current smoking. Other positive predictors of serum vitamin D were GSR in the hypertensive cohort and oily fish consumption in the bone cohort (in the model which used GSR and not season as a covariate).

CHAPTER 13: POST HOC ANALYSIS AND RESULTS

As supplement use may be a surrogate marker for other factors that may determine vitamin D status, a separate analysis to investigate for correlates of supplement use was performed in each cohort.

The relationship between GSR and serum 25(OH)D was explored graphically by plotting both variables in supplemented and non-supplemented groups in all cohorts.

Graphical plots of Timed Up and Go (in quintiles) versus serum 25(OH)D were also constructed in supplemented and non-supplemented groups in each cohort to look for potential threshold effects of vitamin D on this measure of physical frailty. The statistical significance of the relationship was tested in a multiple linear regression model adjusting for age, gender, BMI, season and /or supplement use where appropriate.

Table 13.1: Factors associated with supplement use in each cohort

	Cognitive (N =1330)		Hypertensive (N = 1568)		Bone (N = 1199)	
	β	P-value	β	P-value	β	P-value
Age	0.02	<0.001*	0.05	<0.0001*	0.03	0.006*
Gender (female)	0.37	<0.001*	0.2	0.0005*	0.16	0.07
Body mass index	-0.02	<0.0001*	-0.02	0.075	-0.08	0.016*
Autumn	-0.23	0.48	-0.23	0.017*	0.12	0.27
Spring	0.09	0.36	0.09	0.38	0.03	0.78
Summer	-0.00	0.59	0.006	0.94	0.27	0.81
Timed Up and Go	0.14	0.002*	-0.17	0.006	-0.05	0.31
Education	0.03	0.13	-0.03	0.17	0.04*	0.055
Living alone	0.14	0.016*	0.11	0.13	0.017.	0.81
MMSE	0.09	0.007*	-0.005	0.90	0.00	0.99
CES-D ≤ 16	0.06	0.93	0.24	0.048*	-0.04	0.77
Sun Holiday Travel	na	na	0.10	0.15	0.04	0.40

Statistical significance ($P < 0.05$). Model includes independent variable and all of the remaining covariates above. NB: Sun holiday travel was not adjusted for in the cognitive cohort as it applied to only 5.6% of participants.

Subjects who used supplements in all three cohorts were more likely to be older ($P < 0.01$) and apart from the hypertensive cohort have a lower body mass index ($P < 0.02$). In the cognitive and hypertensive cohort, users were also statistically more likely to be female ($P <0.001$). Some associations were specific to individual cohorts. For example, in the hypertensive cohort supplement users were less likely to be depressed ($P=0.048$) or be recruited during the Autumn ($P = 0.017$). In the cognitive cohort, supplement users had a worse performance on the Timed Up and Go ($P=0.002$) and were more likely to live alone ($P =0.016$) though despite this had a higher MMSE ($P=0.007$).

Monthly variation in serum [25(OH)D] and Global Solar Radiation (GSR) in the Hypertensive Cohort.

Error bars represent one standard error.

P-value: For relatiionship between GSR and 25(OH)D after adjusting for age, gender, BMI, TUG and/or supplement use.

Fig 13.1: Total Cohort (N = 1568)

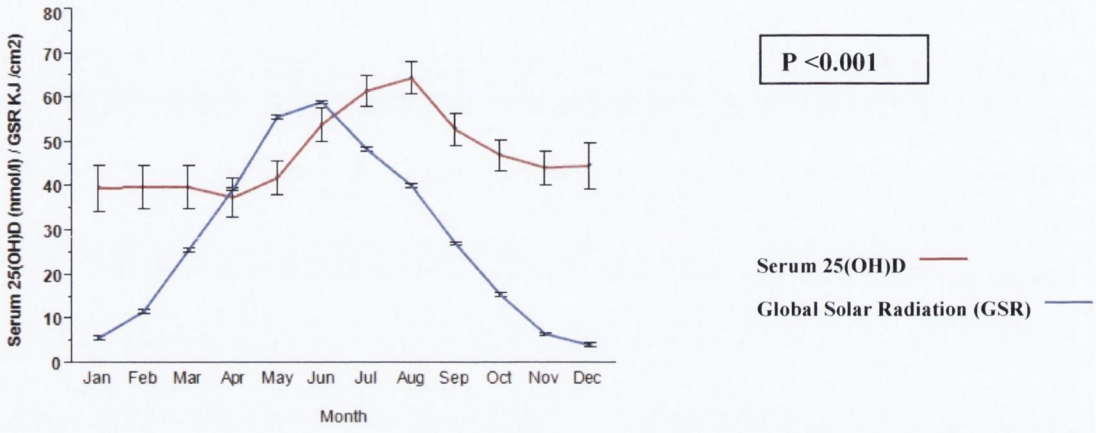


Fig 13.11: Non supplemented (N = 1169)

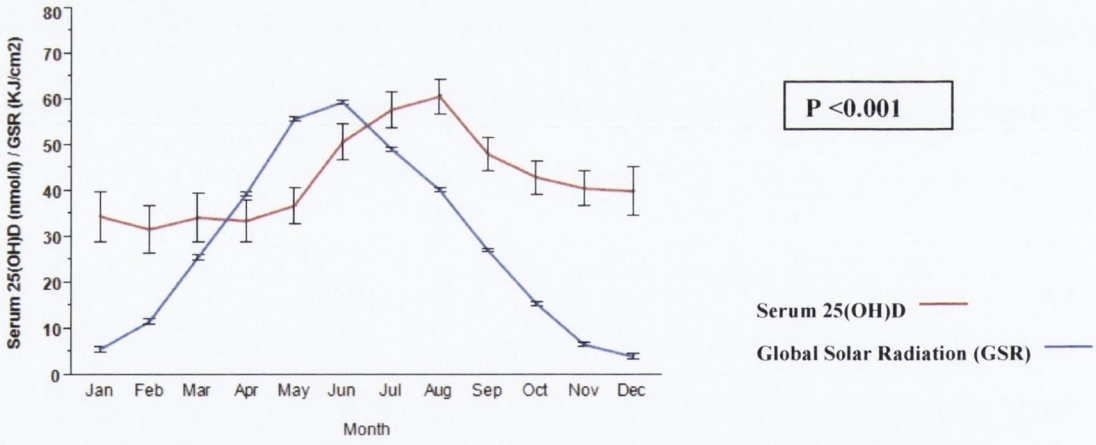
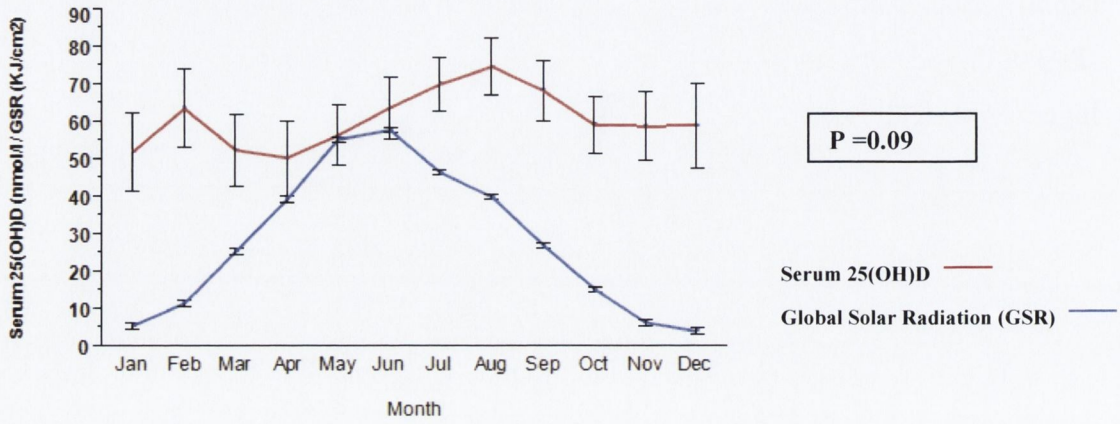


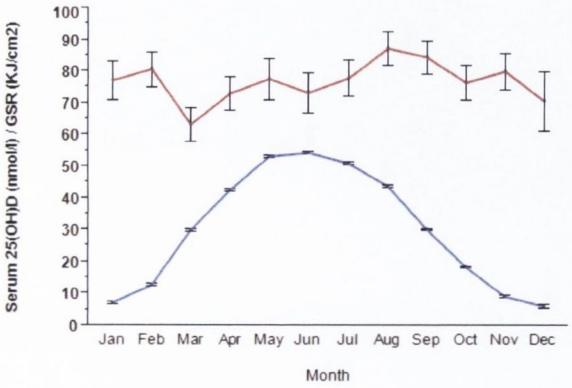
Fig 13.12: Supplemented (N = 399)



Graphical Plot of 25-Hydroxyvitamin D [25(OH)D] and monthly Global Solar Radiation (GSR) in Cognitive and Bone Cohort

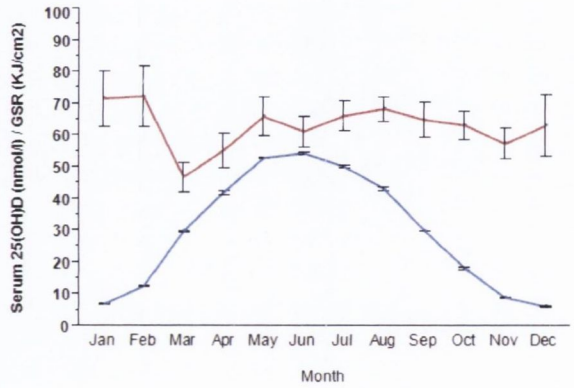
BONE COHORT

Fig 13.2: (Supplemented N=878)



P =NS

Fig 13.21: (Non supplemented N=321)



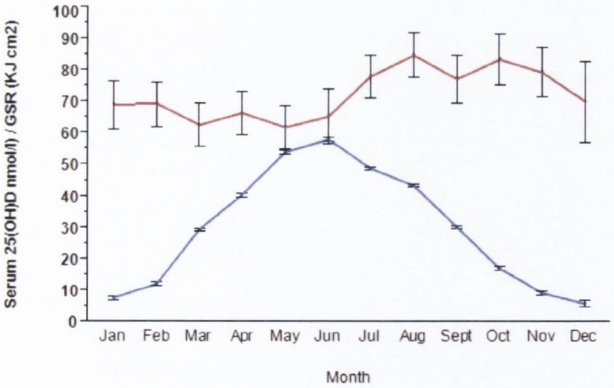
P =NS

Serum 25(OH)D

Global Solar Radiation (GSR)

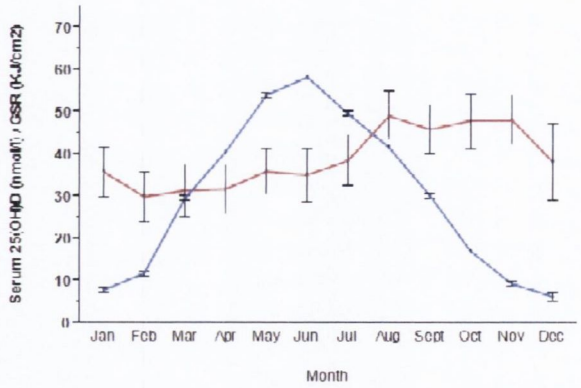
COGNITIVE COHORT

Fig 13.3: Supplemented (N=700)



P =NS

Fig 13.31: Non-supplemented (N=630)



P =NS

Serum 25(OH)D

Global Solar Radiation (GSR)

P- value: For relationship between GSR and 25(OH)D after adjusting for age, gender, season, BMI, TUG and supplement use (where appropriate), NS =Non-significant (P≥ 0.05).

As shown graphically, there is lag period of about one month between increases in global solar radiation and serum 25(OH)D in the hypertensive cohort ($P<0.001$). However, no statistically significant relationship was found in those who were supplemented ($P=0.09$), (see Fig's 13.1-13.12).

In bone cohort, there appears to be a synchronous rise in GSR and serum 25(OH)D during the months of March to May though overall no statistically significant relationship was identified (see Fig's 13.2 & 13.21).

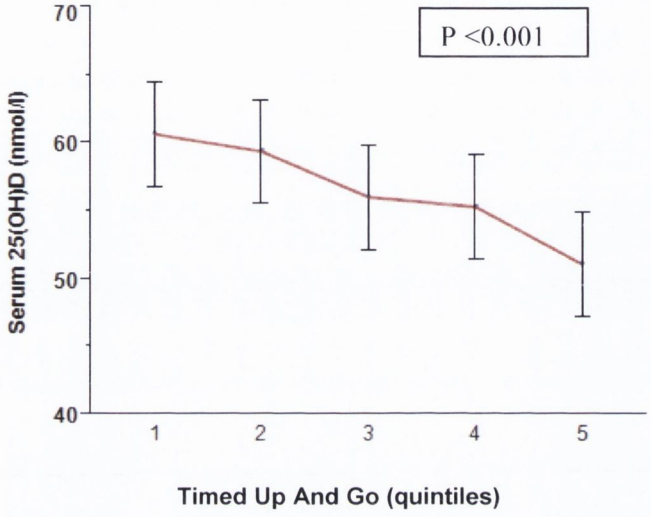
In both users and non-users of supplements in the cognitive cohort, there is a longer lag period between rises in GSR and 25(OH)D though no statistically significantly relationship was found (see Fig's 13.3 & 13.31).

Graphs to show Timed Up and Go versus serum [25(OH)D] in Cognitive cohort

Error bars represent one standard error

P-value: adjusted for age, gender, BMI, season and/or supplement use (where relevant)

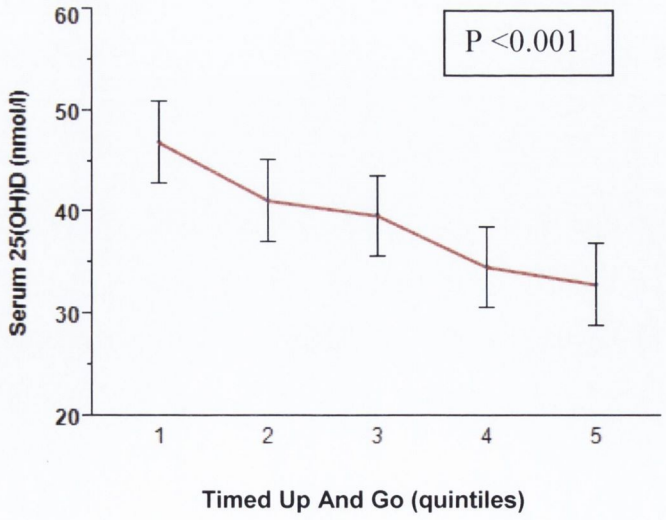
Fig 13.40: Total Cohort (N = 1330)



Quintiles: I (4- 14), II (14-18), III (18-22), IV (22-28), V (>28) seconds

.

Fig 13.41: Non-supplemented (N = 630)



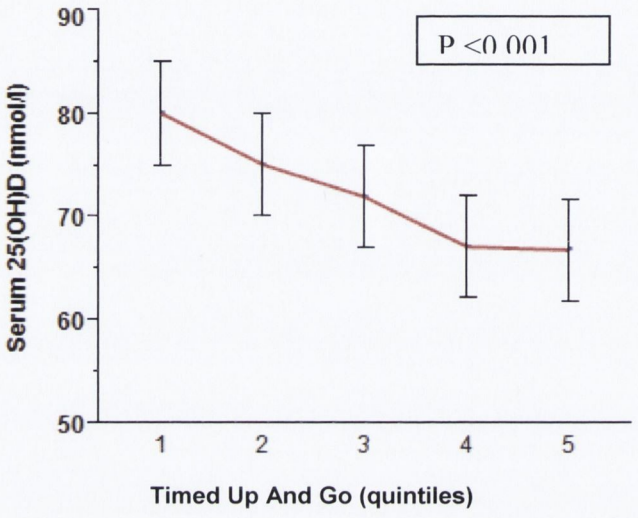
Quintiles: I (4-12), II (12-18), III (18-22), IV (22-28), V (>28) seconds

Graphs to show Timed Up and Go versus serum [25(OH)D] in Cognitive cohort

Error bars represent one standard error

P-value: adjusted for age, gender, BMI, season and supplement use.

Fig 13.42: Supplemented (N = 700)



Quintiles I [(4-16), II (16-20), III (20-24), IV (24-28), V (>28)] seconds

As shown graphically there was a significant inverse relationship between performance on Timed Up and Go in the cognitive cohort (in both supplemented and non-supplemented groups, P <0.001). A rise in serum 25(OH)D of up to 80 nmol/l was associated with better TUG performance (See Fig's 13.40 –13.42).

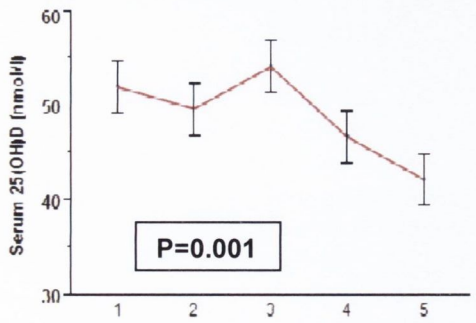
In the hypertensive cohort, serum 25(OH)D levels of approximately 45-50 nmol/l were associated with optimal TUG performance (P <0.001) in non-supplemented subjects. In those who were supplemented a 25(OH)D level of up to about 65 nmol/l was also associated with a better TUG time (P=0.02) (see Fig's, 13.50-13.52). There appeared to be a threshold effect with a TUG time of greater than about 10 seconds being associated with lower serum vitamin D

No difference in serum vitamin D was found across TUG quintiles in the bone cohort (see Fig's 13.6-13.62).

Graphs to show Variation in Timed Up and Go versus serum 25 Hydroxyvitamin D [25(OH)D]

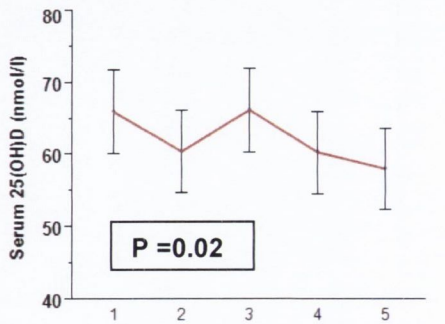
HYPERTENSIVE COHORT

Fig 13.5: Total (N = 1568)



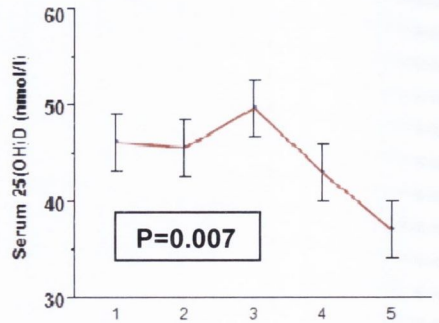
TUG: Q1 (4.5- 7.3), Q2 (7.3-8.7), Q3 (8.7.-10.0), Q4 (10.0-12.1), Q5 (>12.1) sec

Fig 13.51: Supplemented (N = 399)



TUG: Q1 (5.0-7.1), Q2 (7.1-8.5), Q3 (8.5-9.7), Q4 (9.7-11.7), Q5 (>11.7) sec

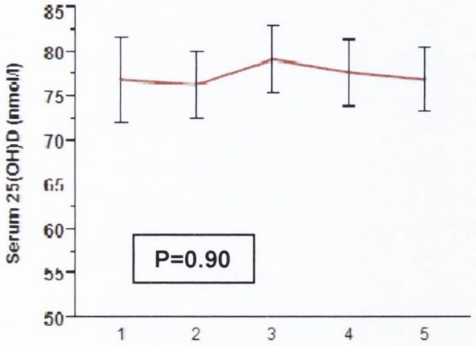
Fig 13.52: Non-supplemented (N = 1169)



TUG: Q1 (4.5-7.4), Q2 (7.4-8.7), Q3 (8.7-10.), Q4 (10.0-12.3), Q5 (>12.3) sec

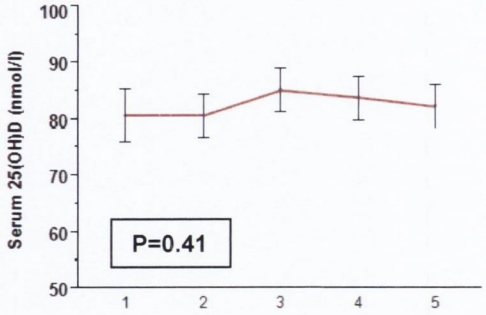
BONE COHORT

Fig 13.6: Total (N=1199)



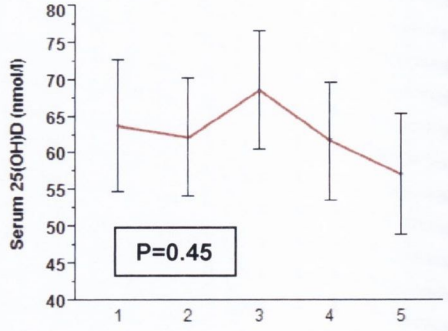
TUG: Q1 (3.0-7.0), Q2 (7.0-8.0),Q3 (8.0-9.6), Q4 (9.6-12.0), Q5 (.>12.0) sec

Fig 13.61: Supplemented (N = 878)



TUG: Q1 (3.0-6.9), Q2 (6.9-8.0), Q3 (8.0-9.7), Q4 (9.7-12.1), Q5 (>12.1) sec

Fig 13.62: Non-supplemented (N = 321)



TUG: Q1 (4.0-7.0), Q2 (7.0-8.0), Q3 (8.0-9.4), Q4 (9.5-12.0), Q5 (>12.0) sec

CHAPTER 14: DISCUSSION

The most important correlates of serum vitamin D in all cohorts were season of measurement and supplement use. Other predictors were body mass index and preference for sunshine when outdoors. However, some factors were not consistently associated with vitamin D across cohorts. These included physical frailty, smoking status, female gender, oily fish consumption and monthly globar solar radiation (GSR) at the time of blood testing. This suggests that specific cohort characteristics may alter the affect of factors determining vitamin D status. Sun holiday travel was a positive predictor of vitamin D as expected, though no conclusions on any potential affects in the cognitive cohort could be drawn due to the small numbers taking vacations. Overall, the models explained approximately between one fifth to one third of the variation in vitamin D status. The effect and potential mechanisms via which the various factors may influence vitamin D are discussed in detail below.

Global Solar Radiation

The total GSR in the previous month was positively associated with serum 25(OH)D but only in the hypertensive cohort (r^2=0.03). This is similar to the findings of a previous study of Irish outpatient subjects residing at a similar latitude (Romero-Ortuno et al., 2011). Results are also in keeping with a recent study that showed a small positive correlation between cumulative solar UV-B irradiance averaged over a 3 month period and 25(OH)D levels (Greenfield et al., 2012). A lag period of about two months between changes in GSR and serum 25(OH)D was identified graphically in a post hoc analysis and may represent the time to reach steady state post UVB exposure. This is also reflected in a large study that found an interval of about 6-8 weeks between GSR and vitamin D levels in 1418 non-supplemented community dwelling Germans (Klenk et al., 2013). Likewise, a two month interval was noted between the vitamin D peak and GSR peak in a study of older Irish adults (Romero-Ortuno et al., 2011).

No relationship was found between serum 25(OH)D and GSR in the cognitive or bone cohort. However, the relationship between UVB irradiation and serum 25(OH)D levels is complex. Factors which directly influence UVB exposure such as time spent outdoors, clothing habits and use of sunscreen are likely to be more important. In

particular, less time spent outdoors for subjects in the cognitive cohort (who were older and frailer) may explain the lack of association. In this regard, younger age and more physical activity have been associated with a stronger relationship between UVB irradiation and 25(OH)D (Greenfield et al., 2012). It is possible that the lag period between UVB irradiation and vitamin D synthesis is greater in the oldest old who may also have a more staggered exposure period due to frailty. Cumulative GSR data for the preceding three as opposed to one month might have related better to vitamin D status in such a group.

Season

Vitamin D was lowest in Spring and highest in Autumn, consistent with large studies of European (Klenk et al., 2013) and US adults (Kasahara et al., 2013). Lowest levels were found in the months of February/March and the highest in the month of September. The seasonal variation in 25(OH)D was significant in all cohorts regardless of supplement use. This conflicts with a previous study of outpatient subjects attending a Dublin clinic which suggested that vitamin D supplements might attenuate any seasonal variation (Romero-Ortuno et al., 2011). The magnitude of the seasonal difference was up to 20 nmol/l, a finding similar to that found in subjects living at the same latitude (Romero-Ortuno -et al., 2011).

Biophysical Factors

Age

Age was not an independent predictor of 25(OH)D status, however, this is consistent with the results of other studies that included mainly older adults (Burgaz et al., 2007; Greenfield et al., 2012). This is probably due to the relatively narrow age range of participants in each cohort. It may be that there is a 'plateau effect' beyond which the negative influence of age on vitamin D diminishes. Whilst a putative mechanism to explain this relates to a reduced level of the vitamin D precursor 7-dehydrocholesterol in the skin, there is little in the way of biological evidence to support a threshold effect for age.

Physical Frailty

Physical frailty (as measured by the Timed Up and Go) was associated with lower serum vitamin D in participants of the cognitive cohort. In a post hoc model where the relationship between TUG quintiles and vitamin D was explored, an increasing level up to and above 80 nmol/l was associated with better TUG performance. In addition, higher TUG quintiles were associated with lower serum 25(OH)D in subjects in the hypertensive cohort but only in a post hoc model which adjusted for age, gender, BMI season and supplement use. In this cohort, there appeared to be a threshold effect with a Timed Up and Go performance above 10 seconds being associated with lower vitamin D status. The overall model looking at correlates of vitamin D included other covariates such as sun holiday travel and depression status. Inclusion of these factors negated the independent relationship between vitamin D and TUG in hypertensive cohort participants. However, physical frailty due to low vitamin D status may account for differences in sun holiday travel and depression which could explain this result.

Other studies exploring the relationship between vitamin D and frailty measures in the elderly have given conflicting results with reports of either an inverse (Gerdhem et al., 2005; Houston et al., 2007) or no association (Mathei et al., 2013). However, in many studies more specific measures including upper or lower limb strength were used (Menant et al., 2012). Few cross sectional studies have specifically looked at the relationship between TUG and vitamin D, though an inverse association has been identified in community dwelling adults aged 70 years or older (Dukas et al., 2005; Kwon et al., 2007). Whether lower vitamin D status in those with a poorer performance on the TUG is fully explained by less UVB exposure is unclear. Subjects who are physically frailer are more likely to have less outdoor activity and hence lower serum vitamin D. Conversely, vitamin D deficiency in itself may lead to a reduction in muscle strength leading to physical disability.

However, our findings in the cognitive cohort are consistent with the results of a randomised controlled trial of 242 elderly subjects (mean age 77 years) that found a 37% reduction in TUG times in association with a rise in 25(OH)D from 55.4 to 84.0 nmol/l (Pfeifer et al., 2008). In addition, other studies have also shown better physical performance with levels of up to 90 nmol/l across a number of other outcome

measures. The finding in a meta-analysis of randomised controlled trials that a level of up to 60 nmol/l is required for falls reduction (Bischoff Ferarri et al., 2006) also strongly suggests a positive role for vitamin D in physical health and function.

Body Mass Index

Lower vitamin D levels were associated with higher BMI in all cohorts, a result that is generally consistent with most studies that have explored this relationship. Several factors have been proposed (as previously discussed) to explain this effect. Sequestration of vitamin D in adipose tissue is one theory that has been hypothesized though greater BMI may also be marker for other lifestyle factors that negatively influence vitamin D. It has also been proposed that low vitamin D status may increase adiposity through increased lipogenesis as a result of elevated parathyroid hormone concentrations (Snijder et al., 2005). It may also be directly involved in cell signalling pathways in adipocytes that could lead to an increased risk of obesity (Vinh Quoc et al., 2013).

Gender

Vitamin D levels were lower in females in the hypertensive and bone cohort. Conversely, in 'older' old participants in the cognitive cohort females had better vitamin D status. Numerous studies have found lower vitamin D status in females though the results are conflicting. It is unclear what other factors that were not adjusted for in our model might account for the conflicting findings.

Lifestyle factors

Supplement use

Supplement use, as expected was associated with significantly higher serum 25(OH)D and was the second most important determinant of vitamin D status. However, a significant proportion of participants still had vitamin D deficiency despite being on supplements. The observed effect of supplementation on 25(OH)D varied between 31.3 nmol/l in the cognitive cohort to 20.1 nmol/l and 15.1 nmol/ in the hypertensive and bone cohort respectively. This compares to a mean increase in 25(OH)D of 23.8 nmol/l with supplement use identified in 546 community dwelling Irish subjects (Romero-Ortuno et al., 2011). Compliance with supplement use has been shown to be

low in studies of older adults (Grant AM et al., 2005) and therefore the influence of supplementation could be underestimated. It is also possible that the smaller effect of supplements in the hypertensive cohort might be explained by poorer compliance given that most were over the counter and non-prescribed. Those on supplements had a statistically higher serum vitamin D across all seasons with the exception of subjects in the bone cohort in the Winter. However, the comparison in this group included a small number not taking supplements (N = 73) and is unlikely to have been underpowered to detect a significant difference. In addition, as the study was not longitudinal, comparisons were made between different cohort participants.

Supplement use may be a surrogate marker for other factors that influence serum vitamin D that could also account for potential differences in the magnitude of the effect. In a post hoc analysis looking at independent correlates of supplement use, those who were supplemented in all cohorts were older had a lower body mass index (apart from the bone cohort). In addition, supplement users in the cognitive cohort were more likely to have a worse TUG performance and those in the hypertensive cohort to suffer with depression. Findings overall, suggests that users of supplements may be frailer particularly in the cognitive cohort and be more likely to have lower vitamin D in the first instance. It also suggests that the observed difference in vitamin D may be largely due to supplement use as opposed to other factors.

Surprisingly, deficiency in non-supplemented subjects in the bone cohort was less prevalent (40.2%). Post hoc analysis of correlates of supplement use, did not identify any specific factors in this group that could not account for the higher than expected vitamin D level. However, the number included was relatively small (N = 321) and may not be representative of the larger population. Likewise, the higher than expected serum vitamin D in subjects in the months of Jan and Feb (as graphically seen) in bone cohort participants may simply be due to chance given the small sample size.

Differences in geophysical factors in the hypertensive cohort may help to explain the observed difference in 25(OH)D in these subjects. As participants were located further North in latitude (55^{o} N versus 53.3^{o} N), the exposure to UVB irradiation may have been less. In this regard, the finding of lower vitamin D status in Scotland versus England, independent of other factors in one study, has been attributed to differences

in latitude (Hypponnen et al., 2007). In addition, a 1^{o} increase in latitude has been associated with a 1 nmol/l increase in 25(OH)D in a study of community dwelling adults (Hagenau et al., 2009). Variation in weather in this location including cloud cover could also have affected UVB irradiation and hence vitamin D levels.

In a post hoc analysis (of the mean seasonal GSR across cohorts), it was found that the mean GSR in Winter and Autumn were lower in the hypertensive compared to either the bone or cognitive cohort. This combined with subjects higher mean BMI may have had a significant negative effect on serum 25(OH)D. It is also possible that genetic differences in vitamin D metabolism (as previously discussed) could account for some of the observed difference.

Dietary Factors

Consumption of oily fish was associated with better vitamin D status in participants in the bone cohort (though only in the model which adjusted for GSR as opposed to season). This discrepancy might be explained by differences in seasonal intake of oily fish. It is unclear why no effect was observed in the other cohorts. Frequency of consumption may be a factor accounting for this, which we were not able to adjust for. Oily fish is a rich source of vitamin D (Lu et al., 2009) and has also been associated with higher serum 25(OH)D in other studies (Burgaz et al., 2007; Nanri et al., 2011). No association was found with margarine consumption, a finding replicated by other studies but it contains relatively small amounts of vitamin D (Burgaz et al., 2007).

Smoking Status

Current smoking status was associated with lower vitamin D levels in the younger old (bone and hypertensive cohort). This finding has been replicated by other studies but no causal explanation has been found (Cutillas-Marco et al., 2012). In a recent study of 6146 community dwelling adults, daily smoking was associated with lower vitamin D levels (Thuesen et al., 2012). It has been suggested that alterations in hepatic metabolism of vitamin D or 1-α-hydroxylation due to smoking could be a potential mechanism (Brot et al., 1999). Alternatively, smoking may simply be a surrogate

marker for other lifestyle factors that impact negatively on serum vitamin D such as lower vitamin D intake (Morabia et al., 2000).

Outside Sunshine Exposure

Preference for spending time in the sun when outside was positively associated with serum 25(OH)D in all cohorts. This finding supports a role for sunshine exposure as means of increasing vitamin D status, even in the frail elderly. In a recent study, albeit of younger adults, a one hour increase in sunlight was found to decrease the risk of vitamin D deficiency by 70% highlighting the important role of UVB exposure (Vallianou et al., 2012).

Sun Holiday Travel

As expected sun holiday travel was correlated with higher vitamin D status as has been previously reported (Burgaz et al., 2007). It is known that sun exposure can lead to production of up to the equivalent 5000 IU of vitamin D3 per day. Whilst a large amount of sun exposure could increase vitamin D reserves it is unclear as to whether the effect could last for up to 6 months. It is also possible that participants who go on sun holiday travel may in general spend more time outdoors.

Study Strength's and Limitations

The study strengths were that it was of relatively large size and accurately accounted for supplement use. We also adjusted for a several factors related to UVB exposure, including global solar radiation, sun holiday travel and preference for sunshine when outside. We measured serum 25(OH)D by LCMS which is the gold standard and our laboratory was DEQAS approved.

However, there were some limitations. Firstly, approximately 5.7% (N=278) of all participants were excluded in our analysis due to missing data which has the potential to alter study results. The study participants were not representative of the general older population to which the findings may not be applicable. We did not have a good measure of non-supplemental dietary vitamin D intake though were able to factor in consumption of oily fish and margarine in our analysis.In addition, no specific or accurate account of outdoor exposure or physical activity was available. Despite this,

we had data for TUG performance which is a good marker of physical frailty. Finally, as the study was cross sectional, no inferences can be made as to causality.

Conclusion

Vitamin D deficiency was prevalent affecting between 40.2-73.2% of non-supplemented older adults. Season and supplement use were the most important determinants of vitamin D status though sun holiday travel and preference for sunshine exposure when outside also appear to play a role. Other modifiable factors included body mass index and in the 'younger' old smoking and oily fish consumption

Results highlight the importance of factoring in season when interpreting 25(OH)D levels. Findings also suggests that careful consideration for checking serum vitamin D should be given to older adults, especially those who are non-supplemented, physically frail and more likely to high prevalence of deficiency.

SECTION 5: VITAMIN D AND COGNITION

CHAPTER 15: INTRODUCTION

An increasing body of evidence supports a role for vitamin D in maintaining normal cognitive function. There is a strong biological plausibility (as previously discussed) underlying the potential mechanisms via which it may achieve this.

Vitamin D is known to cross the blood brain barrier and metabolic pathways exist for its activation in the central nervous system. The vitamin D receptor (VDR) is found in multiple brain areas important in cognition including the hippocampus, frontal, parietal and temporal lobes.

Vitamin D may protect against cognitive decline through an effect on neuro-protection, vasculo-protection, neuronal calcium regulation, inflammation and modulation of vascular risk factors. It has also been specifically implicated in the pathophysiology of Alzheimer's disease and displays anti-amyloid, anti-glutamate and pro-cholinergic properties in animal models.

Most cross sectional studies have found a positive association between serum 25(OH)D and tests of global or specific cognitive function. Longitudinal studies also suggest that vitamin D deficiency may be a risk factor for cognitive decline or dementia. However, the relationship remains controversial and appears to be more consistent in those who are vitamin D deficient (< 50 nmol/l).

It remains unclear as to which areas of cognition are most affected by vitamin D, with studies pointing to a role in executive function, though results are inconsistent. In addition, some studies have included only small sample sizes, had widely different subject groups (normal cognition to dementia), used limited cognitive test measures and adjusted for few confounds. In particular, there is the potential for study results to be explained by 'reverse causality' whereby lower vitamin D levels may be due to reduced sun exposure in those who are cognitively impaired.

Finally, there are few intervention trials of vitamin D and cognition and no large well designed randomised controlled trials have been performed.

We aimed in this study is to identify if serum 25(OH)D is associated with cognitive function (in a variety of cognitive domains) in community dwelling older Irish adults.

CHAPTER 16: METHODOLOGY

Study Population and Design

The study population comprised participants of the TUDA (Trinity, University of Ulster, Dept. of Agriculture) study which has been previously described. This is a large collaborative, cross sectional study designed to create a phenotype/genotype database for three cohorts of community dwelling subjects aged over sixty years. Study data was available for 1800, 1704 and 1404 participants in the hypertensive, cognitive and bone cohort respectively.

Study methods and participant characteristics are as previously outlined (see chapter 7). Participants with MMSE scores below 24 were excluded as several confounds were self-reported. In addition, we specifically aimed to look for the relationship between vitamin D and cognition in non-dementia subjects.

Neuropsychological Assessment

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), (Randolph et al, 1998) was used to assess five cognitive domains: Immediate Memory, Visuospatial, Language, Attention and Delayed Memory, each represented by a score on the respective indices I, II, III, IV and V. It is a relatively brief but comprehensive test of cognition that takes approximately 30 minutes to administer. For each domain, there are two test subcategories which are scored separately and then used to calculate the overall index score. Global cognitive performance in the RBANS was represented by a composite Total Scale. Executive function was specifically measured with the Frontal Assessment Battery (FAB) (Dubois et al., 2000) and a global assessment of cognitive function was also measured with the Mini Mental State Examination (MMSE), (Folstein et al., 1975). (See appendix for neuropsychological tests)

Statistical Analysis

We initially performed an analysis in the entire TUDA study population to identify if there was an interaction between cognitive status, serum vitamin D and each of the three study cohorts. This allowed us to establish if the analysis should be performed separately for each cohort.

All parameters were inspected for normality and those that were significantly skewed were appropriately transformed. All statistical analysis was performed using JMP edition 11.01. (SAS Institute, 2010).

Study Covariates

The total global solar radiation (GSR) which represents the total amount of irradiation received per unit area per month ($KJcm^{-2}$) was used as a surrogate marker of UVB exposure. GSR data was obtained by request from the Irish Meteorological Service. Data from weather stations nearest to study recruitment sites (Dublin Airport) and (Malin Head, Donegal) were used respectively for analysis in the Cognitive / Bone and Hypertensive cohort.

Season was defined according to the standard meteorological definition used in Ireland (Irish Meteorological Service) with the following months comprising Winter (Dec, Jan, Feb), Spring (Mar, Apr, May), Summer (Jun, Jul, Aug), and Autumn (Sept, Oct, Nov).

Timed Up and Go (TUG) measurements were divided into quintiles as it was not possible to measure it in a small proportion of subjects with significant physical disability. These subjects were categorised in the lowest quintile of TUG performance.

Statistical Models

Multiple linear regression models were used to explore the relationship using both serum 25(OH)D and cognitive test scores as continuous variables. Cognitive outcome measures included MMSE and FAB scores, performance on indices I, II, III, IV, V and Total Scale on the RBANS.

In model 1, adjustment was made for well established confounds of 25(OH)D and cognition including age, gender, education, supplement use, body mass index, season, or global solar radiation (as our surrogate marker of UVB exposure). In model 2, further adjustment was made for physical frailty as measured by the timed up and go (TUG) . In further models sun holiday travel, current depression (CES-D≥16), stroke history and serum PTH were included as covariates.

CHAPTER 17: RESULTS

Five hundred and thirty three subjects were excluded after application of study inclusion criteria (MMSE ≥ 24). A further 278 subjects were excluded due to missing or incomplete data, leaving the respective number in the hypertensive, cognitive and bone cohort 1568, 1330 and 1199. For an outline of study cohort participant characteristics see Chapter 8 and Table 8.1.

A significant interaction was identified between vitamin D, cognitive performance as per RBANS index III and cohorts (interaction term in cognitive cohort, β =-0.06, P <0.0001, bone cohort β =-0.07, P <0.0001). Accordingly, analysis was performed separately for each cohort. The results of multiple linear regression analysis are shown in Tables 17.1-17.3).

In the hypertensive cohort (N = 1568), a significant positive relationship was found between 25(OH)D and performance in the FAB (P = 0.01) and RBANS Index I (P = 0.04), Index II (P = 0.036), Index III (P <0.0001) and Total Scale (P = 0.006) after adjustment for age, gender, education, body mass index, season, supplement use and physical frailty (TUG). After further adjustment for sun holiday travel the findings remained significant for FAB (P = 0.027), Index III (P <0.0001) and Total Scale (P = 0.005). Furthermore, when a history of stroke, current depression status and serum PTH were added individually as covariates the positive findings for FAB, Index III and Total Scale remained. Substitution of GSR for season in the models did not alter the study findings (see table 17.1).

In the bone cohort (N = 1199), 25(OH)D was positively associated with Index II (P=0.019) and FAB (P=0.005) after adjusting for age, gender, education, BMI, season, supplement use and TUG. The result remained significant after further inclusion in the model of sun holiday travel and stroke history or serum PTH or depression status Substitution of GSR for season in the models did not alter the study findings (see table 17.12).

No association was identified between 25(OH)D and cognition in the cognitive cohort across all tests and in all models (N = 1330).

Table 17.1: Relationship between 25(OH)D and Cognition in Hypertensive Cohort.

Relationship between 25(OH)D and Cognition in Hypertensive Cohort (N=1568)												
	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6	
Test	β	P-value	β	P-value	β	P-value	β	P-value	β	P-value	β	P-value
MMSE	-0.000	0.74	-0.000	0.56	-0.0015	0.32	-0.000	0.25	-0.001	0.26	-0.001	0.38
FAB (log)	0.000	0.004*	0.000	0.01*	0.000	0.029*	0.00	0.048*	0.000	0.039*	0.000	0.044
Index I	0.040	0.023	0.040	0.04*	0.021	0.25	0.023	0.23	0.025	0.20	0.024	0.21
Index II	0.040	0.056	0.036	0.08	0.019	0.30	0.03	0.14	0.05	0.09	0.039	0.08
Index III	0.06	<0.0001*	0.06	<0.0001*	0.059	<0.0001*	0.054	<0.0001*	0.06	<0.0001*	0.060	<0.0001*
Index IV	0.04	0.04	0.033	0.086	0.028	0.14	0.023	0.26	0.06	0.20	0.026	0.42
Index V	0.006	0.86	-0.000	0.53	-0.037	0.26	-0.03	0.45	-0.02	0.51	-0.027	0.42
Total Scale	0.055	0.002*	0.049	0.006*	0.074	0.005*	0.040	0.038*	0.040	0.025*	0.037	0.044*

Model 1: age, gender, education, BMI, season, supplement use.
Model 2: as per model 1 and Timed Up and Go.
Model 3: as per model 2 and sun holiday travel in last 6 months
Model 4: as per model 3 and depression (CES-D≥ 16)
Model 5: as per model 3 and stroke
Model 6: as per model 3 and serum PTH

NB: When GSR was substituted for season in model 1, the study findings were unchanged.
* Statistically significant (P<0.05).

Table: 17.2: Relationship between 25(OH)D and Cognition in Cognitive Cohort .

Relationship between 25(OH)D and Cognition in Cognitive Cohort (N=1330)										
	Model 1		Model 2		Model 3		Model 4		Model 5	
Test	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**
MMSE	0.022	NS	0.000	NS	0.000	NS	0.002	NS	-0.001	NS
FAB (log)	0.000	NS	0.000	NS	0.000	NS	0.026	NS	0.000	NS
Index I	0.030	NS	0.016	NS	0.021	NS	0.026	NS	0.009	NS
Index II	0.060	NS	0.036	NS	0.037	NS	0.06	NS	0.036	NS
Index III	0.008	NS	0.004	NS	0.000	NS	0.005	NS	-0.004	NS
Index IV	0.03	NS	0.019	NS	0.013	NS	0.030	NS	0.01	NS
Index V	0.01	NS	-0.000	NS	-0.000	NS	-0.002	NS	-0.005	NS
Total Scale	0.11	NS	0.08	NS	0.440	NS	0.09	NS	0/.09	NS

Model 1: age, gender, education, BMI, season, supplement use.
Model 2: as per model 1 and Timed Up and Go.
Model 3: as per model 3 and depression (CES-D≥ 16)
Model 4: as per model 3 and stroke
Model 5: as per model 3 and serum PTH

NB: When GSR was substituted for season in the models 1, the study findings were unchanged.
NS: Non-significant (P ≥0.05)

Table: 17.3: Relationship between 25(OH)D and Cognition in Bone Cohort.

Relationship between 25(OH)D and Cognition in Bone Cohort (N=1199)												
	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6	
Test	β	P-value	β	P-value	β	P-value	β	P-value	β	P-value	β	P-value
MMSE	0.022	0.32	0.000	0.27	0.000	0.32	0.000	0.90	0.002	0.39	-0.001	0.99
FAB (log)	0.000	0.004*	0.000	0.005*	0.000	0.007*	0.000	0.014*	0.026	0.014*	0.000	0.01*
Index I	0.030	0.056	0.016	0.27	0.021	0.94	0.023	0.54	0.026	0.11	0.009	0.56
Index II	0.060	0.0012*	0.040	0.019	0.037	0.027*	0.03	0.045*	0.6	0.002*	0.036	0.038*
Index III	0.008	0.43	-0.004	0.81	-0.000	0.160	-0.06	0.15	0.005	0.66	-0.004	0.73
Index IV	0.03	0.028*	0.019	0.20	0.013	0.400	0.010	0.51	0.030	0.52	0.01	0.39
Index V	0.01	0.48	-0.000	0.53	-0.000	0.65	-0.03	0.57	-0.002	0.70	-0.005	0.73
Total Scale	0.11	0.36	0.08	0.48	0.440	0.380	0.07	0.59	0.09	0.47	0.046	0.71

Model 1: age, gender, education, BMI, season, supplement use.
Model 2: as per model 1 and Timed Up and Go.
Model 3: as per model 2 and sun holiday travel in last 6 months
Model 4: as per model 3 and depression (CES-D≥ 16)
Model 5: as per model 3 and stroke
Model 6: as per model 3 and serum PTH

NB: When GSR was substituted for season in model 1, the study findings were unchanged.
* Statistically significant (P<0.05).

CHAPTER 18: POST HOC ANALYSIS AND RESULTS

The potential relationship between 25(OH)D and each subtest within the RBANS indices were explored: list learning and story recall in Index I, line orientation and figure copy in Index II, category fluency and picture naming in Index III, forward digit span and digit symbol coding in Index IV, word recall, word recognition, figure construction and story recall in Index V. The association was explored in multiple linear regression models adjusting for age, gender, BMI, education, supplement use, sun holiday travel, season or GSR, TUG and individually for depression status (CES-D score $\geq$ 16), presence of stroke, and serum parathyroid (PTH) level.

We also plotted graphically the relationship between cognitive measures and serum 25OH)D (by quintiles) so as to determine potential cut offs in the association.

Table 18.1: Relationship between RBANS Index Subtests and 25(OH)D in Hypertensive Cohort

Relationship between serum 25(OH)D and Cognition in Hypertensive Cohort (N=1568)								
	Model 1		Model 2		Model 3		Model 4	
Cognitive Test	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**
Index 1								
List learning	0.01	0.046*	0.01	NS	0.011	NS	0.01	NS
Story Memory	-	NS	-	NS	-	NS	-	NS
Index II								
Line orientation	-	NS	-	NS	-	NS	-	NS
Figure copy	-	NS	--	NS	-	NS	-	NS
Index III								
Semantic fluency	0.016	0.0003*	-	0.0003*	0.016	0.0009*	0.017	0.0003*
Picture naming	0.0036	0.0002*	-	<0.0001*	0.004	0.0001*	0.005	0.0001*
Index IV								
Digit Span	-		-	NS	-	NS	-	NS
Coding	0.026	0.018*	0.02	0.06	0.024	0.044*	0.025	0.039*
Index V								
List recall	-	NS	-	NS	-	NS	-	NS
Story recall	-	NS	-	NS	-	NS	-	NS
Figure recall	-	NS	-	NS	-	NS	-	NS
Combined recall	-	NS	-	NS	-	NS	-	NS

Model 1: age, gender, BMI, season, supplement use, education, TUG, holiday travel.
Model 2: model 1 + depression (CES-D ≥ 16).
Model 3: model 1 + stroke.
Model 4: model 1 + serum PTH.

NS: Nonsignificant P 0.05, * Statistically significant (P<0.05).

NB: When GSR was substituted for season, the study findings were unchanged except for the result of a positive relationship between 25(OH)D and coding in model 2 (Index IV) (β =0.04, P=0.02).

Table 18.2: Relationship between RBANS Index Subtests and 25(OH)D in Bone Cohort

Relationship between serum 25(OH)D and Cognition in Bone Cohort (N=1199)								
	Model 1		Model 2		Model 3		Model 4	
Cognitive Test	β	P-value	β	P-value	β	P-value	β	P-value
Index 1								
List learning	-	NS	-	NS	-	NS	-	NS
Story Memory	-	NS	-	NS	-	NS	-	NS
Index II								
Line orientation	0.01	0.005*	0.009	0.015*	0.01	0.005*	0.01	0.004*
Figure copy	-0.15	0.003	-0.14	0.006*	-0.16	0.003*	-0.16	0.002*
Index III								
Semantic fluency	-	NS	-	NS	-	NS	-	NS
Picture naming	-	NS	-	NS	-	NS	-	NS
Index IV								
Digit Span	--	NS	-	NS	-	NS	-	NS
Coding	-	NS	-	NS	-	NS	-	NS
Index V								
List recall	-	NS	-	NS	-	NS	-	NS
Story recall	-	NS	-	NS	-	NS	-	NS
Figure recall	-	NS	-	NS	-	NS	-	NS
Combined recall	-	NS	-	NS	-	NS	-	NS

Model 1: age, gender, BMI, season, supplement use, education, TUG, holiday travel.
Model 2: model 1 + depression (CES-D ≥ 16).
Model 3: model 1 + stroke.
Model 4: model 1 + serum PTH.

NS: Nonsignificant P≥ 0.05, * Statistically significant (P<0.05).

NB: When GSR was substituted for season in model 1, the study findings were unchanged

Table 18.3: Relationship between RBANS Index Subtests and 25(OH)D in Cognitive Cohort

Relationship between serum 25(OH)D and Cognition in Cognitive Cohort (N=1330)								
	Model 1		Model 2		Model 3		Model 4	
Cognitive Test	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**
Index 1								
List learning		NS		NS		NS		NS
Story Memory	-	NS	-	NS	-	NS	-	NS
Index II								
Line orientation	-	NS	-	NS	-	NS	-	NS
Figure copy	-	NS	--	NS	-	NS	-	NS
Index III								
Semantic fluency	-	NS	-	NS		NS		NS
Picture naming	-	NS	-	NS		NS		NS
Index IV								
Digit Span	--	NS	-	NS	-	NS	-	NS
Coding	-	NS		NS		NS		NS
Index V								
List recall	-	NS	-	NS	-	NS	-	NS
Story recall	-	NS	-	NS	-	NS	-	NS
Figure recall	-	NS	-	NS	-	NS	-	NS
Combined recall	-	NS	-	NS	-	NS	-	NS

NS: Nonsignificant $P \geq 0.05$

In the post hoc-analysis exploring the relationship between 25(OH)D and Index subtests in the hypertensive cohort, a positive relationship was found with the following: list learning (Index I) ($P = 0.046$), category fluency (Index III), ($P = 0.0003$), picture naming (Index III),($P = 0.002$) and digit symbol coding (Index IV), ($P = 0.018$) after adjustment for age, gender, season, BMI, supplement use, TUG and sun holiday travel. After further adjustment for depression (CES-D $\geq$ 16) the relationship with digit symbol coding ($P = 0.06$) and list learning ($P = 0.06$) became insignificant. However, findings remained significant for picture naming and category fluency (Index III), when depression, stroke and serum PTH where further added into the models. When GSR was substituted into the models instead of season the relationship with FAB and coding remained significant ($P<0.05$) throughout but otherwise there were no change in study findings (see Table 18.1).

In the bone cohort, vitamin D was positively associated with line orientation (Index I) and negatively associated with figure copy (Index II) in all models. In the cognitive cohort, no association was found between 25(OH)D and RBANS subtests in any models (using either GSR or season as a covariate).

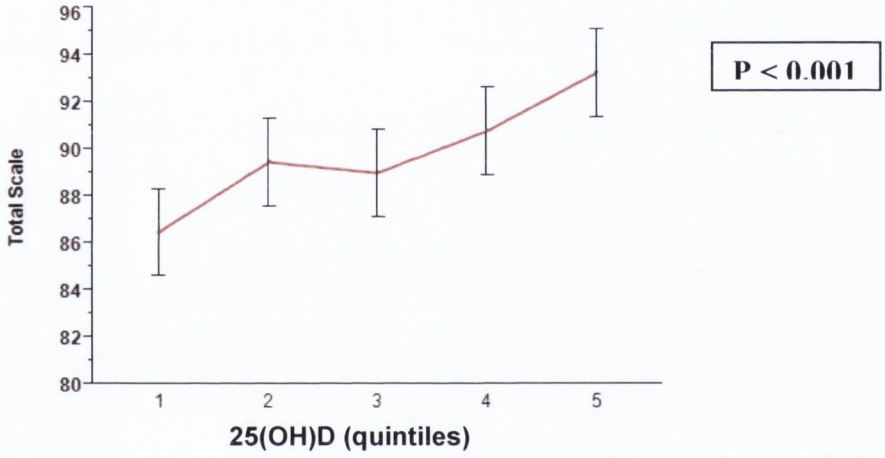
Graphical plots of cognitive performance across 25(OH)D quintiles

Graphical plots show that increasing quintiles of 25(OH)D were associated with higher Total Scale in the RBANS and better performance in the Frontal Assessment Battery. However, there appeared to be a threshold effect for index III and it's subcategories, with only subjects above the third quintile (≥ 50.5 nmol/l) performing better.

Fig 18.1: Total Scale (RBANS) versus quintiles of 25(OH)D in the Hypertensive Cohort

Error bar represents one standard error.

*P value – adjusted for age, gender, BMI, season, GSR, supplement use.



Quintiles: Q1 (1.54-27.4),(27.5-38.8), (38.9-50.5),(50.6-69.8),(69.9-167) nmol/l

Fig 18.2: FAB scores versus quintiles of 25(OH)D in the Hypertensive Cohort

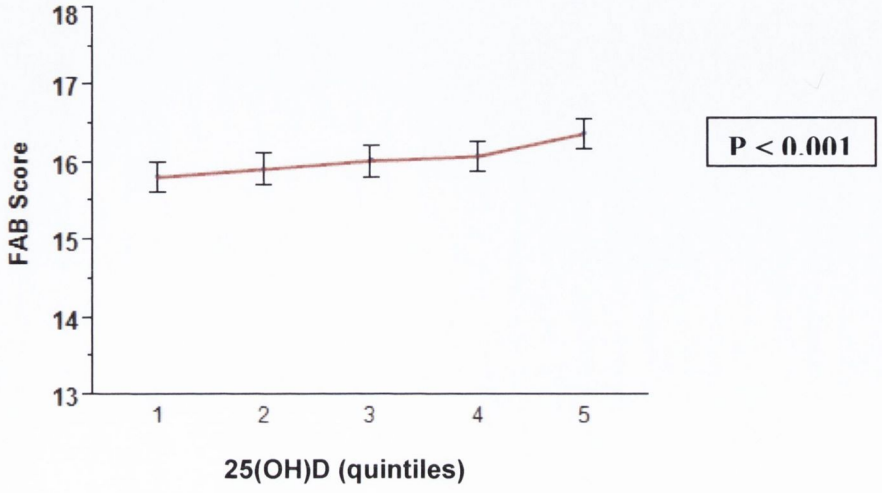
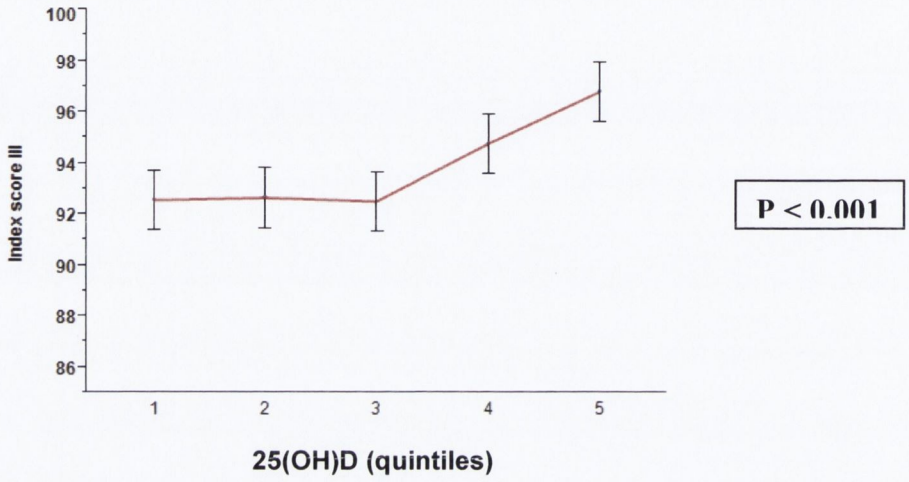


Fig 18.3: Index III (RBANS) versus quintiles of 25(OH)D in the Hypertensive Cohort

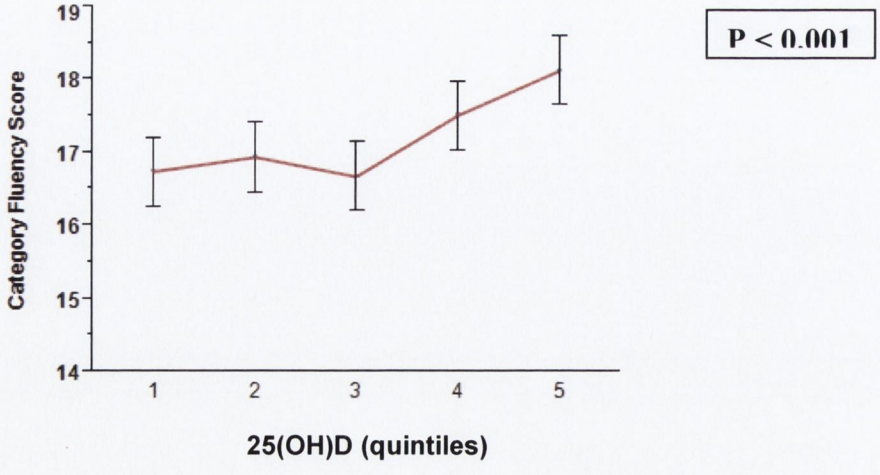
Error bar represents one standard error.

*P value – adjusted for age, gender, BMI, season and supplement use.



Quintiles: (1.54-27.4),(27.5-38.8), (38.9-50.5),(50.6-69.8),(69.9-167) nmol/l

Fig 18.31 Category fluency (Index III subtest, RBANS) versus quintiles of 25(OH)D in the Hypertensive Cohort

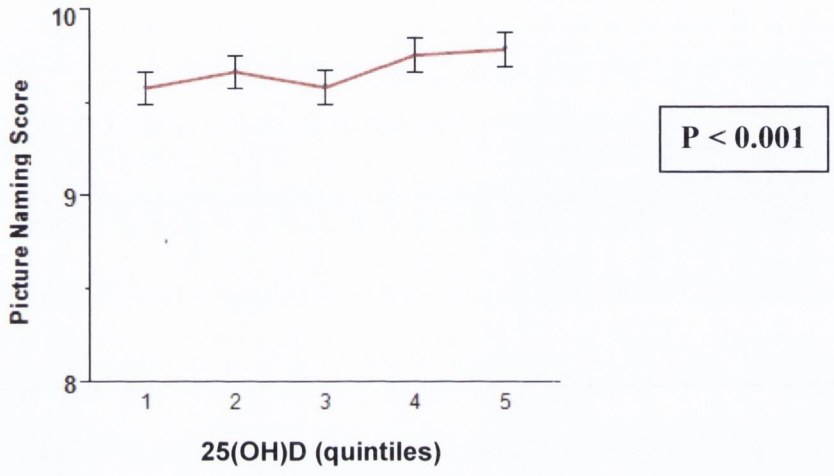


Quintiles: (1.54-27.4),(27.5-38.8), (38.9-50.5),(50.6-69.8),(69.9-167) nmol/l

Fig 18.32: Picture Naming (Index III subtest, RBANS) versus quintiles of 25(OH)D in the Hypertensive Cohort

Error bar represents one standard error.

*P value – adjusted for age, gender, education, BMI, season, supplement use.



Quintiles: (1.54-27.4),(27.5-38.8), (38.9-50.5),(50.6-69.8),(69.9-167) nmol/l

CHAPTER 19: DISCUSSION

Vitamin D was positively associated with some cognitive test measures, but only in younger older adults who were participants of the bone and hypertensive cohort.

Specifically, we found a positive association between vitamin D and two tests of executive function the Frontal Assessment battery and a subcomponent of Index IV on the RBANS (digit symbol coding). The findings remained significant after adjustment for multiple confounds including physical frailty. However, in a post hoc model the association with digit symbol coding became insignificant after adjustment for depression. Despite this, the relationship with FAB scores remained robust even when factoring in current depression or stroke history, both which can contribute to executive dysfunction in their own right. In addition, inclusion of serum PTH as novel covariate did not change the result. High PTH has been implicated as mechanism via which vitamin D deficiency could affect cognition and has been associated with executive function in at least one small study (Jorde et al., 2010). However, our findings suggest an effect of vitamin D independent of serum PTH. Results also suggest a linear relationship with FAB performance across 25(OH)D quintiles with no threshold effect identified.

The finding of an association with executive function are consistent with other large cross sectional studies using different test measures (.Buell et al., 200: Lee et al., 2010). An inverse relationship has also been found between severe vitamin D deficiency and increased risk of decline in executive function in longitudinal studies (Llewellyn et al., 2010) though results are conflicting (Slinin et al., 2010; Slinin et al., 2012).

No association between vitamin D and tests of either verbal or visual memory were identified apart from in one subcomponent (list learning) in the Immediate Memory index (Index I) in the hypertensive cohort. However, the relationship became insignificant after adjusting for current depression. In addition, a large component of this test requires attention and encoding skills and it is therefore not a pure test of memory or retention. Findings are consistent with all other studies to date that have been negative for vitamin D and specific tests of memory (Jorde et al., 2006: Wilkins

et al. 2006; Mc Grath et al., 2007; Lee et al., 2009; Buell et al., 2009; Lewellyn et al., 2010),

We also found a significant association between vitamin D and one measure of global cognition (Total Scale on RBANS), though only in participants of the hypertensive cohort. No association was found with the MMSE. However, it is possible that it was too insensitive as test of cognitive function.. The lack of association with MMSE is also consistent with the results of another large study that found no relationship in 'younger' older adults (Buell et al., 2009). Conversely, the absence of an association in the cognitive cohort is contrary to some other studies that have included older adults (Przyelski et al., 2007: Oudshoorn et al., 2008; Peterson et al., 2012) and used other brief measures of global cognition such as the SPSMQ (Llewellyn et al., 2009) and the AMTS (Skalska et al., 2012).

A positive association was found between 25(OH)D and Index II which measures visuoconstruction and visuospatial domains, in both the hypertensive and bone cohorts. However, the relationship became insignificant after adjustment for sun holiday travel in the hypertensive cohort. In the post hoc analysis exploring the relationship between vitamin D and RBANS Index II subtests, no association was identified in the hypertensive cohort though it may have been underpowered to detect this. However, a statistical significant positive and negative relationship was found between vitamin D and RBANS index II subtests (line orientation and figure copy respectively) in the bone cohort. This conflicting finding and lack of association in other studies to date between vitamin D and visuspatial domains (Lee et al., 2009) suggests that this may have been due to chance and be of limited import.

A strong association was found between 25(OH)D and language domains in the hypertensive cohort. This remained significant in all models and for both language subtests (picture naming and category/semantic fluency for animals). A threshold effect was identified with better performance found in those with serum 25(OH)D above approximately 50 nmol/l. Few studies have looked at language though two found no relationship with phonetic fluency tests (Wilkins et al., 2006; Jorde et al., 2006). However, the subtests we used are different and potentially relate to dysfunction in different brain areas which might be more vulnerable to the effects of

vitamin D. In this regard, two studies have suggested that vitamin D may play a role in semantic language function. A VDR polymorphism has been associated with decline in category fluency in a longitudinal study of US females (Beydoun et al., 2012). In addition, another study found an association between low gestational vitamin D (<50 nmol/l) and an increased risk of a poorer performance on a semantic based language task in children at between up 5-10 years follow up (Whitehouse et al., 2012).

No association was found between 25(OH)D and any of the neuropsychological tests in the cognitive cohort. However, subjects in this cohort had a high level of physical frailty and other co-morbidities and were at the 'older' old end of the age spectrum. It is possible that any relationship with vitamin D could be obscured in a more complex group. It may also be that the relationship is lost with time and that a prolonged period of low vitamin D status earlier in life may contribute more to cognitive impairment. This supports the idea of a 'long latency deficiency' effect and suggests that future randomised controlled trials shoud be performed in 'younger' older adults.

Study Strengths and limitations

There were some limitations to this study. Firstly, approximately 5% of all participants were excluded in our analysis due to missing data which has the potential to alter study results. The study participants were not representative of the general older population to which the findings may not be applicable. We did not have a good measure of non-supplemental dietary vitamin D intake though we were able to factor in accurately supplement use. In addition, no specific or accurate account of outdoor exposure or physical activity was available. Despite this, we had data for TUG performance which is a good marker of physical frailty. Finally, as the study was cross sectional, no inferences can be made as to causality.

Study strengths were that we had a large sample size and adjusted for several confounds. A variety of neuropsychological tests that were sensitive in examining multiple cognitive domains were used. We were able to look at associations with vitamin D in widely varied aged groups. As subjects had MMSE scores of 24 or more, most were likely to have either mild cognitive impairment or normal cognition. By

excluding subjects with dementia, we eliminated the potential for reverse causality due to more advanced cognitive impairment.

Conclusion

Findings suggest that vitamin D may play a role in cognition, specifically in language, and executive domains both only in the 'younger' old. It is possible that vitamin D may exert a greater effect on cognitive function earlier in life and that randomised controlled trials in 'younger' older adults should now be considered.

SECTION 6: VITAMIN D AND BlOOD PRESSURE

CHAPTER 20: INTRODUCTION

Emerging evidence supports a role for vitamin D in the aetiopathology of hypertension. Vitamin D receptors are found throughout the cardiovascular system including the myocardium and vascular smooth muscle cells. Vitamin D is known to down regulate the renin angiotenin aldosterone system (RAAS) and may play a role in maintaining normal vascular endothelial function. In addition, vitamin D's regulation of calcium metabolism, prevention of secondary hyperparathyroidism and potential reno-protective properties have all been implicated as a mechanism by which it may effect blood pressure

Most cross sectional studies show an inverse association between vitamin D and blood pressure or a diagnosis of hypertension. However, only a small number of longitudinal studies have been conducted and results have been inconsistent though the relationship in studies appears to be confined to those who are hypertensive and vitamin D deficient. In addition, few randomised controlled trials have been performed with most not supporting a beneficial effect for vitamin D. However, most have been small and were of short duration, and many were not primarily designed to investigate the effect of vitamin D on blood pressure. Some also included subjects who were normotensive or had normal vitamin D status at baseline and used only small treatment doses.

The purpose of this study is to determine if serum 25(OH)D is associated with systolic or diastolic blood pressure or the presence of hypertension in an older Irish community dwelling population

CHAPTER 21: METHODOLOGY

Study Population and Design

The study population comprised participants of the TUDA (Trinity, University of Ulster, Dept. of Agriculture) study which been previously described. This is a large collaborative, cross sectional study designed to create a phenotype/genotype database for three cohorts of community dwelling subjects aged over sixty years. Study data was available for 1800, 1704 and 1404 participants in the hypertensive, cognitive and bone cohort respectively.

Study methods and participants characteristics are as previously outlined (see chapter 8). Participants with MMSE scores below 24 were excluded in the analysis as several confounds were self-reported. In addition, we specifically aimed to look at the relationship in non-dementia subjects.

Blood Pressure Measurement

Blood pressure was measured with a clinically validated automated blood pressure recording device (Omron 705CP-II) with the subject in a seated position (El Assaad et al., 2003). The arm with the highest initial blood pressure recording was used for subsequent readings, which were taken until diastolic and systolic blood pressure were within 5 mmHg dor two readings or until 5 recordings were performed. Subjects were also asked to report if they had a diagnosis of a hypertension.

Statistical Analysis

We initially performed an analysis in the entire TUDA study population to identify if there was an interaction between a diagnosis of hypertension or mean diastolic or systolic blood pressure and serum vitamin D for each of the three study cohorts. This allowed us to establish if the analysis should be performed separately for each cohort. As interaction terms were nonsignficant ($P.> 0.05$) the cohorts were analysed as as single group.

All parameters were inspected for normality and those that were significantly skewed were appropriately transformed. All statistical analysis was performed using JMP edition 10.0.2 (SAS Institute, 2010).

Study Covariates

Diagnosis of hypertension was based on self report and/or having a mean systsolic and diastolic blood pressure greater than or equal to 140 mmHg and 90 mmHg respectively. This was similar to that used in other studies (Kim et al., 2008). Mean blood pressure was defined as the average of two readings where both systolic and diastolic blood pressure were within 5 mmHg or alternatively of all five readings.

The total global solar radiation (GSR) which represents the total amount of irradiation received per unit area per month ($KJcm^{-2}$) was used as a surrogate marker of UVB exposure. GSR data was obtained by request from the Irish Meteorological Service. Data from weather stations nearest to study recruitment sites (Dublin Airport) and (Malin Head, Donegal) were used respectively for analysis in the Cognitive / Bone and Hypertensive cohort.

Season was defined according to the standard meteorological definition used in Ireland (Irish Meteorological Service) with the following months comprising Winter (Dec, Jan, Feb), Spring (Mar, Apr, May), Summer (Jun, Jul, Aug), and Autumn (Sept, Oct, Nov).

Timed Up and Go (TUG) measurements were divided into quintiles as it was not possible to measure it in a small proportion of subjects with significant physical disability. These subjects were categorised in the lowest quintile of TUG performance.

Statistical Models

A comparative analysis of differences between those who were hypertensive (self report and or/by study criteria) and normotensive was made using the unpaired t and Chi squared test as appropriate. Logistic regression was also used to explore the relationship between serum 25-hydroxyvitamin D and a diagnosis of hypertension (defined as self reported diagnosis and/or mean SBP ≥140 or DBP ≥90 mmHg). The potential relationship with mean systolic and diastolic blood pressure was also explored in multiple-linear regression models. As the vast majority of subjects were taking anti-hypertensives or medications with secondary blood pressure lowering

affects, it was not possible to exclude these partipcants or adjust for their use in the analysis.

In model 1, the analysis incorporated basic demographic factors and established confounds of vitamin D status (age, gender, body mass index, seasons or global solar radiation and factors known to affect blood pressure (smoking and alcohol intake). In model 2, further adjustment was made for physical frailty (Timed Up and Go) and in model 3, sun holiday travel was added as a covariate. Serum PTH and calcium were also included as covariates in models 4 and 5.

CHAPTER 22: RESULTS

No significant interaction term was identified and therefore the analysis was performed in the entire TUDA population. Five hundred and thirty three subjects were excluded after application of study inclusion criteria (MMSE ≥ 24). A further 298 subjects were excluded due to missing or incomplete data, leaving the total study number 4077.

Table 22.1: Characteristics of subjects with and without a diagnosis of hypertension

Characteristics of participants by diagnosis of Hypertension (N = 4077)			
	Hypertensive *(N=3409)	**Non-Hypertensive (N=668)**	**P- value**
Age (yrs)	73.3 ± 8.8	73.6 ± 8.2	0.360 [a]
Gender (Female. %)	66.1	78.5	<0.001 [b]
BMI (kgm-^{2})	28.5 ±5.4	25.2 ± 4.8	<0.001 [a]
TUG (quintiles) (%)	III (20.7), V (19.90)	III (16.0), V (17.1)	<0.02 [b]
Current Alcohol intake,(%)	57.9	64.7	0.020 [b]
Current smoker (%)	11.7	14.7	0.030 [b]
Current or past smoker (%)	53.5	55.4	0.350 [b]
Sun Holiday Travel (%)	17.9	21.4	0.033 [b]
Vitamin D Supplements (%)	44.1	69.1	<0.001 [b]
Mean SBP (mmHg)	148.5 ± 20.5	124.1 ± 9.9	<0.001 [a]
Mean DBP (mmHg)	79.5 ±11.5	72.7 ± 8.5	<0.001 [a]
25(OH)D (nmol/l)	57.5 ± 31.8	71.4 ± 30.7	<0.001 [a]
<25 nmoll (%)	13.5	8.7	<0.001 [b]
25- 49.9 nmol/l (%)	33.9	16.9	<0.001 [b]
50- 74.5 nmol/l (%)	25.1	26.6	0.630 [b]
≥ 75 nmol/l (%)	27.5	47.8	<0.001 [b]

[a] unpaired t-test, [b] Chi Squared test, *Hypertension defined as self reported diagnosis (made by doctor) and/or SBP and DBP ≥ 140 and 90 mmHg respectively. TUG quintiles III (1-12.4), V (≥ 20) seconds.

71.2% (N=2906) of subjects self reported a diagnosis of hypertension. An additional 12.4% (N = 503) of all participants were found to have hypertension when the diagnosis was based on study criteria which included blood pressure readings measured on the assessment day. There was a significant difference in mean systolic and diastolic blood pressure between those with and without hypertension regardless of how it was defined (P <0.001). In those only self-reporting their diagnosis, the mean difference in systolic and diastolic blood pressure was smaller (10.3 mmHg and 6.8 mmHg respectively). However, a significant proportion of subjects (approximately 30%) with a self- reported diagnosis of hypertension had blood pressure readings that were normal on the day of assessment.

Those who were hypertensive (based on study criteria) were more likely to be male (P <0.001), have a higher body mass index (P <0.001) and not take vitamin D supplements (P <0.001) or have a recent sun holiday travel in the last 6 months (P = 0.033). Physical frailty as asssessed by the Timed Up and Go was also more prevalent in those who were hypertensive (P =0.02) though current smoking (P = 0.03) and alcohol drinking (P = 0.02) was less prevalent.

Subjects with hypertension had a lower 25(OH)D (mean difference = 13.4 nmol/l, P <0.001). The prevalence of severe vitamin D deficiency and deficiency was also greater in hypertensive participants (P <0.001). Likewise, those who had a vitamin D level above 75 nmol/l were significantly less likely to be hypertensive (P <0.001). However, no difference in prevalence was found in subjects with a 25(OH)D level between 25-74.9 nmol/l (P = 0.63).

Table 22.2: Serum 25(OH)D and Diagnosis of Hypertension

Serum 25(OH)D and Hypertension (N = 4077)				
	Self reported (N=2906)		Self reported and/or SBP ≥ 140, DBP ≥ 90 mmHg (N= 3409)	
	β	P-value	β	P-value
Model 1	0.005	0.006	0.007	<0.0001
Model 2	0.005	0.004	0.007	<0.0001
Model 3	0.005	0.005	0.007	<0.0001
Model 4	0.005	0.005	0.008	<0.0001
Model 5	0.006	0.004	0.008	<0.0001

Model 1: age, gender, body mass index, season, alcohol intake, smoking status, supplement use.
Model 2: as per model 1 and Timed Up and Go.
Model 3: as per model 2 and sun holiday travel in last 6 months.
Model 4: as per model 3 and serum PTH
Model 5: as per model 3 and serum calcium

When GSR was substituted for season in the above models the study findings were unchanged.

Table 22.3: Serum 25(OH)D and Blood Pressure

Serum 25(OH)D and Hypertension (N = 4077)				
	Systolic Blood Pressure		Diastolic Blood Pressure	
	β	P-value	β	P-value
Model 1	-	NS	-	NS
Model 2	-	NS	-	NS
Model 3	-	NS	-	NS

Model 1: age, gender, body mass index, season, alcohol intake, smoking status, supplement use.
Model 2: as per model 1 and Timed Up and Go.
Model 3: as per model 2 and sun holiday travel in last 6 months.
Model 4: as per model 3 and serum PTH
Model 5: as per model 3 and serum calcium

When GSR was substituted for season in the above models the study findings were unchanged

In a multiple linear regression model, serum 25(OH)D was inversely associated with both a self reported diagnosis of hypertension (P =0.006) and a diagnosis based on both self report and blood pressure readings taken on the day of assessment (P < 0.0001). The association remained significant after adjustment for age, gender, season, body mass index, supplement use, physical frailty (TUG), sun holiday travel, alcohol and smoking status. In addition, further adjustment for serum calcium or PTH did not change the result. No association was found between vitamin D and systolic or diastolic blood pressure. Substitution of season for GSR as a covaraite did not change the study findings.

CHAPTER 24: DISCUSSION

The significant inverse relationship between serum 25(OH)D and a diagnosis of hypertension (self reported and/or based on blood pressure readings) in our study is consistent with most other studies in the literature to date. The relationship remained robust after confounding for multiple covariates including novel factors such as serum calcium and parathyroid hormone. We found no association with systolic or diastolic blood pressure, though a significant proportion of subjects with a diagnosis of hypertension did not have elevated blood pressure on the day of assessment. This can explained by the fact that the majority of participants were taking anti-hypertensive medications, as well as other tablets with blood pressure lowering effects which is likely to obscure any potential relationship.

Vitamin D levels were significantly lower in those who were hypertensive (-13.4 nmol/l), though part of this may be accounted for by non-use of vitamin D supplements, less sun holiday travel and higher body mass index. The relationship was also found to be independent of serum parathyroid hormone and calcium, both which have been implicated as factors to explain how vitamin D might cause hypertension (Jungert et al., 2012).

Whether there is a threshold effect or a cut-off 25(OH)D level for optimum blood pressure control is unclear. The prevalence of hypertension was about twofold greater in those who were vitamin D deficient (47.4% versus 25.6%), however no significant difference was found for those who had a level between 50-74.9 nmol/l. Despite this, those with a 25(OH)D above 75 nmol/l were less likely to be hypertensive (27.5 versus 47.8%). Overall, findings suggest that increasing vitamin D status up to 75 nmol/l may be beneficial.

Whilst many subjects in the study may have obtained their diagnosis of hypertension many years ago, most still remained on antihypertensives. Apart from the potential dynamic effects of vitamin D on the renin angiotensin aldosterone system (RAAS) and endothelial dysfunction, it is possible that deficiency may also create a 'hypertensive disease state' mediated by long-term damage. For example, vitamin D

status has been inversely associated with arterial wall changes including calcification (Zaqura er al., 2012) that could predispose to hypertension.

Study Strength's and Limitations

There were limitations to this study, some of which have been previously discussed. Firstly, approximately 5% of all participants were excluded in our analysis due to missing data which has the potential to alter study results. The study participants were not representative of the general older population to which the findings may not be applicable. We did not have a good measure of non-supplemental dietary vitamin D intake though we were able to factor in accurately supplement use. In addition, no specific or accurate account of outdoor exposure or physical activity was available. We were not able to adjust for anti-hypertensive medication use or other factors affecting blood pressure such as sodium intake or exercise level.

Whilst including the hypertensive cohort in our analysis increased our statistical power, these subjects lived in a different geographical location and other factors including genetic might be a contributory factor to their hypertension. In particular, there may be differential effects of vitamin D in different populations. For example, as previously discussed a VDR polymorphism has been associated with an increased risk of hypertension (Swapna et al., 2011) and also lower plasma renin activity (Vaidya et al., 2011). The greater North Latitude of subjects in this cohort could also contribute to their lower vitamin D status, though we did adjust for global solar radiation which is a surrogate marker of UVB exposure.

Study strengths are that there was a large sample size and we adjusted for multiple confounds of blood pressure and vitamin D. We also had several blood pressure recordings taken on the day which may have avoided misclassifying normotensive paticipants with 'white coat hypertension'.

Conclusion

Serum 25(OH)D was inversely associated with a diagnosis of hypertension but not with systolic or diastolic blood pressure. No clear threshold affect was found in the relationship. Findings support a role for vitamin D deficiency in causing a 'hypertensive disease state' and suggest a possible effect of supplementation on blood

pressure control. However, if vitamin D and anti-hypertensive medications share a common mechanism for lowering blood pressure, then further addition of vitamin D supplements may not prove effective. Further well designed randomised controlled trials with be required trials to explore vitamin D's possible anti-hypertensive effects.

SECTION 7: VITAMIN D AND MOOD

CHAPTER 25: INTRODUCTION

Vitamin D has been implicated in the aetiopathology of depression in recent years. Vitamin D is known to cross the blood brain barrier and metabolic pathways exist for it's activation in the brain. The vitamin D receptor (VDR) is found in multiple brain areas implicated in depression including the prefrontal cortex, amygdala and hypothalamus. Vitamin D may play a role in mood through it's possible effect on the hypothalamic pituitary adrenal (HPA) axis, brain serotonin and serum parathyroid hormone levels. Vitamin D is also known to have neuro-protective and vasculo-protective affects in the brain, which might protect against depression.

Both cross sectional and longitudinal studies exploring the relationship between vitamin D and depression have given conflicting results, though most show an inverse association. A recent systematic review and meta-analysis which included 10 cross sectional and three cohort studies (N=31,424) found that lower vitamin D status was associated with a higher rate of depression (OR 1.31 and 2.21 respectively),(Anglin et al., 2013).

Despite this, randomised controlled trials have given inconsistent results, though some have been of short duration, have included only small sample sizes, used too low a dose of vitamin D or treated those who weren't vitamin D deficient at baseline. Other intervention studies have not been placebo controlled.

The purpose of this study is to determine if serum 25(OH)D is associated with depression or depressive symptoms in community dwelling older Irish adults.

CHAPTER 26: METHODOLOGY

Study Population and Design

The study population comprised participants of the TUDA (Trinity, University of Ulster, Dept. of Agriculture) study which been previously described. This is a large collaborative, cross sectional study designed to create a phenotype/genotype database for three cohorts of community dwelling subjects aged over sixty years. Study data was available for 1800, 1704 and 1404 participants in the hypertensive, cognitive and bone cohort respectively.

Study methods and participant characteristics are as previously outlined (see chapter 8). Participants with MMSE scores below 24 were excluded in the analysis as several confounds were self-reported. In addition, we aimed to look at the potential relationship in non-dementia subjects.

Assessment of Mood

Subjects were administered the Centre for Epidemiologic Studies Depression scale (CES-D) (Radloff et al., 1977), a screening test for depressive symptoms. CES-D is a twenty item questionnaire that has been validated as a screening tool for depression in community dwelling elderly subjects and also has been found to be a robust measure in subjects with mild dementia (Paspassotiropoulos et al., 1999), (Cheng et al., 2008). A score of greater than or equal to 16 out of a possible total of 60 is both specific and sensitive for major depression (Beckmann et al, 1997). Participants were also asked to self-report on a previous history of depression as diagnosed by their general practitioner or physician.

Statistical Analysis

We initially performed an analysis in the entire TUDA study population to identify if there was an interaction between depression (CES-D $\geq$16) or depressive symptoms (CES-D score), serum vitamin D and each of the study cohorts. This enabled us to decide whether each cohort should be examined separately.

All parameters were inspected for normality and if significantly skewed were appropriately transformed. All statistical analysis was performed using JMP edition 10.0.2. (SAS Institute, 2010).

Study Covariates

Subjects with depression were defined as having a CES-D score of greater than or equal to sixteen. Total CES-D score was used as measure of depressive symptoms.

The total global solar radiation (GSR) which represents the total amount of irradiation received per unit area per month ($KJcm^{-2}$) was used as a surrogate marker of UVB exposure. GSR data was obtained by request from the Irish Meteorological Service. Data from weather stations nearest to study recruitment sites (Dublin Airport) and (Malin Head, Donegal) were used respectively for analysis in the Cognitive / Bone and Hypertensive cohort.

Season was defined according to the standard meteorological definition used in Ireland (Irish Meteorological Service) with the following months comprising Winter (Dec, Jan, Feb), Spring (Mar, Apr, May), Summer (Jun, Jul, Aug), and Autumn (Sept, Oct, Nov).

Timed Up and Go (TUG) measurements were divided into quintiles as it was not possible to measure it in a small proportion of subjects with significant physical disability. These subjects were categorised in the lowest quintile of TUG performance.

Statistical Model

Multiple linear and logistic regression analysis was used to explore the relationship between serum 25 hydroxyvitamin D and (CES-D $\geq$ 16) / depressive symptoms (CES-D score) in a variety of models each using vitamin D as a continuous variable.
In model 1, adjustment was made for age, gender, body mass index, season or GSR, supplement use, living alone status, supplement use, and education, In model 2, the above analysis also incorporated sun holiday travel. In model 3, further adjustment was made for physical frailty as assessed by the TUG. Separate and individual adjustment was also made for other covariates in addition to model 3. These included stroke history, MMSE, PSMS serum PTH and prior history of depression in models 4, 5,6 7 & 8 respectively.

CHAPTER 27: RESULTS

Five hundred and thirty three subjects were excluded after application of study inclusion criteria (MMSE ≥ 24). A further 278 subjects were excluded due to missing or incomplete data, leaving the respective number in the hypertensive, cognitive and bone cohort 1568, 1330 and 1199. For an outline of study cohort participant characteristics see Chapter 8 and Table 8.1.

The relationship was not explored in the bone cohort as the proportion of participants that were depressed was only 6.7% (N = 82). As a significant interaction term was identified and so a separate analysis was performed in the cognitive and hypertensive cohorts. The results of multiple linear regression analyses in both cohorts are shown below.

Table 27.1: Serum 25(OH)D and Depression in the Hypertensive Cohort

Hypertensive Cohort (N=1568)				
	CES-D ≥ 16		CES-D Score	
	β	P-value	β	P-value
Model 1	-0.018	0.0004	-0.037	<0.0001
Model 2	-0.016	<0.001	-0.030	0.0001
Model 3	-0.019	0.005	-0.028	0.0015
Model 4	-0.014	0.006	-0.027	0.002
Model 5	-0.014	0.006	-0.027	0.002
Model 6	-0.014	0.007	-0.024	0.004
Model 7	-0.014	0.006	-0.028	0.0009
Model 8	-0.009	0.07	-0.018	0.027

Model 1: age, gender, body mass index, season, living alone, supplement use, education
Model 2: as per model 1 and Timed Up and Go.
Model 3: as per model 2 and sun holiday travel in last 6 months.
Model 4: as per model 3 and stroke history
Model 5: as per model 3 and MMSE
Model 6: as per model 3 and PSMS
Model 7: as per model 3 and serum PTH
Model 8: as per model 3 and prior history of depression

When GSR was substituted for season in the above models the study findings were unchanged.

27.2: Serum 25(OH)D and Depression in the Cognitive Cohort

Cognitive Cohort (N=1330)				
	CES-D ≥ 16		CES-D Score	
	β	P-value	β	P-value
Model 1	-0.007	0.019	-0.009	0.210
Model 2	-0.004	0.100	- 0.005	0.940
Model 3	-	NS	-	NS
Model 4	-	NS	-	NS
Model 5	-	NS	-	NS
Model 6	-	NS	-	NS
Model 7	-	NS	-	NS
Model 8	-	NS	-	NS

NB: No adjustment was made for sun holiday travel as only 6.3% went on holiday.

Model 1: age, gender, body mass index, season, GSR, living alone, supplement use, education
Model 2: as per model 1 and Timed Up and Go..
Model 3: as per model 2 and stroke history
Model 4: as per model 2 and MMSE
Model 5: as per model 2 and PSMS
Model 6: as per model 2 and serum PTH
Model 7: as per model 2 and prior history of depression

When GSR was substituted for season in the above models the study findings were unchanged.

A significant inverse relationship was found between 25(OH)D and depression (CES-D≥ 16) in the cognitive (P = 0.02) and hypertensive (P = 0.0004) cohorts after adjustment for age, gender, education, body mass index, season, living alone status and supplement use.

The relationship with 25(OH)D remained significant in the hypertensive cohort after further adjustment for TUG (P<0.001) and sun holiday travel (P = 0.005). The finding also remained significant after inclusion of stroke history (P = 0.006), PSMS (P = 0.007), MMSE (P = 0.006) and serum PTH (P = 0.006) as covariates However, the association became insignificant when a prior history of depression was included as a confound (P = 0.07).

In the cognitive cohort the relationship between 25(OH)D and depression became insignificant after further adjustment with the Timed Up and Go (TUG), (P = 0.10). In addition, no association was found between 25(OH)D and depressive symptoms (CES-D score) in the cognitive cohort in any of the models. Conversely, a significant

inverse relationship was identified in the hypertensive cohort and the result remained robust in the full statistical model (P = 0.027).

CHAPTER 28: POST HOC ANALYSIS AND RESULTS

The proportion of subjects with current depression who had a history of prior depression was also identified. Graphical plots of mean CES-D scores were made against quintiles of 25(OH)D in each cohort to identify if there were any potential cut-off points in the relationship. As vitamin D has been implicated in seasonal affective disorder, a univariate analysis was also performed to look for the potential relationship between season or global solar radiation and depression (CES-D ≥ 16) or depressive symptoms (CES-D score).

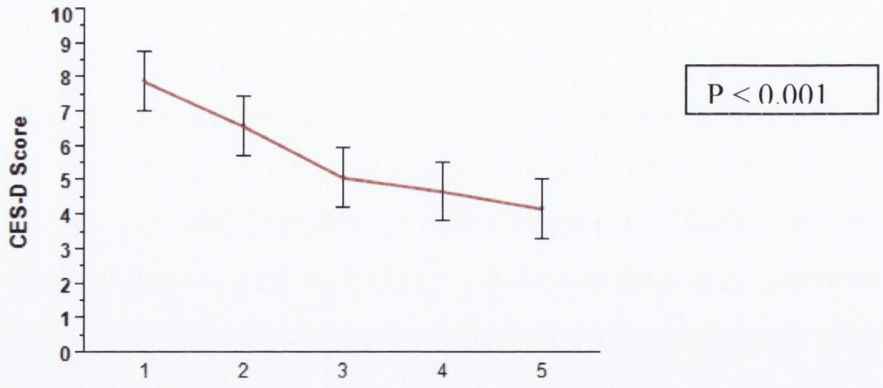
Current Depression and Prior History of Depression

In a post hoc analysis, 72.7% and 47.7% of those in the respective hypertensive and cognitive cohort who had current depression (CES-D ≥16) also reported a previous history of depression. When subjects with a past history of depression were excluded, only 7.3% and 2.7% of the entire cognitive and hypertensive cohort remaining had depression.

Fig 28.1:CES-D Score versus quintiles of 25(OH)D in the Hypertensive Cohort

Error bars represent one standard error

*P-value- adjusted faor age, gender, BMI, season, supplement use, living alone

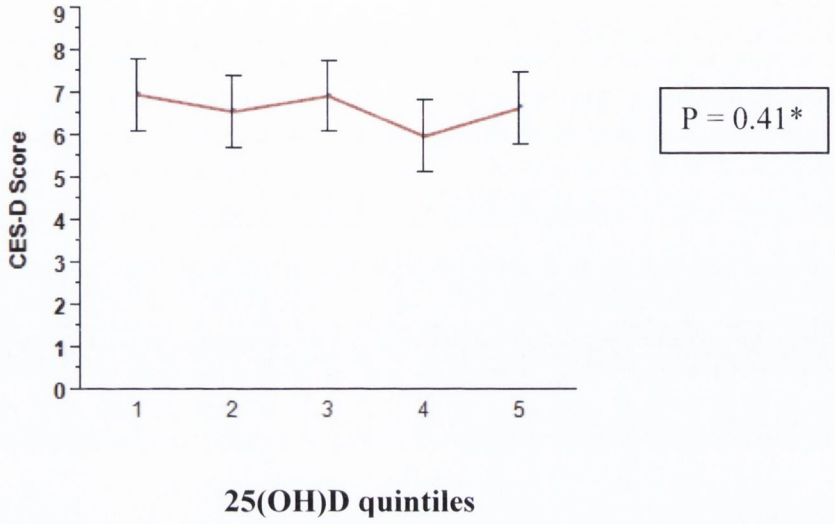


Quintiles: Q1 (1.54-27.4), Q2 (27.5-38.8), Q3 (38.9-50.5), Q4 (50.6-69.8), Q5(69.9-167)

Fig 28.2: CES-D Score versus quintiles of 25(OH)D in the Cognitive Cohort

Error bars represent one standard error

*P-value- adjusted for age, gender, BMI, season, supplement use, living alone



Quintiles: Q1 (5.28-26.3), Q2 (26.4-43.1), Q3 (43.2 –61.3), Q4 (61.4-84.8), Q5(84.9- 185.0)

As graphically shown, most of the improvement in depressive symptoms in the hypertensive cohort occurs when moving from the first to the third quintile of 25(OH)D (see fig 28.1). No significant difference in depressive symptoms was found across all quintiles in the cognitive cohort (P =0.41)

Table 28.1: Mood versus Season / GSR in the Hypertensive & Cognitive Cohort

Mood versus Season / GSR in the Hypertensive & Cognitive Cohort				
	CES-D ≥ 16		CES-D	
	HTN cohort	Cognitve Cohort	HTN cohort	Cognitive cohort
GSR	0.49 [a]	0.66 [a]	0.09 [c]	0.30 [c]
Season	0.12 [b]	0.48 [b]	0.18 [a]	0.81 [a]
[a] Logistic regression, [b] Chi squared test, [c] Univariate regression, GSR-Global Solar Radiation				

No relationship was identified between depression or depressive symptoms and season or global solar radiation in both cohorts.

CHAPTER 29: DISCUSSION

A significant inverse relationship was found between 25(OH)D and depression or depressive symptoms in the hypertensive cohort after adjustment for multiple confounds (age, gender, BMI, season, GSR, supplement use, Timed up and Go, and education, living alone status) However, the relationship with depression but not depressive symptoms became insignificant when factoring in prior history of depression. In the cognitive cohort no association was found with depressive symptoms and that with depression became insignificant after adjustment for physical frailty (TUG).

The majority of subjects (72.7%) with current depression in the hypertensive cohort also had a past history of depression. This suggests that they may have had specific biological or other risk factors that could account for their depression as opposed to their low vitamin D status. However, it is also possible that low vitamin D levels in such participants may have been the aetiological factor for their depression in the first instance. Most studies have not factored in whether participants had a previous history of depression. Hoang et al., found that the association between vitamin D levels and current depressive symptoms (in the largest large study of community dwelling adults to date, N = 12,594) was stronger in those with a history of depression. Despite this, having a history of depression in itself was not associated with lower vitamin D levels (Hoang et al., 2011). This suggests that such a group may be more vulnerable to the effects of vitamin D and could explain the lack of association found in other studies.

Interestingly, the relationship between vitamin D and depressive symptom in the hypertensive cohort remained robust, regardless of whether subjects had a prior history of depression. In addition, the effect was independent of other factors including cognition, physical frailty, functional impairment, stroke history and serum parathyroid levels. It has been suggested that elevated PTH secondary to vitamin D deficiency may play a role in depression (Hoogendijik et al, 2008) though our findings do not support this and are consistent with other studies (Lee et al., 2010). Post hoc graphical analysis suggests that the risk for depressive symptoms is greatest in those with 25(OH)D level below 50 nmol/l. This is consistent with other studies

that found an increased risk in those with 25(OH)D below 50 nmol/l (Milaneschi et al., 2010; Ganji et al., 2010) though results are inconsistent (Stewart et al., 2010).

The association between vitamin D and depression in the cognitive cohort appeared to be explained by physical frailty (TUG). Physical frailty could be an aetiological factor in causing depression (Mezuk et al., 2012). Alternatively, as a surrogate marker of physical disability, it could explain the lower 25(OH)D levels via reduced physical activity and sun exposure, which could subsequently contribute to low mood. Indeed, as previously discussed Timed Up and Go performance was negatively correlated with serum 25(OH)D in cognitive cohort participants. No association was found between vitamin D and depressive symptoms in any of the models in this cohort. However, these participants are older and more complex and include some attending a geriatric day hospital service. It may be that other factors related to medical ill health or disability such as pain may play a greater role in their depressive symptomatology.

It has been proposed that seasonal changes in the photoperiod may give rise to depressive symptoms or Seasonal Affective Disorder, which could be mediated by vitamin D. However, there is little evidence in studies to support this. It is unlikely that vitamin D played any role in that regard in this study, as no direct association was found between season or global solar radiation and depressive symptoms or depression,

Study Strength's and Limitations

There were some limitations to this study similar those that have been previously discussed. Exclusion of up to 5% of participants where there was missing data could have affected our results. The cohorts were 'disease' defined and so the results may not be generalisable to the wider population. We were not able to adjust for physical or outdoor activity, chronic diseases or take account of antidepressant use. Diagnosis of depression was based on a brief assessment which only applies to symptoms in the last week, but has been well validated including in those with cognitive impairment. History of depression was based on patient self report of a doctor diagnosis but was not further defined. In addition, it was not possible to do a separate analysis in those with no history of depression as the sample size was too small..

Study strength's include the fact that it is was of relatively large size and adjustment was made for multiple confounds. In particular, unlike most other studies we were also able to adjust for a previous history of depression.

Conclusion

Vitamin D was associated with low mood in 'younger' old as opposed to 'older' old adults. Levels below 50 nmol/l appear to be associated with more depressive symptoms. Previous history of depression may be more important in those who have current depression than vitamin D itself, though this remains unclear. Lack of association in 'older' old adults may reflect other factors related to ill health that may play a greater role. Findings suggest that randomised controlled trials to examine the potential affect of vitamin D on mood should be perfomed in 'younger' older adults in the future.

SECTION 8: VITAMIN D AND FALLS

CHAPTER 30: INTRODUCTION

Vitamin D deficiency appears to be related to falls as it is associated with proximal muscle weakness (Schott et al., 1976). Furthermore, there is strong biological evidence supporting a role for vitamin D in muscle function. The vitamin D receptor (VDR) and the metabolic pathway for it's activation is found in human muscle cells where it is known to affect metabolism and calcium uptake.

Several studies have shown that vitamin D status is positively associated with muscle strength and physical performance and inversely associated with falls. Vitamin D supplementation has also been shown to improve tests of muscle function and balance and positively impact on muscle fibre composition and morphology in vitamin D deficient older adults (Ceglia et al., 2008). Finally, numerous randomised controlled trials have found that vitamin D reduces either the rate of falls or falls risk. Despite this, the effect of vitamin D on falls appears to depend on several factors and it remains unclear who benefits most. In addition, some cross sectional studies have found no relationship between vitamin D and falls in older adults.

The purpose of this study was to identify if serum 25(OH)D is associated with falls risk or recurrent falls in community dwelling older Irish adults.

CHAPTER 31: METHODOLOGY

Study Population and Design

The study population comprised participants of the TUDA (Trinity, University of Ulster, Dept. of Agriculture) study which been previously described. This is a large collaborative, cross sectional study designed to create a phenotype/genotype database for three cohorts of community dwelling subjects aged over sixty years. Study data was available for 1800, 1704 and 1404 participants in the hypertensive, cognitive and bone cohort respectively.

Participants with MMSE scores below 24 were excluded in the analysis as several confounds were self-reported. In addition, we specifically aimed to explore the relationship with falls in non-dementia subjects.

Study methods are as previously outlined (see chapter 8). Subjects partook in an assessment lasting about 70 minutes. Participants were asked to report if they had a fall in the past year and if so how many. A fall was defined as an event which resulted in a person coming to rest inadvertently on the ground or floor or other lower level. They were also asked if they suffered with dizziness on standing up.

Statistical Analysis

We initially performed an analysis in the entire TUDA study population to identify if there was an interaction between the presence of falls, serum 25(OH)D and cohorts so as to establish if the analysis should be performed separately for each cohort. When the interaction term was significant ($P < 0.05$) cohorts were analysed individually.

All parameters were inspected for normality and if significantly skewed were appropriately transformed. All statistical analysis was performed using JMP edition 10.1 (SAS Institute 2010).

Study Covariates

Falls history was divided into categories (fall versus no fall) and (two or more falls versus one or no fall).

The total global solar radiation (GSR) which represents the total amount of irradiation received per unit area per month ($KJcm^{-2}$) was used as a surrogate marker of UVB exposure. GSR data was obtained by request from the Irish Meteorological Service. Data from weather stations nearest to study recruitment sites (Dublin Airport) and (Malin Head, Donegal) were used respectively for analysis in the Cognitive / Bone and Hypertensive cohort.

Season was defined according to the standard meteorological definition used in Ireland (Irish Meteorological Service) with the following months comprising Winter (Dec, Jan, Feb), Spring (Mar, Apr, May), Summer (Jun, Jul, Aug), and Autumn (Sept, Oct, Nov).

Timed Up and Go (TUG) measurements were divided into quintiles as it was not possible to measure it in a small proportion of subjects with significant physical disability. These subjects were categorised in the lowest quintile of TUG performance.

Statistical Models

The relationship between 25(OH)D and the falls risk or recurrent falls were explored in logistic regression models incorporating several covariates. In model 1, adjustment was made for age, gender, body mass index, season or global solar radiation, and supplement use. In model 2, adjustment was made for sun holiday travel. In subsequent models, further and individual adjustment was made for postural dizziness, depression and physical frailty (TUG).

CHAPTER 32: RESULTS

Five hundred and three subjects were excluded after application of study inclusion criteria (MMSE ≥ 24). A further 278 were excluded due to missing or incomplete data, leaving the respective number in each of the above cohorts 1568, 1330 and 1199. A significant interaction term was identified and so cohorts were analysed separately. For study population characteristics see Table . 8.1.

Table 32.1: 25(OH)D and Falls in the Hypertensive Cohort

Relationship between 25(OH)D and Falls in Hypertensive Cohort (N=1568)				
	Fall versus No Fall		Two or more falls versus one or no fall	
	β	P-value	β	P-value
Model 1	-0.005	0.07	-0.01	0.020
Model 2	-0.004	0.13	-0.01	0.045
Model 3	-0.005	0.13	-0.009	0.057
Model 4	-0.003	0.36	-0.008	0.11
Model 5	-0.005	0.40	-0.008	0.11

Model 1: age, gender, body mass index, season, supplement use
Model 2: model 1 + sun holiday travel
Model 3: model 2 + postural dizziness
Model 4: model 2 + depression (CES-D≥16)
Model 5: model 2 + Timed Up and Go

When GSR was substituted for season, the relationship with two or more falls remained significant in model 2, but otherwise the results were unchanged.

Table 32.2: 25(OH)D and Falls in the Cognitive Cohort

Relationship between 25(OH)D and Falls in Cognitive Cohort (N=1330)				
	Fall versus No Fall		Two or more falls versus one or no fall	
	β	P-value	β	P-value
Model 1	-0.002	0.42	-0.003	0.15
Model 2*	-0.001	0.35	-0.002	0.37
Model 3	-0.002	0.38	-.0.003	0.45
Model 4	--0.002	0.42	--0.004	0.52

Model 1: age, gender, body mass index, season, supplement use..
Model 2: model 1 + postural dizziness
Model 3: model 1 + depression (CES-D≥16)
Model 4: model 1 + Timed Up and Go

When GSR was substituted for season, results were unchanged.

Table 32.3: 25(OH)D and Falls in the Bone Cohort

Relationship between 25(OH)D and Falls in Bone Cohort (N=1199)				
	Fall versus No Fall		Two or more falls versus one or no fall	
	β	P-value	β	P-value
Model 1	0.003	0.18	-0.003	0.15
Model 2*	-0.001	0.35	-0.000	0.81
Model 3	-	NS	-	NS
Model 4	-	NS	-	NS
Model 5	-	NS	-	NS

Model 1: age, gender, body mass index, season, supplement use.
Model 2: model 1 + sun holiday travel
Model 3: model 2 + postural dizziness
Model 4: model 2 + depression (CES-D≥16)
Model 5: model 2 + PTH

When GSR was substituted for season, there was no change in the study findings.

An inverse relationship between vitamin D and falls was found in participants of the hypertensive cohort. Lower vitamin D status in these subjects was associated with an increased risk of having two or more than falls versus one or no fall (P = 0.020). The result remained significant after adjusting for sun holiday travel (P = 0.045) but became negative when postural dizziness and current depression were included as a covariates (P> 0.05). Further adjustment for physical frailty (TUG) did not change the result. In addition, the presence of falls in itself was not inversely associated with lower vitamin D status. No association was found between vitamin D and falls in the either the bone or cognitive cohort (see tables 32.1 –32.3).

CHAPTER 33: POST HOC ANALYSIS & RESULTS

To further explore any potential relationship between vitamin D and falls in the older 'older' adults in the cognitive cohort, an analysis was performed separately in non-users of vitamin D supplements. A standard statistical model adjusting for supplement use cannot take account of differences in compliance or vitamin D dosage at a individual level and could therefore affect results. In addition, our previous analysis of subjects in this cohort revealed that those who used supplements were older and physically frailer which could have the potential to affect results.

POST HOC RESULTS

Table 33.1: Characteristics of fallers in Cognitive Cohort (non-supplemented)

	Fall (N = 300)	No Fall (N = 330)	P-value	Fall > 1 (N = 155)	Fall ≤1 (N = 475)	P-value
25(OH)D (nmol/l)	36.8	41.1	0.02 [a]	37.2	39.7	0.21 [a]
< 25 (%)	35.3	27.6	0.04 [b]	34.2	30.3	0.40 [b]
25 –49.9 (%)	42.3	42.2	0.08 [b]	42.6	42.3	0.26 [b]
50-74.9 (%)	15.0	20.3	0.37 [b]	16.1	18.3	0.66 [b]
Age (years)	79.5	79.6	0.90 [a]	79.0	79.7	0.31 [a]
Gender (female, %)	53.4	60.3	0.10 [b]	54.5	57.9	0.51 [b]
BMI (kgm^{-2})	28.1	27.6	0.24 [a]	28.5	27.6	0.09 [a]
TUG (quintiles)	-	-	0.31 [b]	-	-	0.91 [b]
MMSE	26.9	27.1	0.08 [a]	26.7	27.1	0.02 [a]
FAB	14.7	15.2	0.004 [c]	14.5	15.2	0.007 [c]
CES-D ≥ 16 (%)	14.7	12.1	0.35 [b]	16.8	12.2	0.17 [b]
Stroke (%)	18.0	15.7	0.46 [b]	19.4	16.0	0.52 [b]
Postural Dizziness (%)	38.0	32.7	0.005 [b]	49.4	34.3	0.008 [b]

[a] unpaired t test, [b] Chi squared test, [c] Wilcoxon Rank test

Subjects in the cognitive cohort (who were not taking supplements) and had fallen in the last year had a lower serum 25(OH)D (mean difference 4.3 nmol/l, P = 0.02). They were also more likely to have severe vitamin D deficiency (<25nmol/l, P = 0.04) postural dizziness (P = 0.005) and have poorer performance on the FAB (P = 0.004). No statistical difference in prevalence of stroke, depression or physical frailty as measured by the TUG was identified (see table 33.1).

Conversely, no significant difference in vitamin D levels was found between those with a history of recurrent falls versus one fall or less (P=0.21). Recurrent fallers were also more likely to have postural dizziness (P = 0.008) and be cognitively impaired with lower scores in the MMSE (P = 0.02) and FAB (P = 0.007).

Table 33.2: Vitamin D and Falls in Non-supplemented Cognitive Cohort Subjects

Relationship between 25(OH)D and Falls in Cognitive Cohort (N=630)				
	Fall versus No Fall		Two or more falls versus one or no fall	
	β	P-value	β	P-value
Model 1	-0.009	0.013	-0.006	0.14
Model 2	-0.009	0.008	-0.002	0.10
Model 3	-0.009	0.016	-0.006	0.17
Model 4	-0.008	0.018	-0.006	0.17
Model 5	-0.006	0.024	-0.006	0.19

Model 1: age, gender, body mass index, season
Model 2: model 1+ postural dizziness
Model 3: model 1+ CES-D ≥ 16
Model 4: model 1 + TUG
Model 5: model 1+FAB (log transformed)

When GSR was substituted for season, the study findings were unchanged.

A significant inverse relationship between 25(OH)D and falls was identified after adjustment for age, gender, BMI and season. The finding remained significant after further individual adjustment for postural dizziness, Timed Up and Go, current depression, and FAB performance. Inclusion of GSR instead of season as a covariate did not change the study findings. No significant relationship was identified between vitamin D and recurrent fallers.

CHAPTER 34: DISCUSSION

Vitamin D was inversely associated with falls in participants of the hypertensive and cognitive cohort. In subjects in the hypertensive cohort, lower vitamin D status predicted recurrent falls but not risk of falling. In addition, the relationship became insignificant after further adjustment for postural dizziness, depression or physical frailty. In the cognitive cohort, the association was only identified in those who were not using supplements. However, it remained significant after adjustment for postural dizziness, physical frailty and cognition.

The findings in the hypertensive cohort do no rule out a role for vitamin D in falls. As the inclusion of postural dizziness resulted in the model becoming insignificant, it suggested it was the explanatory variable. However, vitamin D could also be part of the missing link as it has been suggested that it may play a role in orthostatic hypotension (McCarroll et al., 2012). In addtion, vitamin D has also been associated with low mood and physical frailty.

Our previous analysis found a relationship between vitamin D and physical frailty in the cognitive cohort, yet despite this no relationship was found with falls in the full sample analysis. Other studies have also found no association between vitamin D and falls in older adults (Stein et al., 1999; Sambrook et al., 2004; Bolland et al., 2010). though results are conflicting. The association between vitamin D and two or more future falls was paradoxically found to be stronger in younger (<75 years of age) as oppose to 'older' old adults in one prospective study, suggesting that other factors may be more important (Snidjer et al., 2005).

In this regard, the aetiology of falls in the elderly is multi-factorial. Whilst vitamin D may affect physical function other factors which influence falls such gait imbalance secondary to osteoarthritis, orthostatic hypotension, medication use and visual impairment also play a role independent of vitamin D and could therefore obscure any relationship.

Our negative result in the full cohort analysis might be explained by undue weighting being attributed to supplement use in the statistical model, on account of differences in compliance and dosage at an individual level. In addition, as previously discussed

those who were supplemented were older and frailer which probably reflects selection bias to treatment, but had higher serum vitamin D on account of their use. In our separate analysis in non-supplemented subjects which avoided potential problems with the above factors, the relationship was significant. However, it appeared to be confined to those who were vitamin D deficient (< 25nmol/l). Interestingly, no difference in TUG performance was identified between fallers and non-fallers or recurrent versus non-recurrent fallers. This suggests a potential mechanism for vitamin D independent of any physical attributes captured by TUG. No association was found with recurrent falls in the cognitive cohort but the number was small and may have been underpowered to detect a statistically significant difference.

We used a 'self report' of falls in the previous year, however this may not be reliable particularly in cognitive cohort participants. It is likely that some subjects who fell as far back as twelve months previously may not have reported this increasing the likelihood of our results being negative. Whilst recurrent falls may be associated with significant physical impairment, for some subjects a once-off fall in the last year could be accidental and not have any specific aetiopathology. The lack of relationship between vitamin D and the presence of falls in the bone and hypertensive cohorts may in part reflect this.

In particular, participants in the bone cohort through bias selection were more likely to have a falls history but nearly two thirds had only one fall and physical frailty was much less prevalent than in the older cognitive cohort subjects. In addition, only 11.7% (N = 141) of the entire bone cohort participant fells more than once and so it may have also have been underpowered to detect a difference.

Study strength's and Limitations

The number of participants with recurrent falls was relatively small, especially in the bone and also the hypertensive cohort. Falls data may also have been be inaccurate as it was self reported. In addition, we were not able to adjust or take account of other factors known to influence falls, such as psychotropic medication use or visual impairment. The study included a large number of subjects and was able to explore the relationship in three cohorts with significantly different characteristics. We were also able to adjust for multiple confounds of vitamin D status.

Conclusion

Overall, we found a significant relationship between vitamin D and falls in non-supplemented 'older' old adults. The association in the total cognitive cohort was negative as it included those who where frailer and potentially more complex. It is possible that in the 'younger' old adults, vitamin D might be related to recurrent falls though this is unclear.

SECTION 9: THESIS CONCLUSIONS

THESIS CONCLUSIONS

The aims of this study were to investigate for the prevalence of vitamin D deficiency, and its correlates as well as the relevance of vitamin D to falls, mood, high blood pressure and cognition in older community dwelling Irish adults.

We identified that vitamin D deficiency was prevalent, particularly in 'older' old adults who were physically frail and not taking supplements. Season and supplement use were the most important determinants of vitamin D status. This highlights the need to factor in season when interpreting levels, especially in the Spring when vitamin D is lowest and in the Autumn when it is highest. Despite the high prevalence of deficiency, the observed effect of supplementation was substantial. Results also suggest that modification of lifestyle factors such as smoking, oily fish intake and sunshine exposure may be important alternative strategies to improve vitamin D status.

A positive association was found between vitamin D and executive function and language tests, though only in younger study participants. The relationship with executive function has been previously reported. However, this is the largest study to date that has found a specific relationship with tests of naming and semantic fluency.

With regard to blood pressure, in a pooled analysis including all subjects, there was a robust inverse relationship with a diagnosis of hypertension independent of several confounds. The lack of association with systolic and diastolic blood pressure may relate to the lack of study adjustment for anti-hypertensive use.

We also found an inverse association between vitamin D and depressive symptoms that was independent of having a prior history of depression. Again the findings were confined to younger subjects in the hypertensive cohort.

Finally, in our last study, vitamin D was associated with falls risk though only in non supplemented 'older' old adults in the cognitive cohort. No association was found with recurrent fallers but the sample size was smaller and it may have been underpowered to detect a difference in vitamin D. In addition, several other factors

related to falls that were not captured in the analysis could account for this non-significant relationship. Vitamin D might also be related to recurrent fallers in younger 'old' but the relationship appeared to be explained by other factors.

Future Research

A significant finding in two of the above studies which examined the relationship between vitamin D and cognition / depression was the lack of any association in 'older' old subjects. It is possible that long-term deficiency of vitamin D may be a risk factor for specific disease outcomes through modulation of multiple factors. If this is so, then the absence of an association in cross sectional studies does not rule out a role for vitamin D earlier in life. Such a hypothesis of 'long latency disease deficiency' has been previously proposed (Heaney RP et al., 2003).

The potential long-term vasculo-protective, neuro-protective or other effects of vitamin D over many years may be more important in preventing the emergence of disease states like hypertension, cognitive impairment or depression. As vitamin D could modulate multiple risk factors including blood pressure, endothelial dysfunction and inflammation, there could be a small but significant positive cumulative effect over many years. Whilst vitamin D may also have short-term dynamic affects such as on neuro-tramitters, inflammation and the renin-angiotensin system the relative importance of this in later life could be subsumed by the negative long-term effects or damage of deficiency. In addition, other factors with advancing age may take greater importance or negate the potential effects of vitamin D.

Overall, study findings from the TUDA cohorts suggest that optimisation of vitamin D status earlier in life may be more important in preventing or ameliorating vitamin D related diseases.

BIBLIOGRAPHY

Absoud, M., Cummins, C., Lim, M. J., Wassmer, E., & Shaw, N. (2011). Prevalence and predictors of vitamin D insufficiency in children: a Great Britain population based study. *PLoS One, 6*(7), e22179. doi: 10.1371/journal.pone.0022179

Ahmadi S, Mirzaei K, Hossein-Nezhad A, Shariati G (2012). Vitamin D receptor FokI genotype may modify the susceptibility to schizophrenia and bipolar mood disorder by regulation of dopamine D1 receptor gene expression. *Minerva Med, 103*(5):383-91.

Ahn, J., Yu, K., Stolzenberg-Solomon, R., Simon, K. C., McCullough, M. L., Gallicchio, L., . . . Albanes, D. (2010). Genome-wide association study of circulating vitamin D levels. *Hum Mol Genet, 19*(13), 2739-2745. doi: 10.1093/hmg/ddq155

Airaksinen, M. S., Eilers, J., Garaschuk, O., Thoenen, H., Konnerth, A., & Meyer, M. (1997). Ataxia and altered dendritic calcium signaling in mice carrying a targeted null mutation of the calbindin D28k gene. *Proc Natl Acad Sci U S A, 94*(4), 1488-1493.

Akatsu, H., Ogawa, N., Kanesaka, T., Hori, A., Yamamoto, T., Matsukawa, N., & Michikawa, M. (2011). Higher activity of peripheral blood angiotensin-converting enzyme is associated with later-onset of Alzheimer's disease. *J Neurol Sci, 300*(1-2), 67-73. doi: 10.1016/j.jns.2010.09.030

Al Anouti, F., Thomas, J., Abdel-Wareth, L., Rajah, J., Grant, W. B., & Haq, A. (2011). Vitamin D deficiency and sun avoidance among university students at Abu Dhabi, United Arab Emirates. *Dermatoendocrinol, 3*(4), 235-239. doi: 10.4161/derm.3.4.16881

Al Attia HM, Ibrahim MA. Al Attia HM, Ibrahim MA (2012). The high prevalence of vitamin D inadequacy and dress style of women in the sunny UAE. *Arch Osteoporos, 7*(1-2):307-10.

Alborzi, P., Patel, N. A., Peterson, C., Bills, J. E., Bekele, D. M., Bunaye, Z., . . . Agarwal, R. (2008). Paricalcitol reduces albuminuria and inflammation in chronic kidney disease: a randomized double-blind pilot trial. *Hypertension, 52*(2), 249-255. doi: 10.1161/HYPERTENSIONAHA.108.113159

Alexianu, M. E., Robbins, E., Carswell, S., & Appel, S. H. (1998). 1Alpha, 25 dihydroxyvitamin D3-dependent up-regulation of calcium-binding proteins in motoneuron cells. *J Neurosci Res, 51*(1), 58-66.

Allender, P. S., Cutler, J. A., Follmann, D., Cappuccio, F. P., Pryer, J., & Elliott, P. (1996). Dietary calcium and blood pressure: a meta-analysis of randomized clinical trials. *Ann Intern Med, 124*(9), 825-831.

Almeras, L., Eyles, D., Benech, P., Laffite, D., Villard, C., Patatian, A., . . . Feron, F. (2007). Developmental vitamin D deficiency alters brain protein expression in the adult rat: implications for neuropsychiatric disorders. *Proteomics, 7*(5), 769-780. doi: 10.1002/pmic.200600392

Almirall, J., Vaqueiro, M., Bare, M. L., & Anton, E. (2010). Association of low serum 25-hydroxyvitamin D levels and high arterial blood pressure in the elderly. *Nephrol Dial Transplant, 25*(2), 503-509. doi: 10.1093/ndt/gfp470

Aloia, J. F., Patel, M., Dimaano, R., Li-Ng, M., Talwar, S. A., Mikhail, M., . . . Yeh, J. K. (2008). Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. *Am J Clin Nutr, 87*(6), 1952-1958.

Al-Saleh, Y., Al-Daghri, N. M., Alkharfy, K. M., Al-Attas, O. S., Alokail, M. S., Al-Othman, A., . . . Chrousos, G. P. (2013). Normal circulating PTH in Saudi healthy individuals with hypovitaminosis D. *Horm Metab Res, 45*(1), 43-46. doi: 10.1055/s-0032-1323679

Altun, B., & Arici, M. (2006). Salt and blood pressure: time to challenge. *Cardiology, 105*(1), 9-16. doi: 10.1159/000088265

Alvarez, J., Wasse, H., & Tangpricha, V. (2012). Vitamin D supplementation in pre-dialysis chronic kidney disease: A systematic review. *Dermatoendocrinol, 4*(2), 118-127. doi: 10.4161/derm.20014

Anglin, R. E., Samaan, Z., Walter, S. D., & McDonald, S. D. (2013). Vitamin D deficiency and depression in adults: systematic review and meta-analysis. *Br J Psychiatry, 202*, 100-107. doi: 10.1192/bjp.bp.111.106666

Annweiler, C., Schott, A. M., Berrut, G., Fantino, B., & Beauchet, O. (2009). Vitamin D-related changes in physical performance: a systematic review. *J Nutr Health Aging, 13*(10), 893-898.

Annweiler C, Schott AM, Rolland Y, Blain H, Herrmann FR, Beauchet O (2010). Dietary intake of vitamin D and cognition in older women: a large population-based study. *Neurology, 16;*75(20):1810

Annweiler C, Rolland Y, Schott AM, Blain H, Vellas B, Beauchet O (2011). Serum vitamin D deficiency as a predictor of incident non-Alzheimer dementias: a 7-year longitudinal study. J Geriatr Psychiatry Neurol, 22(3):188-95.

Annweiler, C., Fantino, B., Gautier, J., Beaudenon, M., Thiery, S., & Beauchet, O. (2012). Cognitive effects of vitamin D supplementation in older outpatients visiting a memory clinic: a pre-post study. *J Am Geriatr Soc, 60*(4), 793-795. doi: 10.1111/j.1532-5415.2011.03877.x

Annweiler, C., Montero-Odasso, M., Muir, S. W., & Beauchet, O. (2012). Vitamin D and Brain Imaging in the Elderly: Should we Expect Some Lesions Specifically Related to Hypovitaminosis D? *Open Neuroimag J, 6*, 16-18. doi: 10.2174/1874440001206010016

Annweiler, C., Fantino, B., Schott, A. M., Krolak-Salmon, P., Allali, G., & Beauchet, O. (2012). Vitamin D insufficiency and mild cognitive impairment: cross-sectional association. *Eur J Neurol, 19*(7), 1023-1029. doi: 10.1111/j.1468-1331.2012.03675.x

Annweiler, C., Rolland, Y., Schott, A. M., Blain, H., Vellas, B., Herrmann, F. R., & Beauchet, O. (2012). Higher vitamin D dietary intake is associated with lower risk of alzheimer's disease: a 7-year follow-up. *J Gerontol A Biol Sci Med Sci, 67*(11), 1205-1211. doi: 10.1093/gerona/gls107

Annweiler, C., Herrmann, F. R., Fantino, B., Brugg, B., & Beauchet, O. (2012). Effectiveness of the combination of memantine plus vitamin D on cognition in patients with Alzheimer disease: a pre-post pilot study. *Cogn Behav Neurol, 25*(3), 121-127. doi: 10.1097/WNN.0b013e31826df647

Annweiler C, Llewellyn DJ, Beauchet O. Low serum vitamin D concentrations in Alzheimer's disease: a systematic review and meta-analysis (2013). *J Alzheimers Dis, ;33*(3):659-74.

Annweiler, C., Montero-Odasso, M., Hachinski, V., Seshadri, S., Bartha, R., & Beauchet, O. (2013). Vitamin D concentration and lateral cerebral ventricle volume in older adults. *Mol Nutr Food Res, 57*(2), 267-276. doi: 10.1002/mnfr.201200418

Annweiler, C., Muir, S. W., Nabeel, S., Gopaul, K., Beauchet, O., & Montero-Odasso, M. (2013). Higher serum vitamin D concentration is associated with better balance in older adults with supra-optimal vitamin D status. *J Am Geriatr Soc, 61*(1), 163-165. doi: 10.1111/jgs.12053

Annweiler, C., Montero-Odasso, M., Llewellyn, D. J., Richard-Devantoy, S., Duque, G., & Beauchet, O. (2013). Meta-Analysis of Memory and Executive Dysfunctions in Relation to Vitamin D. *J Alzheimers Dis, 37*(1), 147-171. doi: 10.3233/JAD-130452

Aparna, R., Subhashini, J., Roy, K. R., Reddy, G. S., Robinson, M., Uskokovic, M. R., . . . Reddanna, P. (2008). Selective inhibition of cyclooxygenase-2 (COX-2) by 1alpha,25-dihydroxy-16-ene-23-yne-vitamin D3, a less calcemic vitamin D analog. *J Cell Biochem, 104*(5), 1832-1842. doi: 10.1002/jcb.21749

Araki T, Holick MF, Alfonso BD, Charlap E, Romero CM, Rizk D, Newman LG (2011).Vitamin D intoxication with severe hypercalcemia due to manufacturing and labeling errors of two dietary supplements made in the United States. *J Clin Endocrinol Metab ;96*(12):3603-8.

Argiles, A., Lorho, R., Servel, M. F., Couret, I., Chong, G., & Mourad, G. (2002). Blood pressure is correlated with vitamin d(3) serum levels in dialysis patients. *Blood Purif, 20*(4), 370-375. doi: 63106

Armas, L. A., Hollis, B. W., & Heaney, R. P. (2004). Vitamin D2 is much less effective than vitamin D3 in humans. *J Clin Endocrinol Metab, 89*(11), 5387-5391. doi: 10.1210/jc.2004-0360

Armstrong DJ, Meenagh GK, Bickle I, Lee AS, Curran ES, Finch MB (2007). Vitamin D deficiency is associated with anxiety and depression in fibromyalgia. *Clin Rheumatol, 26*:551-554

Arnarson, A., Geirsdottir, O. G., Ramel, A., Jonsson, P. V., Steingrimsdottir, L., & Thorsdottir, I. (2012). [Dietary habits and their association with blood pressure among elderly Icelandic people]. *Laeknabladid, 98*(10), 515-520.

Arregui, A., Perry, E. K., Rossor, M., & Tomlinson, B. E. (1982). Angiotensin converting enzyme in Alzheimer's disease increased activity in caudate nucleus and cortical areas. *J Neurochem, 38*(5), 1490-1492.

Arunabh, S., Pollack, S., Yeh, J., & Aloia, J. F. (2003). Body fat content and 25-hydroxyvitamin D levels in healthy women. *J Clin Endocrinol Metab, 88*(1), 157-161.

Arvold, D. S., Odean, M. J., Dornfeld, M. P., Regal, R. R., Arvold, J. G., Karwoski, G. C., . . . Sjoberg, R. J. (2009). Correlation of symptoms with vitamin D deficiency and symptom response to cholecalciferol treatment: a randomized controlled trial. *Endocr Pract, 15*(3), 203-212.

Asemi, Z., Samimi, M., Tabassi, Z., Shakeri, H., & Esmaillzadeh, A. (2013). Vitamin D Supplementation Affects Serum High-Sensitivity C-Reactive Protein, Insulin Resistance, and Biomarkers of Oxidative Stress in Pregnant Women. *J Nutr*. doi: 10.3945/jn.113.177550

Aung, K., Burnett, J., Smith, S. M., & Dyer, C. B. (2006). Vitamin D deficiency associated with self-neglect in the elderly. *J Elder Abuse Negl, 18*(4), 63-78.

Autier, P., Gandini, S., & Mullie, P. (2012). A systematic review: influence of vitamin D supplementation on serum 25-hydroxyvitamin D concentration. *J Clin Endocrinol Metab, 97*(8), 2606-2613. doi: 10.1210/jc.2012-1238

Avioli, L. V. (1969). Absorption and metabolism of vitamin D3 in man. *Am J Clin Nutr, 22*(4), 437-446.

Baas, D., Prufer, K., Ittel, M. E., Kuchler-Bopp, S., Labourdette, G., Sarlieve, L. L., & Brachet, P. (2000). Rat oligodendrocytes express the vitamin D(3) receptor and respond to 1,25-dihydroxyvitamin D(3). *Glia, 31*(1), 59-68.

Baker, A. R., McDonnell, D. P., Hughes, M., Crisp, T. M., Mangelsdorf, D. J., Haussler, M. R., . . . O'Malley, B. W. (1988). Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proc Natl Acad Sci U S A, 85(10), 3294-3298.

Balabanova S, Richter HP, Antoniadis G, Homoki J, Kremmer N, Hanle J, Teller WM (1984). 25-Hydroxyvitamin D, 24, 25-dihydroxyvitamin D and 1,25-dihydroxyvitamin D in human cerebrospinal fluid. *Klin Wochenschr, 62*(22):1086-90.

Balion, C., Griffith, L. E., Strifler, L., Henderson, M., Patterson, C., Heckman, G., . . . Raina, P. (2012). Vitamin D, cognition, and dementia: a systematic review and meta-analysis. *Neurology, 79*(13), 1397-1405. doi: 10.1212/WNL.0b013e31826c197f

Bambridge K.G, Celio M.R, Roger JH (1992). Calcium binding protein in the nervous system. *Trends Neur Sci 15,*303-308.

Barger-Lux, M. J., Heaney, R. P., Dowell, S., Chen, T. C., & Holick, M. F. (1998). Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. *Osteoporos Int, 8*(3), 222-230.

Barger-Lux MJ, Heaney RP (2002). Effects of above average summer sun exposure on serum 25-hydroxyvitamin D and calcium absorption. *J Clin Endocrinol Metab;87*(11):4952-6.

Barnes, N. (1957). Vitamin D intoxication. *Ill Med J, 112*(4), 182-185.

Barr, R., Macdonald, H., Stewart, A., McGuigan, F., Rogers, A., Eastell, R., . . . Reid, D. M. (2010). Association between vitamin D receptor gene polymorphisms, falls, balance and muscle power: results from two independent studies (APOSS and OPUS). *Osteoporos Int, 21*(3), 457-466. doi: 10.1007/s00198-009-1019-6

Becker, A., Eyles, D. W., McGrath, J. J., & Grecksch, G. (2005). Transient prenatal vitamin D deficiency is associated with subtle alterations in learning and memory functions in adult rats. *Behav Brain Res, 161*(2), 306-312. doi: 10.1016/j.bbr.2005.02.015

Benge, J. F., Perrier, N. D., Massman, P. J., Meyers, C. A., Kayl, A. E., & Wefel, J. S. (2009). Cognitive and affective sequelae of primary hyperparathyroidism and early response to parathyroidectomy. *J Int Neuropsychol Soc, 15*(6), 1002-1011. doi: 10.1017/S1355617709990695

Berg, A. O., Melle, I., Torjesen, P. A., Lien, L., Hauff, E., & Andreassen, O. A. (2010). A cross-sectional study of vitamin D deficiency among immigrants and Norwegians with psychosis compared to the general population. *J Clin Psychiatry, 71*(12), 1598-1604. doi: 10.4088/JCP.09m05299yel

Berninie G, Carrara D, Bacca A, Carli V, Virdis A, Rugani I, Duranti E, Ghiadoni L, Bernin M, Taddei (2013). Effect of acute and chronic vitamin D administration on systemic rennin angiotensin system in essential hypertensives and controls. J Endocrinol Invest; 36(4):216-20.

Berk, M., Jacka, F. N., Williams, L. J., Ng, F., Dodd, S., & Pasco, J. A. (2008). Is this D vitamin to worry about? Vitamin D insufficiency in an inpatient sample. *Aust N Z J Psychiatry, 42*(10), 874-878. doi: 10.1080/00048670802345516

Berk, M., Sanders, K. M., Pasco, J. A., Jacka, F. N., Williams, L. J., Hayles, A. L., & Dodd, S. (2007). Vitamin D deficiency may play a role in depression. *Med Hypotheses, 69*(6), 1316-1319. doi: 10.1016/j.mehy.2007.04.001

Berridge, M. J. (1998). Neuronal calcium signaling. *Neuron, 21*(1), 13-26.

Berry, D., & Hypponen, E. (2011). Determinants of vitamin D status: focus on genetic variations. *Curr Opin Nephrol Hypertens, 20*(4), 331-336. doi: 10.1097/MNH.0b013e328346d6ba

Bertone-Johnson, E. R., Hankinson, S. E., Bendich, A., Johnson, S. R., Willett, W. C., & Manson, J. E. (2005). Calcium and vitamin D intake and risk of incident premenstrual syndrome. *Arch Intern Med, 165*(11), 1246-1252. doi: 10.1001/archinte.165.11.1246

Bertone-Johnson, E. R., Powers, S. I., Spangler, L., Brunner, R. L., Michael, Y. L., Larson, J. C., . . . Manson, J. E. (2011). Vitamin D intake from foods and supplements and depressive symptoms in a diverse population of older women. *Am J Clin Nutr, 94*(4), 1104-1112. doi: 10.3945/ajcn.111.017384

Bertone-Johnson, E. R., Powers, S. I., Spangler, L., Larson, J., Michael, Y. L., Millen, A. E., . . . Manson, J. E. (2012). Vitamin D supplementation and depression in the women's health initiative calcium and vitamin D trial. *Am J Epidemiol, 176*(1), 1-13. doi: 10.1093/aje/kwr482

Beydoun, M. A., Ding, E. L., Beydoun, H. A., Tanaka, T., Ferrucci, L., & Zonderman, A. B. (2012). Vitamin D receptor and megalin gene polymorphisms and their associations with longitudinal cognitive change in US adults. *Am J Clin Nutr, 95*(1), 163-178. doi: 10.3945/ajcn.111.017137

Bikle, D. (2009). Nonclassic actions of vitamin D. *J Clin Endocrinol Metab, 94*(1), 26-34. doi: 10.1210/jc.2008-1454

Bikle, D. D. (2007). Vitamin D insufficiency/deficiency in gastrointestinal disorders. *J Bone Miner Res, 22 Suppl 2*, V50-54. doi: 10.1359/jbmr.07s208

Bills, V. L., Varet, J., Millar, A., Harper, S. J., Soothill, P. W., & Bates, D. O. (2009). Failure to up-regulate VEGF165b in maternal plasma is a first trimester predictive marker for pre-eclampsia. *Clin Sci (Lond), 116*(3), 265-272. doi: 10.1042/CS20080270

Binkley, N., Gemar, D., Engelke, J., Gangnon, R., Ramamurthy, R., Krueger, D., & Drezner, M. K. (2011). Evaluation of ergocalciferol or cholecalciferol dosing, 1,600 IU daily or 50,000 IU monthly in older adults. *J Clin Endocrinol Metab, 96*(4), 981-988. doi: 10.1210/jc.2010-0015

Birkett, N. J. (1998). Comments on a meta-analysis of the relation between dietary calcium intake and blood pressure. *Am J Epidemiol, 148*(3), 223-228; discussion 232-223.

Bischoff, H. A., Borchers, M., Gudat, F., Duermueller, U., Theiler, R., Stahelin, H. B., & Dick, W. (2001). In situ detection of 1,25-dihydroxyvitamin D3 receptor in human skeletal muscle tissue. *Histochem J, 33*(1), 19-24.

Bischoff, H. A., Stahelin, H. B., Dick, W., Akos, R., Knecht, M., Salis, C., . . . Conzelmann, M. (2003). Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. *J Bone Miner Res, 18*(2), 343-351. doi: 10.1359/jbmr.2003.18.2.343

Bischoff, H. A., Stahelin, H. B., Monsch, A. U., Iversen, M. D., Weyh, A., von Dechend, M., . . . Theiler, R. (2003). Identifying a cut-off point for normal mobility: a comparison of the timed 'up and go' test in community-dwelling and institutionalised elderly women. *Age Ageing, 32*(3), 315-320.

Bischoff-Ferrari, H. A., Borchers, M., Gudat, F., Durmuller, U., Stahelin, H. B., & Dick, W. (2004). Vitamin D receptor expression in human muscle tissue decreases with age. *J Bone Miner Res, 19*(2), 265-269. doi: 10.1359/jbmr.2004.19.2.265

Bischoff-Ferrari, H. A., Dawson-Hughes, B., Willett, W. C., Staehelin, H. B., Bazemore, M. G., Zee, R. Y., & Wong, J. B. (2004). Effect of Vitamin D on falls: a meta-analysis. *JAMA, 291*(16), 1999-2006. doi: 10.1001/jama.291.16.1999

Bischoff-Ferrari, H. A., Giovannucci, E., Willett, W. C., Dietrich, T., & Dawson-Hughes, B. (2006). Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr, 84*(1), 18-28.

Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, Wong JB, Egli A, Kiel DP, Henschkowski J (2009). Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. BMJ;339:b3692. doi:

Bischoff-Ferrari, H. (2009). Vitamin D: what is an adequate vitamin D level and how much supplementation is necessary? *Best Pract Res Clin Rheumatol, 23*(6), 789-795. doi: 10.1016/j.berh.2009.09.005

Bischoff-Ferrari, H. A., Shao, A., Dawson-Hughes, B., Hathcock, J., Giovannucci, E., & Willett, W. C. (2010). Benefit-risk assessment of vitamin D supplementation. *Osteoporos Int, 21*(7), 1121-1132. doi: 10.1007/s00198-009-1119-3

Bischoff-Ferrari, H. A., Dawson-Hughes, B., Stocklin, E., Sidelnikov, E., Willett, W. C., Edel, J. O., . . . Egli, A. (2012). Oral supplementation with 25(OH)D3 versus vitamin D3: effects on 25(OH)D levels, lower extremity function, blood pressure, and markers of innate immunity. *J Bone Miner Res, 27*(1), 160-169. doi: 10.1002/jbmr.551

Bjelakovic, G., Gluud, L. L., Nikolova, D., Whitfield, K., Wetterslev, J., Simonetti, R. G., . . . Gluud, C. (2011). Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database Syst Rev*(7), CD007470. doi: 10.1002/14651858.CD007470.pub2

Bjorkman, M. P., Sorva, A. J., & Tilvis, R. S. (2010). Does elevated parathyroid hormone concentration predict cognitive decline in older people? *Aging Clin Exp Res, 22*(2), 164-169. doi: 10.3275/6626

Bjorn Jensen, C., Thorne-Lyman, A. L., Vadgard Hansen, L., Strom, M., Odgaard Nielsen, N., Cohen, A., & Olsen, S. F. (2013). Development and validation of a vitamin D status prediction model in Danish pregnant women: a study of the Danish National Birth Cohort. *PLoS One, 8*(1), e53059. doi: 10.1371/journal.pone.0053059

Black, L. J., Seamans, K. M., Cashman, K. D., & Kiely, M. (2012). An updated systematic review and meta-analysis of the efficacy of vitamin D food fortification. *J Nutr, 142*(6), 1102-1108. doi: 10.3945/jn.112.158014

Bodnar, L. M., Catov, J. M., Simhan, H. N., Holick, M. F., Powers, R. W., & Roberts, J. M. (2007). Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab, 92*(9), 3517-3522. doi: 10.1210/jc.2007-0718

Bohannon, R. W. (2006). Reference values for the timed up and go test: a descriptive meta-analysis. *J Geriatr Phys Ther, 29*(2), 64-68.

Bohr, D. F. (1963). Vascular Smooth Muscle: Dual Effect of Calcium. *Science, 139*(3555), 597-599. doi: 10.1126/science.139.3555.597

Bula, C. M., Huhtakangas, J., Olivera, C., Bishop, J. E., Norman, A. W., & Henry, H. L. (2005). Presence of a truncated form of the vitamin D receptor (VDR) in a strain of VDR-knockout mice. Endocrinology, 146(12), 5581-5586. doi: 10.1210/en.2005-0806

Boland, R. (1986). Role of vitamin D in skeletal muscle function. *Endocr Rev, 7*(4), 434-448.

Boland, R., de Boland, A. R., Marinissen, M. J., Santillan, G., Vazquez, G., & Zanello, S. (1995). Avian muscle cells as targets for the secosteroid hormone 1,25-dihydroxy-vitamin D3. *Mol Cell Endocrinol, 114*(1-2), 1-8.

Bolland MJ & Reid IR (2010). Vitamin D Deficiency and Its Health Consequences in New Zealand. Chapter 30; Vitamin D; Physiology, Molecular Biology and Clinical Applications.

Bolland, M. J., Bacon, C. J., Horne, A. M., Mason, B. H., Ames, R. W., Wang, T. K., . . . Reid, I. R. (2010). Vitamin D insufficiency and health outcomes over 5 y in older women. *Am J Clin Nutr, 91*(1), 82-89. doi: 10.3945/ajcn.2009.28424

Bollerslev, J., Rosen, T., Mollerup, C. L., Nordenstrom, J., Baranowski, M., Franco, C., . . . Group, S. S. (2009). Effect of surgery on cardiovascular risk factors in mild primary hyperparathyroidism. *J Clin Endocrinol Metab, 94*(7), 2255-2261. doi: 10.1210/jc.2008-2742

Bossola, M., Ciciarelli, C., Di Stasio, E., Conte, G. L., Vulpio, C., Luciani, G., & Tazza, L. (2010). Correlates of symptoms of depression and anxiety in chronic hemodialysis patients. *Gen Hosp Psychiatry, 32*(2), 125-131. doi: 10.1016/j.genhosppsych.2009.10.009

Brandenbarg, J., Vrijkotte, T. G., Goedhart, G., & van Eijsden, M. (2012). Maternal early-pregnancy vitamin D status is associated with maternal depressive symptoms in the Amsterdam Born Children and Their Development cohort. *Psychosom Med, 74*(7), 751-757. doi: 10.1097/PSY.0b013e3182639fdb

Breitling LP, Perna L, Müller H, Raum E, Kliegel M, Brenner H (2012). Vitamin D and cognitive functioning in the elderly population in Germany. *Exp Gerontol, 47*(1):122-7.

Brennan, P. J., Greenberg, G., Miall, W. E., & Thompson, S. G. (1982). Seasonal variation in arterial blood pressure. *Br Med J (Clin Res Ed), 285*(6346), 919-923.

Brewer, L. D., Porter, N. M., Kerr, D. S., Landfield, P. W., & Thibault, O. (2006). Chronic 1alpha,25-(OH)2 vitamin D3 treatment reduces Ca2+ -mediated hippocampal biomarkers of aging. *Cell Calcium, 40*(3), 277-286. doi: 10.1016/j.ceca.2006.04.001

Brickman, A. S., Nyby, M. D., Griffiths, R. F., von Hungen, K., & Tuck, M. L. (1993). Racial differences in platelet cytosolic calcium and calcitropic hormones in normotensive subjects. *Am J Hypertens, 6*(1), 46-51.

Brickman, A. S., Nyby, M. D., von Hungen, K., Eggena, P., & Tuck, M. L. (1990). Calcitropic hormones, platelet calcium, and blood pressure in essential hypertension. *Hypertension, 16*(5), 515-522.

Briones, T. L., & Darwish, H. (2012). Vitamin D mitigates age-related cognitive decline through the modulation of pro-inflammatory state and decrease in amyloid burden. *J Neuroinflammation, 9*, 244. doi: 10.1186/1742-2094-9-244

Brock, K., Huang, W. Y., Fraser, D. R., Ke, L., Tseng, M., Stolzenberg-Solomon, R., . . . Graubard, B. (2010). Low vitamin D status is associated with physical inactivity, obesity and low vitamin D intake in a large US sample of healthy middle-aged men and women. *J Steroid Biochem Mol Biol, 121*(1-2), 462-466. doi: 10.1016/j.jsbmb.2010.03.091

Brock, K. E., Graubard, B. I., Fraser, D. R., Weinstein, S. J., Stolzenberg-Solomon, R. Z., Lim, U., . . . Albanes, D. (2010). Predictors of vitamin D biochemical status in a large sample of middle-aged male smokers in Finland. *Eur J Clin Nutr, 64*(3), 280-288. doi: 10.1038/ejcn.2009.137

Brot, C., Jorgensen, N. R., & Sorensen, O. H. (1999). The influence of smoking on vitamin D status and calcium metabolism. *Eur J Clin Nutr, 53*(12), 920-926.

Broulik, P. D., Horky, K., & Pacovsky, V. (1986). Effect of parathyroid hormone on plasma renin activity in humans. *Horm Metab Res, 18*(7), 490-492. doi: 10.1055/s-2007-1012353

Brouwer-Brolsma, E. M., Feskens, E. J., Steegenga, W. T., & de Groot, L. C. (2013). Associations of 25-hydroxyvitamin D with fasting glucose, fasting insulin, dementia and depression in European elderly: the SENECA study. *Eur J Nutr, 52*(3), 917-925. doi: 10.1007/s00394-012-0399-0

Brown, J., Bianco, J. I., McGrath, J. J., & Eyles, D. W. (2003). 1,25-dihydroxyvitamin D3 induces nerve growth factor, promotes neurite outgrowth and inhibits mitosis in embryonic rat hippocampal neurons. Neurosci Lett, 343(2), 139-143.

Brustad, M., Alsaker, E., Engelsen, O., Aksnes, L., & Lund, E. (2004). Vitamin D status of middle-aged women at 65-71 degrees N in relation to dietary intake and exposure to ultraviolet radiation. *Public Health Nutr, 7*(2), 327-335. doi: 10.1079/PHN2003536

Bucher, H. C., Cook, R. J., Guyatt, G. H., Lang, J. D., Cook, D. J., Hatala, R., & Hunt, D. L. (1996). Effects of dietary calcium supplementation on blood pressure. A meta-analysis of randomized controlled trials. *JAMA, 275*(13), 1016-1022.

Buell, J. S., & Dawson-Hughes, B. (2008). Vitamin D and neurocognitive dysfunction: preventing "D"ecline? *Mol Aspects Med, 29*(6), 415-422. doi: 10.1016/j.mam.2008.05.001

Buell JS, Scott TM, Dawson-Hughes B, Dallal GE, Rosenberg IH, Folstein MF, Tucker KL (2009). Vitamin D is associated with cognitive function in elders receiving home health services. *J Gerontol A Biol Sci Med Sci, 64*(8):888-95.

Buell, J. S., Dawson-Hughes, B., Scott, T. M., Weiner, D. E., Dallal, G. E., Qui, W. Q., . . . Tucker, K. L. (2010). 25-Hydroxyvitamin D, dementia, and cerebrovascular pathology in elders receiving home services. *Neurology, 74*(1), 18-26. doi: 10.1212/WNL.0b013e3181beecb7

Bukoski, R. D., Li, J., & Bo, J. (1993). Effect of long-term administration of 1,25 (OH)2 vitamin D3 on blood pressure and resistance artery contractility in the spontaneously hypertensive rat. *Am J Hypertens, 6*(11 Pt 1), 944-950.

Bukoski, R. D., Wang, D. B., & Wagman, D. W. (1990). Injection of 1,25-(OH)2 vitamin D3 enhances resistance artery contractile properties. *Hypertension, 16*(5), 523-531.

Bukoski, R. D., Xue, H., & McCarron, D. A. (1987). Effect of 1,25(OH)2 vitamin D3 and ionized Ca2+ on 45Ca uptake by primary cultures of aortic myocytes of spontaneously hypertensive and Wistar Kyoto normotensive rats. *Biochem Biophys Res Commun, 146*(3), 1330-1335.

Bula CM, Huhtakangas J, Olivera C, Bishop JE, Norman AW, Henry HL. Presence of a truncated form of the vitamin D receptor (VDR) in a strain of VDR-knockout mice. Endocrinology, 146(12):5581-6.

Burgaz, A., Byberg, L., Rautiainen, S., Orsini, N., Hakansson, N., Arnlov, J., . . . Wolk, A. (2011). Confirmed hypertension and plasma 25(OH)D concentrations amongst elderly men. *J Intern Med, 269*(2), 211-218. doi: 10.1111/j.1365-2796.2010.02309.x

Burgaz, A., Orsini, N., Larsson, S. C., & Wolk, A. (2011). Blood 25-hydroxyvitamin D concentration and hypertension: a meta-analysis. *J Hypertens, 29*(4), 636-645. doi: 10.1097/HJH.0b013e32834320f9

Burgess, E. D., Hawkins, R. G., & Watanabe, M. (1990). Interaction of 1,25-dihydroxyvitamin D and plasma renin activity in high renin essential hypertension. *Am J Hypertens, 3*(12 Pt 1), 903-905.

Burne, T. H., Becker, A., Brown, J., Eyles, D. W., Mackay-Sim, A., & McGrath, J. J. (2004). Transient prenatal Vitamin D deficiency is associated with hyperlocomotion in adult rats. Behav Brain Res, 154(2), 549-555. doi: 10.1016/j.bbr.2004.03.023

Calvo, M. S., & Park, Y. K. (1996). Changing phosphorus content of the U.S. diet: potential for adverse effects on bone. *J Nutr, 126*(4 Suppl), 1168S-1180S.

Calvo M. S, Whiting SJ, Barton CN (2004). Vitamin D fortification in the United States and Canada: current status and data needs. *Am J Clin Nutr, 80*(6 Suppl):1710S-6S.

Calvo M. S, Whiting SJ, Barton CN (2005). Vitamin D intake: a global perspective of current status. *J Nutr, 135*(2):310-6.

Cameron, I. D., Gillespie, L. D., Robertson, M. C., Murray, G. R., Hill, K. D., Cumming, R. G., & Kerse, N. (2012). Interventions for preventing falls in older people in care facilities and hospitals. *Cochrane Database Syst Rev, 12*, CD005465. doi: 10.1002/14651858.CD005465.pub3

Cardus, A., Panizo, S., Encinas, M., Dolcet, X., Gallego, C., Aldea, M., . . . Valdivielso, J. M. (2009). 1,25-dihydroxyvitamin D3 regulates VEGF production through a vitamin D response element in the VEGF promoter. *Atherosclerosis, 204*(1), 85-89. doi: 10.1016/j.atherosclerosis.2008.08.020

Caro, Y., Negron, V., & Palacios, C. (2012). Association between vitamin D levels and blood pressure in a group of Puerto Ricans. *P R Health Sci J, 31*(3), 123-129.

Caron-Jobin, M., Morisset, A. S., Tremblay, A., Huot, C., Legare, D., & Tchernof, A. (2011). Elevated serum 25(OH)D concentrations, vitamin D, and calcium intakes are associated with reduced adipocyte size in women. *Obesity (Silver Spring), 19*(7), 1335-1341. doi: 10.1038/oby.2011.90

Carrara, D., Bernini, M., Bacca, A., Rugani, I., Duranti, E., Virdis, A., . . . Bernini, G. (2013). Cholecalciferol administration blunts the systemic renin-angiotensin system in essential hypertensives with hypovitaminosis D. *J Renin Angiotensin Aldosterone Syst*. doi: 10.1177/1470320312471149

Carthy, E. P., Yamashita, W., Hsu, A., & Ooi, B. S. (1989). 1,25-Dihydroxyvitamin D3 and rat vascular smooth muscle cell growth. *Hypertension, 13*(6 Pt 2), 954-959.

Cashman, K. D., Seamans, K. M., Lucey, A. J., Stocklin, E., Weber, P., Kiely, M., & Hill, T. R. (2012). Relative effectiveness of oral 25-hydroxyvitamin D3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults. Am J Clin Nutr, 95(6), 1350-1356. doi: 10.3945/ajcn.111.031427

Cashman, K. D., Fitzgerald, A. P., Kiely, M., & Seamans, K. M. (2011). A systematic review and meta-regression analysis of the vitamin D intake-serum 25-hydroxyvitamin D relationship to inform European recommendations. *Br J Nutr, 106*(11), 1638-1648. doi: 10.1017/S0007114511005058

Cashman, K. D., Muldowney, S., McNulty, B., Nugent, A., FitzGerald, A. P., Kiely, M., . . . Flynn, A. (2013). Vitamin D status of Irish adults: findings from the National Adult Nutrition Survey. *Br J Nutr, 109*(7), 1248-1256. doi: 10.1017/S0007114512003212

Cass, W. A., Smith, M. P., & Peters, L. E. (2006). Calcitriol protects against the dopamine- and serotonin-depleting effects of neurotoxic doses of methamphetamine. *Ann N Y Acad Sci, 1074*, 261-271. doi: 10.1196/annals.1369.023

Cassidy-Bushrow, A. E., Peters, R. M., Johnson, D. A., Li, J., & Rao, D. S. (2012). Vitamin D nutritional status and antenatal depressive symptoms in African American women. *J Womens Health (Larchmt), 21*(11), 1189-1195. doi: 10.1089/jwh.2012.3528

Ceglia, L. (2008). Vitamin D and skeletal muscle tissue and function. *Mol Aspects Med, 29*(6), 407-414. doi: 10.1016/j.mam.2008.07.002

Ceglia, L., & Harris, S. S. (2013). Vitamin D and its role in skeletal muscle. *Calcif Tissue Int, 92*(2), 151-162. doi: 10.1007/s00223-012-9645-y

Centers for Disease Control (1999). Achievements in public health,1900-1999. Safer and Healthier foods. MMWR Morb Mortal Wkly Rep: 48:905-13.

Cervilla, J. A., & Prince, M. J. (1997). Cognitive impairment and social distress as different pathways to depression in the elderly: a cross-sectional study. *Int J Geriatr Psychiatry, 12*(10), 995-1000.

Chan, R., Chan, D., Woo, J., Ohlsson, C., Mellstrom, D., Kwok, T., & Leung, P. (2011). Association between serum 25-hydroxyvitamin D and psychological health in older Chinese men in a cohort study. *J Affect Disord, 130*(1-2), 251-259. doi: 10.1016/j.jad.2010.10.029

Chan, R., Chan, D., Woo, J., Ohlsson, C., Mellstrom, D., Kwok, T., & Leung, P. (2012). Serum 25-hydroxyvitamin D and parathyroid hormone levels in relation to blood pressure in a cross-sectional study in older Chinese men. *J Hum Hypertens, 26*(1), 20-27. doi: 10.1038/jhh.2010.126

Chao, M. V. (1994). The p75 neurotrophin receptor. *J Neurobiol, 25*(11), 1373-1385. doi: 10.1002/neu.480251106

Chao, M. V., & Hempstead, B. L. (1995). p75 and Trk: a two-receptor system. *Trends Neurosci, 18*(7), 321-326.

Chapuy, M. C., Preziosi, P., Maamer, M., Arnaud, S., Galan, P., Hercberg, S., & Meunier, P. J. (1997). Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int, 7*(5), 439-443.

Chard, P. S., Jordan, J., Marcuccilli, C. J., Miller, R. J., Leiden, J. M., Roos, R. P., & Ghadge, G. D. (1995). Regulation of excitatory transmission at hippocampal synapses by calbindin D28k. *Proc Natl Acad Sci U S A, 92*(11), 5144-5148.

Chen TC, Lu Z, Holick MF. Photobiology of Vitmain D. *Vitamin D :Physiology, Molecular Biology, and Clinical Applications*. 2nd Edition, Humana Press.

Cheng, S., Massaro, J. M., Fox, C. S., Larson, M. G., Keyes, M. J., McCabe, E. L., . . . Wang, T. J. (2010). Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. *Diabetes, 59*(1), 242-248. doi: 10.2337/db09-1011

Cheng, S. T., & Chan, A. C. (2008). Detecting depression in Chinese adults with mild dementia: findings with two versions of the Center for Epidemiologic Studies Depression Scale. *Psychiatry Res, 159*(1-2), 44-49. doi: 10.1016/j.psychres.2007.06.023

Chesney, R. W., & Hedberg, G. (2009). Rickets in lion cubs at the London Zoo in 1889: some new insights. *Pediatrics, 123*(5), e948-950. doi: 10.1542/peds.2008-3504

Cheung, C. L., Lau, K. S., Sham, P. C., Tan, K. C., & Kung, A. W. (2013). Genetic variant in vitamin D binding protein is associated with serum 25-hydroxyvitamin D and vitamin D insufficiency in southern Chinese. *J Hum Genet*. doi: 10.1038/jhg.2013.84

Chiba, Y., Satoh, K., Ueda, S., Kanazawa, N., Tamura, Y., & Horiuchi, T. (2007). Marked improvement of psychiatric symptoms after parathyroidectomy in elderly primary hyperparathyroidism. *Endocr J, 54*(3), 379-383.

Choi, H. S., Oh, H. J., Choi, H., Choi, W. H., Kim, J. G., Kim, K. M., . . . Lim, S. K. (2011). Vitamin D insufficiency in Korea--a greater threat to younger generation: the Korea National Health and Nutrition Examination Survey (KNHANES) 2008. *J Clin Endocrinol Metab, 96*(3), 643-651. doi: 10.1210/jc.2010-2133

Chowdhury, R., Stevens, S., Ward, H., Chowdhury, S., Sajjad, A., & Franco, O. H. (2012). Circulating vitamin D, calcium and risk of cerebrovascular disease: a systematic review and meta-analysis. *Eur J Epidemiol, 27*(8), 581-591. doi: 10.1007/s10654-012-9729-z

Christophersen, N. S., Gronborg, M., Petersen, T. N., Fjord-Larsen, L., Jorgensen, J. R., Juliusson, B., . . . Brundin, P. (2007). Midbrain expression of Delta-like 1 homologue is regulated by GDNF and is associated with dopaminergic differentiation. *Exp Neurol, 204*(2), 791-801. doi: 10.1016/j.expneurol.2007.01.014

Chua, G. T., Chan, Y. C., & Cheng, S. W. (2011). Vitamin D status and peripheral arterial disease: evidence so far. *Vasc Health Risk Manag, 7*, 671-675. doi: 10.2147/VHRM.S24876

Chua, G. T., & Wong, R. Y. (2011). Association Between Vitamin D Dosing Regimen and Fall Prevention in Long-term Care Seniors. *Can Geriatr J, 14*(4), 93-99. doi: 10.57700/cgj.v14i4.23

Chung, M., Balk, E. M., Brendel, M., Ip, S., Lau, J., Lee, J., . . . Trikalinos, T. A. (2009). Vitamin D and calcium: a systematic review of health outcomes. *Evid Rep Technol Assess (Full Rep)*(183), 1-420.

Clemens, T. L., Adams, J. S., Henderson, S. L., & Holick, M. F. (1982). Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. *Lancet, 1*(8263), 74-76.

Coelho, R., Silva, C., Maia, A., Prata, J., & Barros, H. (1999). Bone mineral density and depression: a community study in women. *J Psychosom Res, 46*(1), 29-35.

Compston, J. E., & Horton, L. W. (1978). Oral 25-hydroxyvitain D3 in treatment of osteomalacia associated with ileal resection and cholestyramine therapy. *Gastroenterology, 74*(5 Pt 1), 900-902.

Coon, A. L., Wallace, D. R., Mactutus, C. F., & Booze, R. M. (1999). L-type calcium channels in the hippocampus and cerebellum of Alzheimer's disease brain tissue. *Neurobiol Aging, 20*(6), 597-603.

Cooper, R., & Rotimi, C. (1994). Hypertension in populations of West African origin: is there a genetic predisposition? *J Hypertens, 12*(3), 215-227.

Cornet, A., Baudet, C., Neveu, I., Baron-Van Evercooren, A., Brachet, P., & Naveilhan, P. (1998). 1,25-Dihydroxyvitamin D3 regulates the expression of VDR and NGF gene in Schwann cells in vitro. *J Neurosci Res, 53*(6), 742-746.

Cosenso-Martin LN, Vilela-Martin JF (2011). Is there an association between vitamin D and hypertension? *Recent Pat Cardiovasc Drug Discov*; 6(2):140-7.

Costa, E. M., Blau, H. M., & Feldman, D. (1986). 1,25-dihydroxyvitamin D3 receptors and hormonal responses in cloned human skeletal muscle cells. *Endocrinology, 119*(5), 2214-2220.

Costa, E. M., & Feldman, D. (1986). Homologous up-regulation of the 1,25 (OH)2 vitamin D3 receptor in rats. *Biochem Biophys Res Commun, 137*(2), 742-747.

Cutillas-Marco, E., Fuertes-Prosper, A., Grant, W. B., & Morales-Suarez-Varela, M. (2012). Vitamin D deficiency in South Europe: effect of smoking and aging. *Photodermatol Photoimmunol Photomed, 28*(3), 159-161. doi: 10.1111/j.1600-0781.2012.00649.x

Cutillas-Marco, E., Fuertes-Prosper, A., Grant, W. B., & Morales-Suarez-Varela, M. (2012). Vitamin D deficiency in South Europe: effect of smoking and aging. *Photodermatol Photoimmunol Photomed, 28*(3), 159-161. doi: 10.1111/j.1600-0781.2012.00649.x

Dam, T. T., von Muhlen, D., & Barrett-Connor, E. L. (2009). Sex-specific association of serum vitamin D levels with physical function in older adults. *Osteoporos Int, 20*(5), 751-760. doi: 10.1007/s00198-008-0749-1

Davey, G. K., Spencer, E. A., Appleby, P. N., Allen, N. E., Knox, K. H., & Key, T. J. (2003). EPIC-Oxford: lifestyle characteristics and nutrient intakes in a cohort of 33 883 meat-eaters and 31 546 non meat-eaters in the UK. *Public Health Nutr, 6*(3), 259-269. doi: 10.1079/PHN2002430

Dawson-Hughes, B., Heaney, R. P., Holick, M. F., Lips, P., Meunier, P. J., & Vieth, R. (2005). Estimates of optimal vitamin D status. *Osteoporos Int, 16*(7), 713-716. doi: 10.1007/s00198-005-1867-7

Dawson-Hughes, B. (2012). Serum 25-hydroxyvitamin D and muscle atrophy in the elderly. *Proc Nutr Soc, 71*(1), 46-49. doi: 10.1017/S0029665111003260

Dawson-Hughes, B. (2013). What is the optimal dietary intake of vitamin D for reducing fracture risk? *Calcif Tissue Int, 92*(2), 184-190. doi: 10.1007/s00223-012-9606-5

de Boer, I. H., Ioannou, G. N., Kestenbaum, B., Brunzell, J. D., & Weiss, N. S. (2007). 25-Hydroxyvitamin D levels and albuminuria in the Third National Health and Nutrition Examination Survey (NHANES III). *Am J Kidney Dis, 50*(1), 69-77. doi: 10.1053/j.ajkd.2007.04.015

de Boland, A. R., & Boland, R. (1985). Suppression of 1,25-dihydroxy-vitamin D3-dependent calcium transport by protein synthesis inhibitors and changes in phospholipids in skeletal muscle. *Biochim Biophys Acta, 845*(2), 237-241.

de Boland, A. R., & Boland, R. L. (1987). Rapid changes in skeletal muscle calcium uptake induced in vitro by 1,25-dihydroxyvitamin D3 are suppressed by calcium channel blockers. *Endocrinology, 120*(5), 1858-1864.

De Servi, B., La Porta, C. A., Bontempelli, M., & Comolli, R. (2002). Decrease of TGF-beta1 plasma levels and increase of nitric oxide synthase activity in leukocytes as potential biomarkers of Alzheimer's disease. *Exp Gerontol, 37*(6), 813-821.

de Viragh, P. A., Haglid, K. G., & Celio, M. R. (1989). Parvalbumin increases in the caudate putamen of rats with vitamin D hypervitaminosis. *Proc Natl Acad Sci U S A, 86*(10), 3887-3890.

Dean, A. J., Bellgrove, M. A., Hall, T., Phan, W. M., Eyles, D. W., Kvaskoff, D., & McGrath, J. J. (2011). Effects of vitamin D supplementation on cognitive and emotional functioning in young adults--a randomised controlled trial. *PLoS One, 6*(11), e25966. doi: 10.1371/journal.pone.0025966

Demir, M., Uyan, U., & Melek, M. (2012). The Effects of Vitamin D Deficiency on the Atrial Fibrillation. *Clin Appl Thromb Hemost.* doi: 10.1177/1076029612453762

Demir, M., Gunay, T., Ozmen, G., & Melek, M. (2013). Relationship between vitamin D deficiency and nondipper hypertension. *Clin Exp Hypertens, 35*(1), 45-49. doi: 10.3109/10641963.2012.689045

Dhesi JK, Bearne LM, Moniz C, Hurley MV, Jackson SH, Swift CG, Allain TJ (2002). Neuromuscular and psychomotor function in elderly subjects who fall and the relationship with vitamin D status. *J Bone Miner Res, 17*(5):891-7.

Dhesi, J. K., Jackson, S. H., Bearne, L. M., Moniz, C., Hurley, M. V., Swift, C. G., & Allain, T. J. (2004). Vitamin D supplementation improves neuromuscular function in older people who fall. *Age Ageing, 33*(6), 589-595. doi: 10.1093/ageing/afh209

Dickenson, J. M., & Hill, S. J. (1992). Intracellular calcium measurements in response to histamine stimulation in DDT1 MF-2 cells. *Biochem Soc Trans, 20*(1), 19S.

Dickinson, H. O., Nicolson, D. J., Cook, J. V., Campbell, F., Beyer, F. R., Ford, G. A., & Mason, J. (2006). Calcium supplementation for the management of primary hypertension in adults. *Cochrane Database Syst Rev*(2), CD004639. doi: 10.1002/14651858.CD004639.pub2

Didriksen A, Grimnes G, Hutchinson MS, Kjærgaard M, Svartberg J, Joakimsen RM, Jorde R. The serum 25-hydroxyvitamin D response to vitamin D supplementation is related to genetic factors, body mass index and baseline levels (2013). *Eur J Endocrinol.* [Epub ahead of print]

Drittanti LN, Boland RL, de Boland AR. Induction of specific proteins in cultured skeletal muscle cells by 1,25-dihydroxyvitamin D-3. *Biochim Biophys Acta, 15*;1012(1):16-23

Dolinsky, D. H., Armstrong, S., Mangarelli, C., & Kemper, A. R. (2013). The association between vitamin D and cardiometabolic risk factors in children: a systematic review. *Clin Pediatr (Phila), 52*(3), 210-223. doi: 10.1177/0009922812470742

Dong, Y., Stallmann-Jorgensen, I. S., Pollock, N. K., Harris, R. A., Keeton, D., Huang, Y., . . . Zhu, H. (2010). 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, 95*(10), 4584-4591. doi: 10.1210/jc.2010-0606

Dorheim, M. A., Tracey, W. R., Pollock, J. S., & Grammas, P. (1994). Nitric oxide synthase activity is elevated in brain microvessels in Alzheimer's disease. *Biochem Biophys Res Commun, 205*(1), 659-665. doi: 10.1006/bbrc.1994.2716

Dorjgochoo, T., Ou Shu, X., Xiang, Y. B., Yang, G., Cai, Q., Li, H., . . . Zheng, W. (2012). Circulating 25-hydroxyvitamin D levels in relation to blood pressure parameters and hypertension in the Shanghai Women's and Men's Health Studies. *Br J Nutr, 108*(3), 449-458. doi: 10.1017/S0007114511005745

Do Thi, N. A., Saillour, P., Ferrero, L., Paunio, T., & Mallet, J. (2007). Does neuronal expression of GDNF effectively protect dopaminergic neurons in a rat model of Parkinson's disease? *Gene Ther*, 14(5), 441-450. doi: 10.1038/sj.gt.3302844

Drevets, W. C., Price, J. L., & Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct, 213*(1-2), 93-118. doi: 10.1007/s00429-008-0189-x

Driscoll, R. H., Jr., Meredith, S. C., Sitrin, M., & Rosenberg, I. H. (1982). Vitamin D deficiency and bone disease in patients with Crohn's disease. *Gastroenterology, 83*(6), 1252-1258.

Drittanti, L. N., Boland, R. L., & de Boland, A. R. (1989). Induction of specific proteins in cultured skeletal muscle cells by 1,25-dihydroxyvitamin D-3. *Biochim Biophys Acta, 1012*(1), 16-23.

Dror Y, Giveon SM, Hoshen M, Feldhamer I, Balicer RD, Feldman BS (2013). Vitamin D levels for preventing actue coronary syndrome and mortality:evidence of a nonlinear association. *J Clin Endocrinol Metab 98*(5):2160-7.

Dubois, B., Slachevsky, A., Litvan, I., & Pillon, B. (2000). The FAB: a Frontal Assessment Battery at bedside. *Neurology, 55*(11), 1621-1626.

Dukas, L., Staehelin, H. B., Schacht, E., & Bischoff, H. A. (2005). Better functional mobility in community-dwelling elderly is related to D-hormone serum levels and to daily calcium intake. *J Nutr Health Aging, 9*(5), 347-351.

Dumas, T. C., Powers, E. C., Tarapore, P. E., & Sapolsky, R. M. (2004). Overexpression of calbindin D(28k) in dentate gyrus granule cells alters mossy fiber presynaptic function and impairs hippocampal-dependent memory. *Hippocampus, 14*(6), 701-709. doi: 10.1002/hipo.10210

Dumville, J. C., Miles, J. N., Porthouse, J., Cockayne, S., Saxon, L., & King, C. (2006). Can vitamin D supplementation prevent winter-time blues? A randomised trial among older women. *J Nutr Health Aging, 10*(2), 151-153.

Dunn, P. M. (1998). Francis Glisson (1597-1677) and the "discovery" of rickets. *Arch Dis Child Fetal Neonatal Ed, 78*(2), F154-155.

Dunn, P. M. (1999). Professor Armand Trousseau (1801-67) and the treatment of rickets. *Arch Dis Child Fetal Neonatal Ed, 80*(2), F155-157.

Duprez, D., De Buyzere, M., De Backer, T., & Clement, D. (1993). Relationship between vitamin D and the regional blood flow and vascular resistance in moderate arterial hypertension. *J Hypertens Suppl, 11*(5), S304-305.

Duprez, D., de Buyzere, M., de Backer, T., & Clement, D. (1994). Relationship between vitamin D3 and the peripheral circulation in moderate arterial primary hypertension. *Blood Press, 3*(6), 389-393.

Dursun, E., Gezen-Ak, D., & Yilmazer, S. (2011). A novel perspective for Alzheimer's disease: vitamin D receptor suppression by amyloid-beta and preventing the amyloid-beta induced alterations by vitamin D in cortical neurons. *J Alzheimers Dis, 23*(2), 207-219. doi: 10.3233/JAD-2010-101377

Dursun, E., Gezen-Ak, D., & Yilmazer, S. (2013). Beta amyloid suppresses the expression of the vitamin d receptor gene and induces the expression of the vitamin d catabolic enzyme gene in hippocampal neurons. *Dement Geriatr Cogn Disord, 36*(1-2), 76-86. doi: 10.1159/000350319

Dursun, E., Gezen-Ak, D., & Yilmazer, S. (2013). A New Mechanism for Amyloid-beta Induction of iNOS: Vitamin D-VDR Pathway Disruption. *J Alzheimers Dis, 36*(3), 459-474. doi: 10.3233/JAD-130416

Dursun, S. (2010). Vitamin D for mental health and cognition. *CMAJ, 182*(17), 1886. doi: 10.1503/cmaj.110-2125

Eagles, J. M. (2003). Seasonal affective disorder. *Br J Psychiatry, 182*, 174-176.

Earthman, C. P., Beckman, L. M., Masodkar, K., & Sibley, S. D. (2012). The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications. *Int J Obes (Lond), 36*(3), 387-396. doi: 10.1038/ijo.2011.119

Ebashi, S. (1985). Ca2+ in biological systems. *Experientia, 41*(8), 978-981.

Ebashi, S., & Endo, M. (1968). Calcium ion and muscle contraction. *Prog Biophys Mol Biol, 18*, 123-183.

Ekbom, K., Hed, R., Kirstein, L., & Astroem, K. E. (1964). Weakness of Proximal Limb Muscles, Probably Due to Myopathy after Partial Gastrectomy. Preliminary Report. *Acta Med Scand, 176*, 493-496.

Elamin, M. B., Abu Elnour, N. O., Elamin, K. B., Fatourechi, M. M., Alkatib, A. A., Almandoz, J. P., . . . Montori, V. M. (2011). Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. *J Clin Endocrinol Metab, 96*(7), 1931-1942. doi: 10.1210/jc.2011-0398

Ellsworth-Bowers, E. R., & Corwin, E. J. (2012). Nutrition and the psychoneuroimmunology of postpartum depression. *Nutr Res Rev, 25*(1), 180-192. doi: 10.1017/S0954422412000091

Engelman, C. D., Fingerlin, T. E., Langefeld, C. D., Hicks, P. J., Rich, S. S., Wagenknecht, L. E., . . . Norris, J. M. (2008). Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans. *J Clin Endocrinol Metab, 93*(9), 3381-3388. doi: 10.1210/jc.2007-2702

Engelson O, Brustad M, Asknes L, Lund E (2005). Daily duration of vitamin D synthesis in human skin with relation to latitude, total ozone, altitude, ground cover, aerosols and cloud thickness. *Photochem Photobiol, 81*(6):1287-90.

Ertek, S., Akgul, E., Cicero, A. F., Kutuk, U., Demirtas, S., Cehreli, S., & Erdogan, G. (2012). 25-Hydroxy vitamin D levels and endothelial vasodilator function in normotensive women. *Arch Med Sci, 8*(1), 47-52. doi: 10.5114/aoms.2012.27280

Ertek, S., Francesco Cicero, A., & Erdogan, G. (2011). The relationship between calcium metabolism, insulin-like growth factor-1 and pulse pressure in normotensive, normolipidaemic and non-diabetic patients. *Arch Med Sci, 7*(5), 776-780. doi: 10.5114/aoms.2011.25551

Eskandari F, Martinez PE Martinez PE, Torvik S, Phillips TM, ...Cizza G (2007). Low bone mass in premenopausal women with depression. Arch Int Med, 167:2329-2336.

Eskenazi, B., Fenster, L., & Sidney, S. (1991). A multivariate analysis of risk factors for preeclampsia. *JAMA, 266*(2), 237-241.

Etgen, T., Sander, D., Bickel, H., Sander, K., & Forstl, H. (2012). Vitamin D deficiency, cognitive impairment and dementia: a systematic review and meta-analysis. *Dement Geriatr Cogn Disord, 33*(5), 297-305. doi: 10.1159/000339702

Eyles, D., Brown, J., Mackay-Sim, A., McGrath, J., & Feron, F. (2003). Vitamin D3 and brain development. *Neuroscience, 118*(3), 641-653.

Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ (2005). Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. *J Chem Neuroanat, 29*(1):21-30.

Eyles, D. W., Rogers, F., Buller, K., McGrath, J. J., Ko, P., French, K., & Burne, T. H. (2006). Developmental vitamin D (DVD) deficiency in the rat alters adult behaviour independently of HPA function. *Psychoneuroendocrinology, 31*(8), 958-964. doi: 10.1016/j.psyneuen.2006.05.006

Eyles, D., Almeras, L., Benech, P., Patatian, A., Mackay-Sim, A., McGrath, J., & Feron, F. (2007). Developmental vitamin D deficiency alters the expression of genes encoding mitochondrial, cytoskeletal and synaptic proteins in the adult rat brain. *J Steroid Biochem Mol Biol, 103*(3-5), 538-545. doi: 10.1016/j.jsbmb.2006.12.096

Eyles, D. W., Feron, F., Cui, X., Kesby, J. P., Harms, L. H., Ko, P., . . . Burne, T. H. (2009). Developmental vitamin D deficiency causes abnormal brain development. *Psychoneuroendocrinology, 34 Suppl 1*, S247-257. doi: 10.1016/j.psyneuen.2009.04.015

Eyles, D. W., Burne, T. H., & McGrath, J. J. (2013). Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. *Front Neuroendocrinol, 34*(1), 47-64. doi: 10.1016/j.yfrne.2012.07.001

Fardella, C., & Rodriguez-Portales, J. A. (1995). Intracellular calcium and blood pressure: comparison between primary hyperparathyroidism and essential hypertension. *J Endocrinol Invest, 18*(11), 827-832.

Fazeli, P. K., Mendes, N., Russell, M., Herzog, D. B., Klibanski, A., & Misra, M. (2013). Bone density characteristics and major depressive disorder in adolescents. *Psychosom Med, 75*(2), 117-123. doi: 10.1097/PSY.0b013e3182821e91

FDA (1934). Committee on Foods, American Medical Association. Vitamin D fortified pasteurised milk. J Am Med Assoc:103:1069.

Fernandes de Abreu D.A. , Eyles D., Feron F. (2009). Vitamin D, a neuro-immunomodulator: implications for neurodegenerative and autoimmune diseases. Psychoneuroendocrinology;34S, S265-S277.

Feron, F., Burne, T. H., Brown, J., Smith, E., McGrath, J. J., Mackay-Sim, A., & Eyles, D. W. (2005). Developmental Vitamin D3 deficiency alters the adult rat brain. *Brain Res Bull, 65*(2), 141-148. doi: 10.1016/j.brainresbull.2004.12.007

Fierro, L., & Llano, I. (1996). High endogenous calcium buffering in Purkinje cells from rat cerebellar slices. *J Physiol, 496 (Pt 3)*, 617-625.

Fieser LF, Fieser M. Vitamin D. In: Steroids. 1st ed. New York: Reinhold Publishing Corporation;1959. p. 90-168

Flicker, L., Mead, K., MacInnis, R. J., Nowson, C., Scherer, S., Stein, M. S., . . . Wark, J. D. (2003). Serum vitamin D and falls in older women in residential care in Australia. *J Am Geriatr Soc, 51*(11), 1533-1538.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res, 12*(3), 189-198.

Foo, L. H., Zhang, Q., Zhu, K., Ma, G., Hu, X., Greenfield, H., & Fraser, D. R. (2009). Low vitamin D status has an adverse influence on bone mass, bone turnover, and muscle strength in Chinese adolescent girls. *J Nutr, 139*(5), 1002-1007. doi: 10.3945/jn.108.102053

Foo, L. H., Zhang, Q., Zhu, K., Ma, G., Trube, A., Greenfield, H., & Fraser, D. R. (2009). Relationship between vitamin D status, body composition and physical exercise of adolescent girls in Beijing. *Osteoporos Int, 20*(3), 417-425. doi: 10.1007/s00198-008-0667-2

Forman, J. P., Bischoff-Ferrari, H. A., Willett, W. C., Stampfer, M. J., & Curhan, G. C. (2005). Vitamin D intake and risk of incident hypertension: results from three large prospective cohort studies. *Hypertension, 46*(4), 676-682. doi: 10.1161/01.HYP.0000182662.82666.37

Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, Curhan GC (2007). Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension, 49*(5):1063-9.

Forman JP, Curhan GC, Taylor EN. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension among young women (2008). *Hypertension, 52*(5):828-32.

Forman, J. P., Scott, J. B., Ng, K., Drake, B. F., Suarez, E. G., Hayden, D. L., . . . Chan, A. T. (2013). Effect of vitamin D supplementation on blood pressure in blacks. *Hypertension, 61*(4), 779-785. doi: 10.1161/HYPERTENSIONAHA.111.00659

Forouhi, N. G., Luan, J., Cooper, A., Boucher, B. J., & Wareham, N. J. (2008). Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. *Diabetes, 57*(10), 2619-2625. doi: 10.2337/db08-0593

Forouhi, N. G., Ye, Z., Rickard, A. P., Khaw, K. T., Luben, R., Langenberg, C., & Wareham, N. J. (2012). Circulating 25-hydroxyvitamin D concentration and the risk of type 2 diabetes: results from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort and updated meta-analysis of prospective studies. *Diabetologia, 55*(8), 2173-2182. doi: 10.1007/s00125-012-2544-y

Forrest, K. Y., & Stuhldreher, W. L. (2011). Prevalence and correlates of vitamin D deficiency in US adults. *Nutr Res, 31*(1), 48-54. doi: 10.1016/j.nutres.2010.12.001

Forsythe, L. K., Livingstone, M. B., Barnes, M. S., Horigan, G., McSorley, E. M., Bonham, M. P., . . . Wallace, J. M. (2012). Effect of adiposity on vitamin D status and the 25-hydroxycholecalciferol response to supplementation in healthy young and older Irish adults. *Br J Nutr, 107*(1), 126-134. doi: 10.1017/S0007114511002662

Fraser, A., Williams, D., & Lawlor, D. A. (2010). Associations of serum 25-hydroxyvitamin D, parathyroid hormone and calcium with cardiovascular risk factors: analysis of 3 NHANES cycles (2001-2006). *PLoS One, 5*(11), e13882. doi: 10.1371/journal.pone.0013882

Fraser, W. D., & Milan, A. M. (2013). Vitamin D assays: past and present debates, difficulties, and developments. *Calcif Tissue Int, 92*(2), 118-127. doi: 10.1007/s00223-012-9693-3

Frost, M., Abrahamsen, B., Nielsen, T. L., Hagen, C., Andersen, M., & Brixen, K. (2010). Vitamin D status and PTH in young men: a cross-sectional study on associations with bone mineral density, body composition and glucose metabolism. *Clin Endocrinol (Oxf), 73*(5), 573-580. doi: 10.1111/j.1365-2265.2010.03847.x

Fu, G. K., Lin, D., Zhang, M. Y., Bikle, D. D., Shackleton, C. H., Miller, W. L., & Portale, A. A. (1997). Cloning of human 25-hydroxyvitamin D-1 alpha-hydroxylase and mutations causing vitamin D-dependent rickets type 1. *Mol Endocrinol, 11*(13), 1961-1970.

Fu, L., Yun, F., Oczak, M., Wong, B. Y., Vieth, R., & Cole, D. E. (2009). Common genetic variants of the vitamin D binding protein (DBP) predict differences in response of serum 25-hydroxyvitamin D [25(OH)D] to vitamin D supplementation. *Clin Biochem, 42*(10-11), 1174-1177. doi: 10.1016/j.clinbiochem.2009.03.008

Ganji, V., Milone, C., Cody, M. M., McCarty, F., & Wang, Y. T. (2010). Serum vitamin D concentrations are related to depression in young adult US population: the Third National Health and Nutrition Examination Survey. *Int Arch Med, 3*, 29. doi: 10.1186/1755-7682-3-29

Gannage-Yared, M. H., Chedid, R., Khalife, S., Azzi, E., Zoghbi, F., & Halaby, G. (2009). Vitamin D in relation to metabolic risk factors, insulin sensitivity and adiponectin in a young Middle-Eastern population. *Eur J Endocrinol, 160*(6), 965-971. doi: 10.1530/EJE-08-0952

Ganten, D., Hermann, K., Unger, T., & Lang, R. E. (1983). The tissue renin-angiotensin systems: focus on brain angiotensin, adrenal gland and arterial wall. *Clin Exp Hypertens A, 5*(7-8), 1099-1118.

Garber, A. J. (1983). Effects of parathyroid hormone on skeletal muscle protein and amino acid metabolism in the rat. *J Clin Invest, 71*(6), 1806-1821.

Garcia, V. C., Schuch, N. J., Catania, A. S., Gouvea Ferreira, S. R., & Martini, L. A. (2013). Parathyroid hormone has an important role in blood pressure regulation in vitamin D-insufficient individuals. *Nutrition, 29*(9), 1147-1151. doi: 10.1016/j.nut.2013.03.022

Garcia-Canton, C., Bosch, E., Auyanet, I., Ramirez, A., Rossique, P., Culebras, C., . . . Checa, M. D. (2010). [25 hydroxyvitamin D levels and cardiovascular risk in a cohort of patients with advanced chronic kidney disease]. *Nefrologia, 30*(4), 435-442. doi: 10.3265/Nefrologia.pre2010.Mar.10288

Garcion, E., Thanh, X. D., Bled, F., Teissier, E., Dehouck, M. P., Rigault, F., . . . Darcy, F. (1996). 1,25-Dihydroxyvitamin D3 regulates gamma 1 transpeptidase activity in rat brain. *Neurosci Lett, 216*(3), 183-186.

Garcion, E., Nataf, S., Berod, A., Darcy, F., & Brachet, P. (1997). 1,25-Dihydroxyvitamin D3 inhibits the expression of inducible nitric oxide synthase in rat central nervous system during experimental allergic encephalomyelitis. *Brain Res Mol Brain Res, 45*(2), 255-267.

Garland, C. F., & Garland, F. C. (1980). Do sunlight and vitamin D reduce the likelihood of colon cancer? *Int J Epidemiol, 9*(3), 227-231.

Garland, C. F., Gorham, E. D., Mohr, S. B., & Garland, F. C. (2009). Vitamin D for cancer prevention: global perspective. *Ann Epidemiol, 19*(7), 468-483. doi: 10.1016/j.annepidem.2009.03.021

Garland, C. F., French, C. B., Baggerly, L. L., & Heaney, R. P. (2011). Vitamin D supplement doses and serum 25-hydroxyvitamin D in the range associated with cancer prevention. *Anticancer Res, 31*(2), 607-611.

Gennari, C., Nami, R., Bianchini, C., Pavese, G., Lucani, B., & Perrone, A. F. (1986). Renal excretion of calcium in human hypertension. *Am J Nephrol, 6 Suppl 1*, 124-127.

Gennari, C., Nami, R., & Gonnelli, S. (1995). Hypertension and primary hyperparathyroidism: the role of adrenergic and renin-angiotensin-aldosterone systems. *Miner Electrolyte Metab, 21*(1-3), 77-81.

Gepner, A. D., Ramamurthy, R., Krueger, D. C., Korcarz, C. E., Binkley, N., & Stein, J. H. (2012). A prospective randomized controlled trial of the effects of vitamin D supplementation on cardiovascular disease risk. *PLoS One, 7*(5), e36617. doi: 10.1371/journal.pone.0036617

Gerdhem, P., Ringsberg, K. A., Obrant, K. J., & Akesson, K. (2005). Association between 25-hydroxy vitamin D levels, physical activity, muscle strength and fractures in the prospective population-based OPRA Study of Elderly Women. *Osteoporos Int, 16*(11), 1425-1431. doi: 10.1007/s00198-005-1860-1

Geusens, P., Vandevyver, C., Vanhoof, J., Cassiman, J. J., Boonen, S., & Raus, J. (1997). Quadriceps and grip strength are related to vitamin D receptor genotype in elderly nonobese women. *J Bone Miner Res, 12*(12), 2082-2088. doi: 10.1359/jbmr.1997.12.12.2082

Gezen-Ak, D., Dursun, E., Ertan, T., Hanagasi, H., Gurvit, H., Emre, M., . . . Yilmazer, S. (2007). Association between vitamin D receptor gene polymorphism and Alzheimer's disease. *Tohoku J Exp Med, 212*(3), 275-282.

Gezen-Ak, D., Dursun, E., Bilgic, B., Hanagasi, H., Ertan, T., Gurvit, H., . . . Yilmazer, S. (2012). Vitamin D receptor gene haplotype is associated with late-onset Alzheimer's disease. *Tohoku J Exp Med, 228*(3), 189-196.

Gillespie, L. D., Robertson, M. C., Gillespie, W. J., Sherrington, C., Gates, S., Clemson, L. M., & Lamb, S. E. (2012). Interventions for preventing falls in older people living in the community. *Cochrane Database Syst Rev, 9*, CD007146. doi: 10.1002/14651858.CD007146.pub3

Ginde, A. A., Liu, M. C., & Camargo, C. A., Jr. (2009). Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. *Arch Intern Med, 169*(6), 626-632. doi: 10.1001/archinternmed.2008.604

Giuliani, D. L., & Boland, R. L. (1984). Effects of vitamin D3 metabolites on calcium fluxes in intact chicken skeletal muscle and myoblasts cultured in vitro. *Calcif Tissue Int, 36*(2), 200-205.

Gloth, F. M., 3rd, Alam, W., & Hollis, B. (1999). Vitamin D vs broad spectrum phototherapy in the treatment of seasonal affective disorder. *J Nutr Health Aging, 3*(1), 5-7.

Goel, R. K., & Lal, H. (2011). Role of vitamin d supplementation in hypertension. *Indian J Clin Biochem, 26*(1), 88-90. doi: 10.1007/s12291-010-0092-0

Gonzalez-Gross, M., Valtuena, J., Breidenassel, C., Moreno, L. A., Ferrari, M., Kersting, M., . . . Group, H. S. (2012). Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study. *Br J Nutr, 107*(5), 755-764. doi: 10.1017/S0007114511003527

Gracious, B. L., Finucane, T. L., Friedman-Campbell, M., Messing, S., & Parkhurst, M. N. (2012). Vitamin D deficiency and psychotic features in mentally ill adolescents: a cross-sectional study. *BMC Psychiatry, 12*, 38. doi: 10.1186/1471-244X-12-38

Grant A.M, Avenell, A, Campbell, M. K, McDonald, A.M, MacLennan, G S, McPherson, G C....Wallace, W.A (2005). Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. *Lancet, 365*(9471):1621–1628.

Grant, W. B. (2011). An estimate of the global reduction in mortality rates through doubling vitamin D levels. *Eur J Clin Nutr, 65*(9), 1016-1026. doi: 10.1038/ejcn.2011.68

Greenfield, J. A., Park, P. S., Farahani, E., Malik, S., Vieth, R., McFarlane, N. A., . . . Knight, J. A. (2012). Solar ultraviolet-B radiation and vitamin D: a cross-sectional population-based study using data from the 2007 to 2009 Canadian Health Measures Survey. *BMC Public Health, 12*, 660. doi: 10.1186/1471-2458-12-660

Grey, A., Lucas, J., Horne, A., Gamble, G., Davidson, J. S., & Reid, I. R. (2005). Vitamin D repletion in patients with primary hyperparathyroidism and coexistent vitamin D insufficiency. *J Clin Endocrinol Metab, 90*(4), 2122-2126. doi: 10.1210/jc.2004-1772

Griffin, F. C., Gadegbeku, C. A., & Sowers, M. R. (2011). Vitamin D and subsequent systolic hypertension among women. *Am J Hypertens, 24*(3), 316-321. doi: 10.1038/ajh.2010.226

Griffith, L. E., Guyatt, G. H., Cook, R. J., Bucher, H. C., & Cook, D. J. (1999). The influence of dietary and nondietary calcium supplementation on blood pressure: an updated metaanalysis of randomized controlled trials. *Am J Hypertens, 12*(1 Pt 1), 84-92.

Grobbee, D. E., Hackeng, W. H., Birkenhager, J. C., & Hofman, A. (1988). Raised plasma intact parathyroid hormone concentrations in young people with mildly raised blood pressure. *Br Med J (Clin Res Ed), 296*(6625), 814-816.

Grober, U., Holick, M. F., & Kisters, K. (2011). [Vitamin D and drugs]. *Med Monatsschr Pharm, 34*(10), 377-387.

Grossmann, R. E., & Tangpricha, V. (2010). Evaluation of vehicle substances on vitamin D bioavailability: a systematic review. *Mol Nutr Food Res, 54*(8), 1055-1061. doi: 10.1002/mnfr.200900578

Groves, N. J., Kesby, J. P., Eyles, D. W., McGrath, J. J., Mackay-Sim, A., & Burne, T. H. (2013). Adult vitamin D deficiency leads to behavioural and brain neurochemical alterations in C57BL/6J and BALB/c mice. *Behav Brain Res, 241*, 120-131. doi: 10.1016/j.bbr.2012.12.001

Grubbs, E. G., Rafeeq, S., Jimenez, C., Feng, L., Lee, J. E., Evans, D. B., & Perrier, N. D. (2008). Preoperative vitamin D replacement therapy in primary hyperparathyroidism: safe and beneficial? *Surgery, 144*(6), 852-858; discussion 858-859. doi: 10.1016/j.surg.2008.06.032

Grundberg, E., Brandstrom, H., Ribom, E. L., Ljunggren, O., Mallmin, H., & Kindmark, A. (2004). Genetic variation in the human vitamin D receptor is associated with muscle strength, fat mass and body weight in Swedish women. *Eur J Endocrinol, 150*(3), 323-328.

Giuliani DL, Boland RL (1984). Effects of vitamin D3 metabolites on calcium fluxes in intact chicken skeletal muscle and myoblasts cultured in vitro. *Calcif Tissue Int, 36*(2):200-5

Gu, Y., Schupf, N., Cosentino, S. A., Luchsinger, J. A., & Scarmeas, N. (2012). Nutrient intake and plasma beta-amyloid. *Neurology, 78*(23), 1832-1840. doi: 10.1212/WNL.0b013e318258f7c2

Gupta, A. K., Brashear, M. M., & Johnson, W. D. (2011). Prediabetes and prehypertension in healthy adults are associated with low vitamin D levels. *Diabetes Care, 34*(3), 658-660. doi: 10.2337/dc10-1829

Guy R.A.(1923). The history of cod liver oil as a remedy. Am J Dis Child;26:112-116.*22*(10), 877-882. doi: 10.1016/j.numecd.2012.01.006

Hagenau, T., Vest, R., Gissel, T. N., Poulsen, C. S., Erlandsen, M., Mosekilde, L., & Vestergaard, P. (2009). Global vitamin D levels in relation to age, gender, skin pigmentation and latitude: an ecologic meta-regression analysis. *Osteoporos Int, 20*(1), 133-140. doi: 10.1007/s00198-008-0626-y

Hagiwara H, Furuhashi H, Nakaya K, Nakamura Y. Effects of vitamin D3 and related compounds on angiotensin converting enzyme activity of endothelial cells and on release of plasminogen activator from them (1998). *Chem Pharm Bull (Tokyo), 36*(12):4858-64.

Hansen, A. L., Dahl, L., Bakke, L., & Thayer, J. F. (2011). Vitamin D and executive function: a preliminary report. *Percept Mot Skills, 113*(2), 677-685.

Hariharan, S., Hong, S. Y., Hsu, A., MacCarthy, E. P., Gartside, P. S., & Ool, B. S. (1991). Effect of 1,25-dihydroxyvitamin D3 on mesangial cell proliferation. *J Lab Clin Med, 117*(5), 423-429.

Harms LR, Burne TH, Eyles DW, McGrath JJ (2011). Vitamin D and the brain. *Best Pract Res Clin Endocrinol Metab, 25*(4):657-69.

Harris, R. A., Carnes, D. L., & Forte, L. R. (1981). Reduction of brain calcium after consumption of diets deficient in calcium or vitamin D. J Neurochem, 36(2), 460-466.

Harris, R. A., Pedersen-White, J., Guo, D. H., Stallmann-Jorgensen, I. S., Keeton, D., Huang, Y., . . . Dong, Y. (2011). Vitamin D3 supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. *Am J Hypertens, 24*(5), 557-562. doi: 10.1038/ajh.2011.12

Harris, S., & Dawson-Hughes, B. (1993). Seasonal mood changes in 250 normal women. *Psychiatry Res, 49*(1), 77-87.

Hathcock, J. N., Shao, A., Vieth, R., & Heaney, R. (2007). Risk assessment for vitamin D. *Am J Clin Nutr, 85*(1), 6-18.

Haussler, M. R., Thomson, W. W., & Norman, A. W. (1969). The isolation of purified nuclear and chromatin fractions from chick intestinal mucosa. *Exp Cell Res, 58*(2), 234-242.

Hayes, C. E., Nashold, F. E., Spach, K. M., & Pedersen, L. B. (2003). The immunological functions of the vitamin D endocrine system. *Cell Mol Biol (Noisy-le-grand), 49*(2), 277-300.

He, J. L., & Scragg, R. K. (2011). Vitamin D, parathyroid hormone, and blood pressure in the National Health and Nutrition Examination Surveys. *Am J Hypertens, 24*(8), 911-917. doi: 10.1038/ajh.2011.73

Heaney, R. P. (2003). Long-latency deficiency disease: insights from calcium and vitamin D. *Am J Clin Nutr, 78*(5), 912-919.

Heaney, R. P. (2003). Vitamin D depletion and effective calcium absorption. J Bone Miner Res, 18(7), 1342; author reply 1343. doi: 10.1359/jbmr.2003.18.7.1342

Heaney, R. P. (2005). The Vitamin D requirement in health and disease. *J Steroid Biochem Mol Biol, 97*(1-2), 13-19. doi: 10.1016/j.jsbmb.2005.06.020

Heaney, R. P. (2011). Serum 25-hydroxyvitamin D is a reliable indicator of vitamin D status. *Am J Clin Nutr, 94*(2), 619-620; author reply 620. doi: 10.3945/ajcn.111.019539

Heaney, R. P., & Holick, M. F. (2011). Why the IOM recommendations for vitamin D are deficient. *J Bone Miner Res, 26*(3), 455-457. doi: 10.1002/jbmr.328

Heaney, R. P., Recker, R. R., Grote, J., Horst, R. L., & Armas, L. A. (2011). Vitamin D(3) is more potent than vitamin D(2) in humans. *J Clin Endocrinol Metab, 96*(3), E447-452. doi: 10.1210/jc.2010-2230

Heaney, R. P., Vieth, R., & Hollis, B. W. (2011). Vitamin D efficacy and safety. *Arch Intern Med, 171*(3), 266; author reply 267. doi: 10.1001/archinternmed.2010.528

Hedman, I., Grimby, G., & Tisell, L. E. (1984). Improvement of muscle strength after treatment for hyperparathyroidism. *Acta Chir Scand, 150*(7), 521-524.

Hemming, M. L., & Selkoe, D. J. (2005). Amyloid beta-protein is degraded by cellular angiotensin-converting enzyme (ACE) and elevated by an ACE inhibitor. *J Biol Chem, 280*(45), 37644-37650. doi: 10.1074/jbc.M508460200

Herman, T., Giladi, N., & Hausdorff, J. M. (2011). Properties of the 'timed up and go' test: more than meets the eye. *Gerontology, 57*(3), 203-210. doi: 10.1159/000314963

Herran, A., Amado, J. A., Garcia-Unzueta, M. T., Vazquez-Barquero, J. L., Perera, L., & Gonzalez-Macias, J. (2000). Increased bone remodeling in first-episode major depressive disorder. *Psychosom Med, 62*(6), 779-782.

Hess A. F, Unger LJ (1921). Cure of infantile rickets by sunlight. JAMA 77:39.

Hess, A. F. (1922). The Prevention and Cure of Rickets by Sunlight. *Am J Public Health (N Y), 12*(2), 104-107.

Hess, A. F., Unger, L. J., & Pappenheimer, A. M. (1922). Experimental Rickets in Rats : Vii. The Prevention of Rickets by Sunlight, by the Rays of the Mercury Vapor Lamp, and by the Carbon Arc Lamp. *J Exp Med, 36*(4), 427-446

Hess, A. F. (1924). On the Induction of Antirachitic Properties in Rations by Exposure to Light. *Science, 60*(1551), 269. doi: 10.1126/science.60.1551.269

Hess A. .F, Lewis JM, Rivkin H. Newer aspects of the therapeutics of viosterol (irradiate egosterol (1930). *JAMA*;94:1885.

Hilger, J., Friedel, A., Herr, R., Rausch, T., Roos, F., Wahl, D. A., . . . Hoffmann, K. (2013). A systematic review of vitamin D status in populations worldwide. *Br J Nutr*, 1-23. doi: 10.1017/S0007114513001840

Hill, T. R., Cotter, A. A., Mitchell, S., Boreham, C. A., Dubitzky, W., Murray, L., . . . Cashman, K. D. (2008). Vitamin D status and its determinants in adolescents from the Northern Ireland Young Hearts 2000 cohort. *Br J Nutr, 99*(5), 1061-1067. doi: 10.1017/S0007114507842826

Hill, T. R., Flynn, A., Kiely, M., & Cashman, K. D. (2006). Prevalence of suboptimal vitamin D status in young, adult and elderly Irish subjects. *Ir Med J, 99*(2), 48-49.

Hill, T. R., O'Brien, M. M., Lamberg-Allardt, C., Jakobsen, J., Kiely, M., Flynn, A., & Cashman, K. D. (2006). Vitamin D status of 51-75-year-old Irish women: its determinants and impact on biochemical indices of bone turnover. *Public Health Nutr, 9*(2), 225-233.

Hintzpeter, B., Mensink, G. B., Thierfelder, W., Muller, M. J., & Scheidt-Nave, C. (2008). Vitamin D status and health correlates among German adults. *Eur J Clin Nutr, 62*(9), 1079-1089. doi: 10.1038/sj.ejcn.1602825

Hirani V, Naganathan V, Cumming RG, Blyth F, Le Couteur DG, Handelsman DJ, Waite LM, Seibel MJ (2013). Associations between frailty and serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations in older Australian men: the Concord Health and Ageing in Men Project. *J Gerontol A Biol Sci Med Sci, 68*(9):1112-21. doi:

Hirasawa T, Nakamura T, Mizushima A, Morita M, Ezawa I, Miyakawa H, Kudo Y (2000). Adverse effects of an active fragment of parathyroid hormone on rat hippocampal organotypic cultures. *Br J Pharmacol, 129*(1):21-8.

Ho, L., Pieroni, C., Winger, D., Purohit, D. P., Aisen, P. S., & Pasinetti, G. M. (1999). Regional distribution of cyclooxygenase-2 in the hippocampal formation in Alzheimer's disease. *J Neurosci Res, 57*(3), 295-303.

Hoang, M. T., Defina, L. F., Willis, B. L., Leonard, D. S., Weiner, M. F., & Brown, E. S. (2011). Association between low serum 25-hydroxyvitamin D and depression in a large sample of healthy adults: the Cooper Center longitudinal study. *Mayo Clin Proc, 86*(11), 1050-1055. doi: 10.4065/mcp.2011.0208

Hogberg, G., Gustafsson, S. A., Hallstrom, T., Gustafsson, T., Klawitter, B., & Petersson, M. (2012). Depressed adolescents in a case-series were low in vitamin D and depression was ameliorated by vitamin D supplementation. *Acta Paediatr, 101*(7), 779-783. doi: 10.1111/j.1651-2227.2012.02655.x

Holick, M. F. (1981). The cutaneous photosynthesis of previtamin D3: a unique photoendocrine system. J Invest Dermatol, 77(1), 51-58.

Holick M. F, Matsuoka LY, Wortsman J (1989). Age, vitamin D, and solar ultraviolet. Lancet, ;2(8671):1104-5.

Holick M. F. (1990). Vitamin D and the skin: photobiology, phsyiology and therpeutic efficacy for psoriasis, In: Heersche JNM and Kanis JA (eds) Bone and mineral research. Elsevier, Amsterdam, pp313-366.

Holick, M. F. (2006). Resurrection of vitamin D deficiency and rickets. J Clin Invest, 116(8), 2062-2072. doi: 10.1172/JCI29449

Holick, M. F. (2007). Vitamin D deficiency. *N Engl J Med, 357*(3), 266-281. doi: 10.1056/NEJMra070553

Holick, M. F. (2009). Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol, 19*(2), 73-78. doi: 10.1016/j.annepidem.2007.12.001

Holick, M. F. (2010). The D-lemma: to screen or not to screen for 25-hydroxyvitamin D concentrations. Clin Chem, 56(5), 729-731. doi: 10.1373/clinchem.2009.139253

Holick, M. F. (2011). The D-batable Institute of Medicine report: a D-lightful perspective. *Endocr Pract, 17*(1), 143-149.

Holick, M. F. (2011). The IOM D-lemma. *Public Health Nutr, 14*(5), 939-941. doi: 10.1017/S1368980011000590

Holick, M. F. (2012). Evidence-based D-bate on health benefits of vitamin D revisited. *Dermatoendocrinol, 4*(2), 183-190. doi: 10.4161/derm.20015

Hollis B. W (1984). Comparison of equilibrium and disequilibrium assay conditions for ergocalciferol, cholecalciferol and their major metabolites. *J Steroid Biochem, 21*(1):81-6.

Holmberg, I., Berlin, T., Ewerth, S., & Bjorkhem, I. (1986). 25-Hydroxylase activity in subcellular fractions from human liver. Evidence for different rates of mitochondrial hydroxylation of vitamin D2 and D3. *Scand J Clin Lab Invest, 46*(8), 785-790. doi: 10.3109/00365518609084051

Hoogendijk, W. J., Lips, P., Dik, M. G., Deeg, D. J., Beekman, A. T., & Penninx, B. W. (2008). Depression is associated with decreased 25-hydroxyvitamin D and increased parathyroid hormone levels in older adults. *Arch Gen Psychiatry, 65*(5), 508-512. doi: 10.1001/archpsyc.65.5.508

Hoogendoorn EH, White KE, Econs MJ, Hermus AR. Hypophosphatemia, osteomalacia and proximal muscle weakness treated by surgery (2003). *Clin Endocrinol (Oxf), 58*(6):796-7.

Horst, R. L., Reinhardt, T. A., Ramberg, C. F., Koszewski, N. J., & Napoli, J. L. (1986). 24-Hydroxylation of 1,25-dihydroxyergocalciferol. An unambiguous deactivation process. *J Biol Chem, 261*(20), 9250-9256.

Hosseinpour, F., Ellfolk, M., Norlin, M., & Wikvall, K. (2007). Phenobarbital suppresses vitamin D3 25-hydroxylase expression: a potential new mechanism for drug-induced osteomalacia. *Biochem Biophys Res Commun, 357*(3), 603-607. doi: 10.1016/j.bbrc.2007.03.177

Houghton, L. A., & Vieth, R. (2006). The case against ergocalciferol (vitamin D2) as a vitamin supplement. *Am J Clin Nutr, 84*(4), 694-697.

Houston, D. K., Tooze, J. A., Neiberg, R. H., Hausman, D. B., Johnson, M. A., Cauley, J. A., . . . Health, A. B. C. S. (2012). 25-hydroxyvitamin D status and change in physical performance and strength in older adults: the Health, Aging, and Body Composition Study. *Am J Epidemiol, 176*(11), 1025-1034. doi: 10.1093/aje/kws147

Hyppönen E, Power C (2007). Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr ;85*(3):860-8.

Hu, J., Igarashi, A., Kamata, M., & Nakagawa, H. (2001). Angiotensin-converting enzyme degrades Alzheimer amyloid beta-peptide (A beta); retards A beta aggregation, deposition, fibril formation; and inhibits cytotoxicity. *J Biol Chem, 276*(51), 47863-47868. doi: 10.1074/jbc.M104068200

Huldschinsky K (1919). Curing Rickets by atrtifical UV-radiation. Heiling von Rachitid durch kunstliche Honensonne. Dtsch Med Wochenschr 45:712-713.

Hulter, H. N., Melby, J. C., Peterson, J. C., & Cooke, C. R. (1986). Chronic continuous PTH infusion results in hypertension in normal subjects. *J Clin Hypertens, 2*(4), 360-370.

Hvarfner, A., Bergstrom, R., Morlin, C., Wide, L., & Ljunghall, S. (1987). Relationships between calcium metabolic indices and blood pressure in patients with essential hypertension as compared with a healthy population. *J Hypertens, 5*(4), 451-456.

Hypponen, E., Boucher, B. J., Berry, D. J., & Power, C. (2008). 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. *Diabetes, 57*(2), 298-305. doi: 10.2337/db07-1122

Ibi, M., Sawada, H., Nakanishi, M., Kume, T., Katsuki, H., Kaneko, S., . . . Akaike, A. (2001). Protective effects of 1 alpha,25-(OH)(2)D(3) against the neurotoxicity of glutamate and reactive oxygen species in mesencephalic culture. *Neuropharmacology, 40*(6), 761-771.

Institute of Medicine (2011). Dietary Reference Intakes for Calcium and Vitamin D. The National Academies Pres: Washington DC:2011

Ito, M., Koyama, H., Ohshige, A., Maeda, T., Yoshimura, T., & Okamura, H. (1994). Prevention of preeclampsia with calcium supplementation and vitamin D3 in an antenatal protocol. *Int J Gynaecol Obstet, 47*(2), 115-120.

Itzhaky, D., Amital, D., Gorden, K., Bogomolni, A., Arnson, Y., & Amital, H. (2012). Low serum vitamin D concentrations in patients with schizophrenia. *Isr Med Assoc J, 14*(2), 88-92.

Jablonski, K. L., Chonchol, M., Pierce, G. L., Walker, A. E., & Seals, D. R. (2011). 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. *Hypertension, 57*(1), 63-69. doi: 10.1161/HYPERTENSIONAHA.110.160929

Jacka, F. N., Pasco, J. A., Henry, M. J., Kotowicz, M. A., Dodd, S., Nicholson, G. C., & Berk, M. (2005). Depression and bone mineral density in a community sample of perimenopausal women: Geelong Osteoporosis Study. *Menopause, 12*(1), 88-91.

Jackson, C., Gaugris, S., Sen, S. S., & Hosking, D. (2007). The effect of cholecalciferol (vitamin D3) on the risk of fall and fracture: a meta-analysis. *QJM, 100*(4), 185-192. doi: 10.1093/qjmed/hcm005

Jaddou, H. Y., Batieha, A. M., Khader, Y. S., Kanaan, S. H., El-Khateeb, M. S., & Ajlouni, K. M. (2012). Depression is associated with low levels of 25-hydroxyvitamin D among Jordanian adults: results from a national population survey. *Eur Arch Psychiatry Clin Neurosci, 262*(4), 321-327. doi: 10.1007/s00406-011-0265-8

Janssen, H. C., Emmelot-Vonk, M. H., Verhaar, H. J., & van der Schouw, Y. T. (2013). Vitamin d and muscle function: is there a threshold in the relation? *J Am Med Dir Assoc, 14*(8), 627 e613-628. doi: 10.1016/j.jamda.2013.05.012

Jespersen, B., Randlov, A., Abrahamsen, J., Fogh-Andersen, N., Olsen, N. V., & Kanstrup, I. L. (1998). Acute cardiovascular effect of 1,25-dihydroxycholecalciferol in essential hypertension. *Am J Hypertens, 11*(6 Pt 1), 659-666.

Jia, Z., & Nemere, I. (1999). Immunochemical studies on the putative plasmalemmal receptor for 1,25-dihydroxyvitamin D3 II. Chick kidney and brain. *Steroids, 64*(8), 541-550.

Jiang, P., Zhang, W. Y., Li, H. D., Cai, H. L., Liu, Y. P., & Chen, L. Y. (2013). Stress and vitamin D: Altered vitamin D metabolism in both the hippocampus and myocardium of chronic unpredictable mild stress exposed rats. *Psychoneuroendocrinology*. doi: 10.1016/j.psyneuen.2013.03.017

Joborn, C., Hetta, J., Niklasson, F., Rastad, J., Wide, L., Agren, H., . . . Ljunghall, S. (1991). Cerebrospinal fluid calcium, parathyroid hormone, and monoamine and purine metabolites and the blood-brain barrier function in primary hyperparathyroidism. *Psychoneuroendocrinology, 16*(4), 311-322.

Joffres, M. R., Reed, D. M., & Yano, K. (1987). Relationship of magnesium intake and other dietary factors to blood pressure: the Honolulu heart study. *Am J Clin Nutr, 45*(2), 469-475.

Jorde, R., & Bonaa, K. H. (2000). Calcium from dairy products, vitamin D intake, and blood pressure: the Tromso Study. *Am J Clin Nutr, 71*(6), 1530-1535.

Jorde, R., Sundsfjord, J., Haug, E., & Bonaa, K. H. (2000). Relation between low calcium intake, parathyroid hormone, and blood pressure. *Hypertension, 35*(5), 1154-1159.

Jorde R, Svartberg J, Sundsfjord J. Serum parathyroid hormone as a predictor of increase in systolic blood pressure in men (2005). *J Hypertens, 23*(9):1639-44.

Jorde, R., Waterloo, K., Saleh, F., Haug, E., & Svartberg, J. (2006). Neuropsychological function in relation to serum parathyroid hormone and serum 25-hydroxyvitamin D levels. The Tromso study. *J Neurol, 253*(4), 464-470. doi: 10.1007/s00415-005-0027-5

Jorde, R., Sneve, M., Figenschau, Y., Svartberg, J., & Waterloo, K. (2008). Effects of vitamin D supplementation on symptoms of depression in overweight and obese subjects: randomized double blind trial. *J Intern Med, 264*(6), 599-609. doi: 10.1111/j.1365-2796.2008.02008.x

Jorde, R., Figenschau, Y., Emaus, N., Hutchinson, M., & Grimnes, G. (2010). Serum 25-hydroxyvitamin D levels are strongly related to systolic blood pressure but do not predict future hypertension. *Hypertension, 55*(3), 792-798. doi: 10.1161/HYPERTENSIONAHA.109.143990

Jorde, R., Sneve, M., Emaus, N., Figenschau, Y., & Grimnes, G. (2010). Cross-sectional and longitudinal relation between serum 25-hydroxyvitamin D and body mass index: the Tromso study. *Eur J Nutr, 49*(7), 401-407. doi: 10.1007/s00394-010-0098-7

Jorde, R., Sneve, M., Hutchinson, M., Emaus, N., Figenschau, Y., & Grimnes, G. (2010). Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study. *Am J Epidemiol, 171*(8), 903-908. doi: 10.1093/aje/kwq005

Jorde, R., Sneve, M., Torjesen, P., & Figenschau, Y. (2010). No improvement in cardiovascular risk factors in overweight and obese subjects after supplementation with vitamin D3 for 1 year. *J Intern Med, 267*(5), 462-472. doi: 10.1111/j.1365-2796.2009.02181.x

Jorde, R., & Grimnes, G. (2011). Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. *Prog Lipid Res, 50*(4), 303-312. doi: 10.1016/j.plipres.2011.05.001

Jorgensen, S. P., Hvas, C. L., Agnholt, J., Christensen, L. A., Heickendorff, L., & Dahlerup, J. F. (2013). Active Crohn's disease is associated with low vitamin D levels. *J Crohns Colitis*. doi: 10.1016/j.crohns.2013.01.012

Jouvenceau, A., Potier, B., Poindessous-Jazat, F., Dutar, P., Slama, A., Epelbaum, J., & Billard, J. M. (2002). Decrease in calbindin content significantly alters LTP but not NMDA receptor and calcium channel properties. *Neuropharmacology, 42*(4), 444-458.

Judd, S. E., Nanes, M. S., Ziegler, T. R., Wilson, P. W., & Tangpricha, V. (2008). Optimal vitamin D status attenuates the age-associated increase in systolic blood pressure in white Americans: results from the third National Health and Nutrition Examination Survey. *Am J Clin Nutr, 87*(1), 136-141.

Judd S. E, Raiser S. N, Kumari M, Tangpricha V. 1,25-dihydroxyvitamin D3 reduces systolic blood pressure in hypertensive adults: a pilot feasibility study (2010). *J Steroid Biochem Mol Biol. 121*(1-2):445-7.

Jungert, A., Roth, H. J., & Neuhauser-Berthold, M. (2012). Serum 25-hydroxyvitamin D3, parathyroid hormone and blood pressure in an elderly cohort from Germany: a cross-sectional study. *Nutr Metab (Lond), 9*(1), 20. doi: 10.1186/1743-7075-9-20

Juzeniene, A., & Moan, J. (2012). Beneficial effects of UV radiation other than via vitamin D production. *Dermatoendocrinol, 4*(2), 109-117. doi: 10.4161/derm.20013

Kalueff, A. V., Lou, Y. R., Laaksi, I., & Tuohimaa, P. (2004). Increased anxiety in mice lacking vitamin D receptor gene. Neuroreport, 15(8), 1271-1274.

Kalueff, A. V., Minasyan, A., Keisala, T., Kuuslahti, M., Miettinen, S., & Tuohimaa, P. (2006). The vitamin D neuroendocrine system as a target for novel neurotropic drugs. CNS Neurol Disord Drug Targets, 5(3), 363-371.

Kalsbeek, A., van Heerikhuize, J. J., Wortel, J., & Buijs, R. M. (1998). Restricted daytime feeding modifies suprachiasmatic nucleus vasopressin release in rats. *J Biol Rhythms, 13*(1), 18-29.

Kalyani, R. R., Stein, B., Valiyil, R., Manno, R., Maynard, J. W., & Crews, D. C. (2010). Vitamin D treatment for the prevention of falls in older adults: systematic review and meta-analysis. *J Am Geriatr Soc, 58*(7), 1299-1310. doi: 10.1111/j.1532-5415.2010.02949.x

Kang, H., & Schuman, E. M. (1995). Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science, 267(5204), 1658-1662.

Kasahara, A. K., Singh, R. J., & Noymer, A. (2013). Vitamin D (25OHD) Serum Seasonality in the United States. *PLoS One, 8*(6), e65785. doi: 10.1371/journal.pone.0065785

Kassowitz M (1897). Tetanie und autointoxication im kindersalter. Wien Med Presse;97:139.

Kawashima, H. (1988). 1,25-Dihydroxyvitamin D3 stimulates Ca-ATPase in a vascular smooth muscle cell line. *Biochem Biophys Res Commun, 150*(3), 1138-1143.

Ke, L., Graubard, B. I., Albanes, D., Fraser, D. R., Weinstein, S. J., Virtamo, J., & Brock, K. E. (2013). Hypertension, pulse, and other cardiovascular risk factors and vitamin d status in finnish men. *Am J Hypertens, 26*(8), 951-956. doi: 10.1093/ajh/hpt051

Keisala, T., Minasyan, A., Jarvelin, U., Wang, J., Hamalainen, T., Kalueff, A. V., & Tuohimaa, P. (2007). Aberrant nest building and prolactin secretion in vitamin D receptor mutant mice. J Steroid Biochem Mol Biol, 104(3-5), 269-273. doi: 10.1016/j.jsbmb.2007.03.031

Kelleher, C. C., Harrington, J., & Friel, S. (2002). Measures of self-reported morbidity according to age, gender and general medical services eligibility in the national survey of lifestyles, attitudes and nutrition. *Ir J Med Sci, 171*(3), 134-138.

Kenny AM, Biskup B, Biskup B, Robbins B, Marcella G, Burleson JA (2003). Effects of vitamin D supplementation on strength, physical function, and health perception in older, community dwelling men. *J Am Geriatr Soc, 51*(12):1762-7.

Khoraminya, N., Tehrani-Doost, M., Jazayeri, S., Hosseini, A., & Djazayery, A. (2013). Therapeutic effects of vitamin D as adjunctive therapy to fluoxetine in patients with major depressive disorder. *Aust N Z J Psychiatry, 47*(3), 271-275. doi: 10.1177/0004867412465022

Kim, M. K., Il Kang, M., Won Oh, K., Kwon, H. S., Lee, J. H., Lee, W. C., . . . Son, H. Y. (2010). The association of serum vitamin D level with presence of metabolic syndrome and hypertension in middle-aged Korean subjects. *Clin Endocrinol (Oxf), 73*(3), 330-338. doi: 10.1111/j.1365-2265.2010.03798.x

Kimball, S. M., Ursell, M. R., O'Connor, P., & Vieth, R. (2007). Safety of vitamin D3 in adults with multiple sclerosis. *Am J Clin Nutr, 86*(3), 645-651.

Kimura, M., Sawada, K., Miyagawa, T., Kuwada, M., Katayama, K., & Nishizawa, Y. (1998). Role of glutamate receptors and voltage-dependent calcium and sodium channels in the extracellular glutamate/aspartate accumulation and subsequent neuronal injury induced by oxygen/glucose deprivation in cultured hippocampal neurons. *J Pharmacol Exp Ther, 285*(1), 178-185.

Kishimoto, H., Tsumura, K., Fujioka, S., Uchimoto, S., & Morii, H. (1993). Chronic parathyroid hormone administration reverses the antihypertensive effect of calcium loading in young spontaneously hypertensive rats. *Am J Hypertens, 6*(3 Pt 1), 234-240.

Kjaergaard, M., Joakimsen, R., & Jorde, R. (2011). Low serum 25-hydroxyvitamin D levels are associated with depression in an adult Norwegian population. *Psychiatry Res, 190*(2-3), 221-225. doi: 10.1016/j.psychres.2011.06.024

Kjaergaard, M., Waterloo, K., Wang, C. E., Almas, B., Figenschau, Y., Hutchinson, M. S., . . . Jorde, R. (2012). Effect of vitamin D supplement on depression scores in people with low levels of serum 25-hydroxyvitamin D: nested case-control study and randomised clinical trial. *Br J Psychiatry, 201*(5), 360-368. doi: 10.1192/bjp.bp.111.104349

Klenk, J., Rapp, K., Denkinger, M. D., Nagel, G., Nikolaus, T., Peter, R., . . . Rothenbacher, D. (2013). Seasonality of vitamin D status in older people in Southern Germany: implications for assessment. *Age Ageing, 42*(3), 404-408. doi: 10.1093/ageing/aft042

Knippenberg, S., Bol, Y., Damoiseaux, J., Hupperts, R., & Smolders, J. (2011). Vitamin D status in patients with MS is negatively correlated with depression, but not with fatigue. *Acta Neurol Scand, 124*(3), 171-175. doi: 10.1111/j.1600-0404.2010.01447.x

Kokot F, Pietrek J, Srokowska S, Wartenberg W, Kuska J, Jedrychowska M, Duda G, Zielińska K, Wartenberg Z, Kuźmiak M (1981). 25-hydroxyvitamin D in patients with essential hypertension. *Clin Nephrol, 16*(4):188-92.

Kolsch, H., Jessen, F., Freymann, N., Kreis, M., Hentschel, F., Maier, W., & Heun, R. (2005). ACE I/D polymorphism is a risk factor of Alzheimer's disease but not of vascular dementia. *Neurosci Lett, 377*(1), 37-39. doi: 10.1016/j.neulet.2004.11.062

Komuro, H., & Rakic, P. (1996). Intracellular Ca2+ fluctuations modulate the rate of neuronal migration. *Neuron, 17*(2), 275-285.

Kong, J., Qiao, G., Zhang, Z., Liu, S. Q., & Li, Y. C. (2008). Targeted vitamin D receptor expression in juxtaglomerular cells suppresses renin expression independent of parathyroid hormone and calcium. *Kidney Int, 74*(12), 1577-1581. doi: 10.1038/ki.2008.452

Kong, J., Kim, G. H., Wei, M., Sun, T., Li, G., Liu, S. Q., . . . Li, Y. C. (2010). Therapeutic effects of vitamin D analogs on cardiac hypertrophy in spontaneously hypertensive rats. *Am J Pathol, 177*(2), 622-631. doi: 10.2353/ajpath.2010.091292

Korsching, S., Auburger, G., Heumann, R., Scott, J., & Thoenen, H. (1985). Levels of nerve growth factor and its mRNA in the central nervous system of the rat correlate with cholinergic innervation. *EMBO J, 4*(6), 1389-1393.

Kota, S. K., Kota, S. K., Jammula, S., Meher, L. K., Panda, S., Tripathy, P. R., & Modi, K. D. (2011). Renin-angiotensin system activity in vitamin D deficient, obese individuals with hypertension: An urban Indian study. *Indian J Endocrinol Metab, 15 Suppl 4*, S395-401. doi: 10.4103/2230-8210.86985

Krause, R., Buhring, M., Hopfenmuller, W., Holick, M. F., & Sharma, A. M. (1998). Ultraviolet B and blood pressure. *Lancet, 352*(9129), 709-710. doi: 10.1016/S0140-6736(05)60827-6

Kremer, R., Campbell, P. P., Reinhardt, T., & Gilsanz, V. (2009). Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. *J Clin Endocrinol Metab, 94*(1), 67-73. doi: 10.1210/jc.2008-1575

Kristal-Boneh, E., Harari, G., Green, M. S., & Ribak, J. (1996). Summer-winter variation in 24 h ambulatory blood pressure. *Blood Press Monit, 1*(2), 87-94.

Kristoffersson, A., Bostrom, A., & Soderberg, T. (1992). Muscle strength is improved after parathyroidectomy in patients with primary hyperparathyroidism. *Br J Surg, 79*(2), 165-168.

Kruger, I. M., Kruger, M. C., Doak, C. M., Schutte, A. E., Huisman, H. W., Van Rooyen, J. M., . . . Kruger, A. (2013). The association of 25(OH)D with blood pressure, pulse pressure and carotid-radial pulse wave velocity in African women. *PLoS One, 8*(1), e54554. doi: 10.1371/journal.pone.0054554

Kuhlmann, A., Haas, C. S., Gross, M. L., Reulbach, U., Holzinger, M., Schwarz, U., . . . Amann, K. (2004). 1,25-Dihydroxyvitamin D3 decreases podocyte loss and podocyte hypertrophy in the subtotally nephrectomized rat. *Am J Physiol Renal Physiol, 286*(3), F526-533. doi: 10.1152/ajprenal.00316.2003

Kulah, E., Dursun, A., Aktunc, E., Acikgoz, S., Aydin, M., Can, M., & Dursun, A. (2007). Effects of angiotensin-converting enzyme gene polymorphism and serum vitamin D levels on ambulatory blood pressure measurement and left ventricular mass in Turkish hypertensive population. *Blood Press Monit, 12*(4), 207-213. doi: 10.1097/MBP.0b013e32813fa371

Kumar, J., Muntner, P., Kaskel, F. J., Hailpern, S. M., & Melamed, M. L. (2009). Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001-2004. *Pediatrics, 124*(3), e362-370. doi: 10.1542/peds.2009-0051

Kuningas, M., Mooijaart, S. P., Jolles, J., Slagboom, P. E., Westendorp, R. G., & van Heemst, D. (2009). VDR gene variants associate with cognitive function and depressive symptoms in old age. *Neurobiol Aging, 30*(3), 466-473. doi: 10.1016/j.neurobiolaging.2007.07.001

Kunutsor, S. K., Apekey, T. A., & Steur, M. (2013). Vitamin D and risk of future hypertension: meta-analysis of 283,537 participants. *Eur J Epidemiol, 28*(3), 205-221. doi: 10.1007/s10654-013-9790-2

Kurtz, T. W., Portale, A. A., & Morris, R. C., Jr. (1986). Evidence for a difference in vitamin D metabolism between spontaneously hypertensive and Wistar-Kyoto rats. *Hypertension, 8*(11), 1015-1020.

Kwasky, A. N., & Groh, C. J. (2012). Vitamin D and depression: is there a relationship in young women? *J Am Psychiatr Nurses Assoc, 18*(4), 236-243. doi: 10.1177/1078390312452451

Laird, E., Ward, M., McSorley, E., Strain, J. J., & Wallace, J. (2010). Vitamin D and bone health: potential mechanisms. *Nutrients, 2*(7), 693-724. doi: 10.3390/nu2070693

Lasaite L, Gailyte I, Puzinas P, Preiska RT, Kazanavicius G (2011). Vitamin D deficiency is related to worse emotional state, *Cent Eur Jr Med, 6*(5); 558-566

Lambert, G. W., Reid, C., Kaye, D. M., Jennings, G. L., & Esler, M. D. (2002). Effect of sunlight and season on serotonin turnover in the brain. *Lancet, 360*(9348), 1840-1842.

Lansdowne, A. T., & Provost, S. C. (1998). Vitamin D3 enhances mood in healthy subjects during winter. *Psychopharmacology (Berl), 135*(4), 319-323.

Lardner, E., Fitzgibbon, M., Wilson, S., Griffin, D., & Mulkerrin, E. (2011). Hypovitaminosis D in a healthy female population, aged from 40 to 85 years, in the west of Ireland. *Ir J Med Sci, 180*(1), 115-119. doi: 10.1007/s11845-010-0550-y

Larsen, T., Mose, F. H., Bech, J. N., Hansen, A. B., & Pedersen, E. B. (2012). Effect of cholecalciferol supplementation during winter months in patients with hypertension: a randomized, placebo-controlled trial. *Am J Hypertens, 25*(11), 1215-1222. doi: 10.1038/ajh.2012.111

Lauridsen, A. L., Vestergaard, P., Hermann, A. P., Brot, C., Heickendorff, L., Mosekilde, L., & Nexo, E. (2005). Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. *Calcif Tissue Int, 77*(1), 15-22. doi: 10.1007/s00223-004-0227-5

Lawson, D. E., Douglas, J., Lean, M., & Sedrani, S. (1986). Estimation of vitamin D3 and 25-hydroxyvitamin D3 in muscle and adipose tissue of rats and man. *Clin Chim Acta, 157*(2), 175-181.

Ledda, F., Paratcha, G., Sandoval-Guzman, T., & Ibanez, C. F. (2007). GDNF and GFRalpha1 promote formation of neuronal synapses by ligand-induced cell adhesion. Nat Neurosci, 10(3), 293-300. doi: 10.1038/nn1855

Lee, D. M., Rutter, M. K., O'Neill, T. W., Boonen, S., Vanderschueren, D., Bouillon, R., . . . European Male Ageing Study, G. (2009). Vitamin D, parathyroid hormone and the metabolic syndrome in middle-aged and older European men. *Eur J Endocrinol, 161*(6), 947-954. doi: 10.1530/EJE-09-0496

Lee, D. M., Tajar, A., Ulubaev, A., Pendleton, N., O'Neill, T. W., O'Connor, D. B., . . . group, E. s. (2009). Association between 25-hydroxyvitamin D levels and cognitive performance in middle-aged and older European men. *J Neurol Neurosurg Psychiatry, 80*(7), 722-729. doi: 10.1136/jnnp.2008.165720

Lee, D. M., Tajar, A., O'Neill, T. W., O'Connor, D. B., Bartfai, G., Boonen, S., . . . group, E. s. (2011). Lower vitamin D levels are associated with depression among community-dwelling European men. *J Psychopharmacol, 25*(10), 1320-1328. doi: 10.1177/0269881110379287

Lehmann, D. J., Refsum, H., Warden, D. R., Medway, C., Wilcock, G. K., & Smith, A. D. (2011). The vitamin D receptor gene is associated with Alzheimer's disease. *Neurosci Lett, 504*(2), 79-82. doi: 10.1016/j.neulet.2011.08.057

Leo, C., & Chen, J. D. (2000). The SRC family of nuclear receptor coactivators. *Gene, 245*(1), 1-11.

Li, Y. C., Amling, M., Pirro, A. E., Priemel, M., Meuse, J., Baron, R., . . . Demay, M. B. (1998). Normalization of mineral ion homeostasis by dietary means prevents hyperparathyroidism, rickets, and osteomalacia, but not alopecia in vitamin D receptor-ablated mice. Endocrinology, 139(10), 4391-4396.

Li, L., Yin, X., Yao, C., Zhu, X., & Wu, X. (2012). Vitamin D, parathyroid hormone and their associations with hypertension in a Chinese population. *PLoS One, 7*(8), e43344. doi: 10.1371/journal.pone.0043344

Li, Y. C., Kong, J., Wei, M., Chen, Z. F., Liu, S. Q., & Cao, L. P. (2002). 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest, 110*(2), 229-238. doi: 10.1172/JCI15219

Li, Y. C., Qiao, G., Uskokovic, M., Xiang, W., Zheng, W., & Kong, J. (2004). Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. *J Steroid Biochem Mol Biol, 89-90*(1-5), 387-392. doi: 10.1016/j.jsbmb.2004.03.004

Lind L, Wengle B, Ljunghall S. Blood pressure is lowered by vitamin D (alphacalcidol) during long-term treatment of patients with intermittent hypercalcaemia (1987). A double-blind, placebo-controlled study. *Acta Med Scand, 222*(5):423-7.

Lind L, Wengle B, Wide L, Sörensen OH, Ljunghall S (1988). Hypertension in primary hyperparathyroidism--reduction of blood pressure by long-term treatment with vitamin D (alphacalcidol). A double-blind, placebo-controlled study. *Am J Hypertens,1*(4 Pt 1):397-402.

Lind, L., Pollare, T., Hvarfner, A., Lithell, H., Sorensen, O. H., & Ljunghall, S. (1989). Long-term treatment with active vitamin D (alphacalcidol) in middle-aged men with impaired glucose tolerance. Effects on insulin secretion and sensitivity, glucose tolerance and blood pressure. *Diabetes Res, 11*(3), 141-147.

Lind, L., Wengle, B., Wide, L., & Ljunghall, S. (1989). Reduction of blood pressure during long-term treatment with active vitamin D (alphacalcidol) is dependent on plasma renin activity and calcium status. A double-blind, placebo-controlled study. *Am J Hypertens, 2*(1), 20-25.

Lind, L., Wengle, B., Lithell, H., & Ljunghall, S. (1992). Plasma ionized calcium and cardiovascular risk factors in mild primary hyperparathyroidism: effects of long-term treatment with active vitamin D (alphacalcidol). *J Intern Med, 231*(4), 427-432.

Lind, L., Hanni, A., Lithell, H., Hvarfner, A., Sorensen, O. H., & Ljunghall, S. (1995). Vitamin D is related to blood pressure and other cardiovascular risk factors in middle-aged men. *Am J Hypertens, 8*(9), 894-901. doi: 10.1016/0895-7061(95)00154-H

Lips, P., Binkley, N., Pfeifer, M., Recker, R., Samanta, S., Cohn, D. A., . . . Papanicolaou, D. A. (2010). Once-weekly dose of 8400 IU vitamin D(3) compared with placebo: effects on neuromuscular function and tolerability in older adults with vitamin D insufficiency. *Am J Clin Nutr, 91*(4), 985-991. doi: 10.3945/ajcn.2009.28113

Liu, L. J., Lv, J. C., Shi, S. F., Chen, Y. Q., Zhang, H., & Wang, H. Y. (2012). Oral calcitriol for reduction of proteinuria in patients with IgA nephropathy: a randomized controlled trial. *Am J Kidney Dis, 59*(1), 67-74. doi: 10.1053/j.ajkd.2011.09.014

Liu, S., Song, Y., Ford, E. S., Manson, J. E., Buring, J. E., & Ridker, P. M. (2005). Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care, 28*(12), 2926-2932.

Liu, Z. M., Woo, J., Wu, S. H., & Ho, S. C. (2013). The role of vitamin d in blood pressure, endothelial and renal function in postmenopausal women. *Nutrients, 5*(7), 2590-2610. doi: 10.3390/nu5072590

Llewellyn DJ, Langa KM, Lang IA. Serum 25-hydroxyvitamin D concentration and cognitive impairment (2009). *J Geriatr Psychiatry Neurol,22(3):*188-95.

Llewellyn DJ, Lang IA, Langa KM, Muniz-Terrera G, Phillips CL, Cherubini A, Ferrucci L, Melzer D. (2010). Vitamin D and risk of cognitive decline in elderly persons. *Arch Intern Med, 170*(13):1135-41.

Llewellyn DJ, Lang IA, Langa KM, Melzer D. Vitamin D and cognitive impairment in the elderly U.S. population (2011*). J Gerontol A Biol Sci Med Sci.;66*(1):59-65.

London, G., Marchais, S., Guerin, A., & Metivier, F. (2007). [Arterial hypertension, chronic renal insufficiency and dialysis]. *Nephrol Ther, 3 Suppl 3*, S156-161.

Looker, A. C., Pfeiffer, C. M., Lacher, D. A., Schleicher, R. L., Picciano, M. F., & Yetley, E. A. (2008). Serum 25-hydroxyvitamin D status of the US population: 1988-1994 compared with 2000-2004. *Am J Clin Nutr, 88*(6), 1519-1527. doi: 10.3945/ajcn.2008.26182

Lou, Y. R., Molnar, F., Perakyla, M., Qiao, S., Kalueff, A. V., St-Arnaud, R., . . . Tuohimaa, P. (2010). 25-Hydroxyvitamin D(3) is an agonistic vitamin D receptor ligand. *J Steroid Biochem Mol Biol, 118*(3), 162-170. doi: 10.1016/j.jsbmb.2009.11.011

Lu, Z. R., Chen, T. C., & Holick, M. F. (1992). [Preparation of milligram quantity of vitamin D3 isomers by a two-step high performance liquid chromatographic method]. *Yao Xue Xue Bao, 27*(5), 369-374.

Lu'o'ng, K. V., & Nguyen, L. T. (2011). The beneficial role of vitamin D in Alzheimer's disease. *Am J Alzheimers Dis Other Demen, 26*(7), 511-520. doi: 10.1177/1533317511429321

Lurie, S. J., Gawinski, B., Pierce, D., & Rousseau, S. J. (2006). Seasonal affective disorder. *Am Fam Physician, 74*(9), 1521-1524.

Luth, H. J., Holzer, M., Gartner, U., Staufenbiel, M., & Arendt, T. (2001). Expression of endothelial and inducible NOS-isoforms is increased in Alzheimer's disease, in APP23 transgenic mice and after experimental brain lesion in rat: evidence for an induction by amyloid pathology. *Brain Res, 913*(1), 57-67.

MacGregor, G. A., & Cappuccio, F. P. (1993). The kidney and essential hypertension: a link to osteoporosis? *J Hypertens, 11*(8), 781-785.

Mackenzie, S. M., & Connell, J. (2006). Hypertension and the expanding role of aldosterone. *Curr Hypertens Rep, 8*(3), 255-261.

MacLaughlin, J., & Holick, M. F. (1985). Aging decreases the capacity of human skin to produce vitamin D3. *J Clin Invest, 76*(4), 1536-1538. doi: 10.1172/JCI112134

Maddock, J., Berry, D. J., Geoffroy, M. C., Power, C., & Hypponen, E. (2013). Vitamin D and common mental disorders in mid-life: Cross-sectional and prospective findings. *Clin Nutr*. doi: 10.1016/j.clnu.2013.01.006

Madrigal, J. L., Hurtado, O., Moro, M. A., Lizasoain, I., Lorenzo, P., Castrillo, A., . . . Leza, J. C. (2002). The increase in TNF-alpha levels is implicated in NF-kappaB activation and inducible nitric oxide synthase expression in brain cortex after immobilization stress. *Neuropsychopharmacology, 26*(2), 155-163. doi: 10.1016/S0893-133X(01)00292-5

Major, G. C., Alarie, F., Dore, J., Phouttama, S., & Tremblay, A. (2007). Supplementation with calcium + vitamin D enhances the beneficial effect of weight loss on plasma lipid and lipoprotein concentrations. *Am J Clin Nutr, 85*(1), 54-59.

Mak, R. H. (1992). Intravenous 1,25 dihydroxycholecalciferol corrects glucose intolerance in hemodialysis patients. *Kidney Int, 41*(4), 1049-1054.

Mallette, L. E., Patten, B. M., & Engel, W. K. (1975). Neuromuscular disease in secondary hyperparathyroidism. *Ann Intern Med, 82*(4), 474-483.

Margolis, K. L., Martin, L. W., Ray, R. M., Kerby, T. J., Allison, M. A., Curb, J. D., . . . Women's Health Initiative, I. (2012). A prospective study of serum 25-hydroxyvitamin D levels, blood pressure, and incident hypertension in postmenopausal women. *Am J Epidemiol, 175*(1), 22-32. doi: 10.1093/aje/kwr274

Margolis, K. L., Ray, R. M., Van Horn, L., Manson, J. E., Allison, M. A., Black, H. R., . . . Women's Health Initiative, I. (2008). Effect of calcium and vitamin D supplementation on blood pressure: the Women's Health Initiative Randomized Trial. *Hypertension, 52*(5), 847-855. doi: 10.1161/HYPERTENSIONAHA.108.114991

Martins, D., Wolf, M., Pan, D., Zadshir, A., Tareen, N., Thadhani, R., . . . Norris, K. (2007). 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, 167*(11), 1159-1165. doi: 10.1001/archinte.167.11.1159

Masoumi A, Goldenson B, Ghirmai S, Avagyan H, Zaghi J, Abel K, Zheng X, Espinosa-Jeffrey A, Mahanian M, Liu PT, Hewison M, Mizwickie M, Cashman J, Fiala M.J (2009). 1alpha,25-dihydroxyvitamin D3 interacts with curcuminoids to stimulate amyloid-beta clearance by macrophages of Alzheimer's disease patients. *Alzheimers Dis.17*(3):703-17.

Massry, S. G., & Smogorzewski, M. (1995). The mechanisms responsible for the PTH-induced rise in cytosolic calcium in various cells are not uniform. *Miner Electrolyte Metab, 21*(1-3), 13-28.

Mastaglia, S. R., Mautalen, C. A., Parisi, M. S., & Oliveri, B. (2006). Vitamin D2 dose required to rapidly increase 25OHD levels in osteoporotic women. *Eur J Clin Nutr, 60*(5), 681-687. doi: 10.1038/sj.ejcn.1602369

Mateus-Hamdan L, Beauchet O, Bouvard B, Legrand E, Fnatino B, Annweiler C. High parathyroid hormone, but not low vitamin D concentrations, expose elderly inpatients to hypertension. *Ger Gerontol Int, 13*(3):783-91

Matheï C, Van Pottelbergh G, Vaes B, Adriaensen W, Gruson D, Degryse JM. No relation between vitamin D status and physical performance in the oldest old: results from the Belfrail study (2013). *Age Ageing, 42*(2):186-90

Mathieu, C., Gysemans, C., Giulietti, A., & Bouillon, R. (2005). Vitamin D and diabetes. *Diabetologia, 48*(7), 1247-1257. doi: 10.1007/s00125-005-1802-7

Matsuoka, L. Y., Ide, L., Wortsman, J., MacLaughlin, J. A., & Holick, M. F. (1987). Sunscreens suppress cutaneous vitamin D3 synthesis. *J Clin Endocrinol Metab, 64*(6), 1165-1168.

Matsuoka LY, Wortsman J, Dannenberg MJ, Hollis BW, Lu Z, Holick MF. Clothing prevents ultraviolet-B radiation-dependent photosynthesis of vitamin D3 (1992). *J Clin Endocrinol Metab* ;75(4):1099-103.7-90.

Mavroeidi, A., Aucott, L., Black, A. J., Fraser, W. D., Reid, D. M., & Macdonald, H. M. (2013). Seasonal variation in 25(OH)D at Aberdeen (57 degrees N) and bone health indicators--could holidays in the sun and cod liver oil supplements alleviate deficiency? *PLoS One, 8*(1), e53381. doi: 10.1371/journal.pone.0053381

Maxmen, A. (2011). Nutrition advice: the vitamin D-lemma. *Nature, 475*(7354), 23-25. doi: 10.1038/475023a

May, H. T., Bair, T. L., Lappe, D. L., Anderson, J. L., Horne, B. D., Carlquist, J. F., & Muhlestein, J. B. (2010). Association of vitamin D levels with incident depression among a general cardiovascular population. *Am Heart J, 159*(6), 1037-1043. doi: 10.1016/j.ahj.2010.03.017

McCann, J. C., & Ames, B. N. (2008). Is there convincing biological or behavioral evidence linking vitamin D deficiency to brain dysfunction? *FASEB J, 22*(4), 982-1001. doi: 10.1096/fj.07-9326rev

McCarroll, K. G., Robinson, D. J., Coughlan, A., Healy, M., Kenny, R. A., & Cunningham, C. (2012). Vitamin D and orthostatic hypotension. *Age Ageing, 41*(6), 810-813. doi: 10.1093/ageing/afs088

McCarron, D. A., Pingree, P. A., Rubin, R. J., Gaucher, S. M., Molitch, M., & Krutzik, S. (1980). Enhanced parathyroid function in essential hypertension: a homeostatic response to a urinary calcium leak. *Hypertension, 2*(2), 162-168.

McCullough, M. L., Weinstein, S. J., Freedman, D. M., Helzlsouer, K., Flanders, W. D., Koenig, K., . . . Shu, X. O. (2010). Correlates of circulating 25-hydroxyvitamin D: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. *Am J Epidemiol, 172*(1), 21-35. doi: 10.1093/aje/kwq113

McCollum, E.V., Simmonds, N., Becker, J.E. and Shipley, P.G. (1922). Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. J. Biol. Chem. 53:293-312

McCollum, E. V. (*1957*) *A History of Nutrition 1957* Houghton Mifflin Co. Boston, MA

McDuffie, J. R., Calis, K. A., Booth, S. L., Uwaifo, G. I., & Yanovski, J. A. (2002). Effects of orlistat on fat-soluble vitamins in obese adolescents. *Pharmacotherapy, 22*(7), 814-822.

McGrath, J., Scragg, R., Chant, D., Eyles, D., Burne, T., & Obradovic, D. (2007). No association between serum 25-hydroxyvitamin D3 level and performance on psychometric tests in NHANES III. Neuroepidemiology, 29(1-2), 49-54. doi: 10.1159/000108918

McGrath JJ, Féron FP, Burne TH, Mackay-Sim A, Eyles DW (2004). Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. J Steroid Biochem Mol Biol, 89-90(1-5):557-60

McKenna, M. J., & Murray, B. F. (2013). Vitamin D dose response is underestimated by Endocrine Society's Clinical Practice Guideline. *Endocr Connect, 2*(2), 87-95. doi: 10.1530/EC-13-0008

McLachlan, D. R., Wong, L., Bergeron, C., & Baimbridge, K. G. (1987). Calmodulin and calbindin D28K in Alzheimer disease. *Alzheimer Dis Assoc Disord, 1*(3), 171-179.

Mellanby T (1918), The part played by an "accesory factor" in the production of experimental rickets. J Physiol 52:11-14.

Menant, J. C., Close, J. C., Delbaere, K., Sturnieks, D. L., Trollor, J., Sachdev, P. S., . . . Lord, S. R. (2012). Relationships between serum vitamin D levels, neuromuscular and neuropsychological function and falls in older men and women. *Osteoporos Int, 23*(3), 981-989. doi: 10.1007/s00198-011-1637-7

Meng, J. E., Hovey, K. M., Wactawski-Wende, J., Andrews, C. A., Lamonte, M. J., Horst, R. L., . . . Millen, A. E. (2012). Intraindividual variation in plasma 25-hydroxyvitamin D measures 5 years apart among postmenopausal women. *Cancer Epidemiol Biomarkers Prev, 21*(6), 916-924. doi: 10.1158/1055-9965.EPI-12-0026

Menkes, D. B., Lancaster, K., Grant, M., Marsh, R. W., Dean, P., & du Toit, S. A. (2012). Vitamin D status of psychiatric inpatients in New Zealand's Waikato region. *BMC Psychiatry, 12*, 68. doi: 10.1186/1471-244X-12-68

Mezuk, B., Edwards, L., Lohman, M., Choi, M., & Lapane, K. (2012). Depression and frailty in later life: a synthetic review. *Int J Geriatr Psychiatry, 27*(9), 879-892. doi: 10.1002/gps.2807

Michelson, D., Stratakis, C., Hill, L., Reynolds, J., Galliven, E., Chrousos, G., & Gold, P. (1996). Bone mineral density in women with depression. *N Engl J Med, 335*(16), 1176-1181. doi: 10.1056/NEJM199610173351602

Michos, E. D., Streeten, E. A., Ryan, K. A., Rampersaud, E., Peyser, P. A., Bielak, L. F., . . . Post, W. (2009). Serum 25-hydroxyvitamin d levels are not associated with subclinical vascular disease or C-reactive protein in the old order amish. *Calcif Tissue Int, 84*(3), 195-202. doi: 10.1007/s00223-008-9209-3

Milaneschi, Y., Shardell, M., Corsi, A. M., Vazzana, R., Bandinelli, S., Guralnik, J. M., & Ferrucci, L. (2010). Serum 25-hydroxyvitamin D and depressive symptoms in older women and men. *J Clin Endocrinol Metab, 95*(7), 3225-3233. doi: 10.1210/jc.2010

Milaneschi Y, Hoogendijk W, Lips P, Heijboer AC, Schoevers R, van Hemert AM, Beekman AT, Smit JH, Penninx BW (2013). The association between low vitamin D and depressive disorders. *Mol Psychiatry* [Epub ahead of print0347

Miller AH, Maletic V, Raison CL (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry, 1*;65(9):732-41.

Minami, J., Kawano, Y., Ishimitsu, T., Yoshimi, H., & Takishita, S. (1996). Seasonal variations in office, home and 24 h ambulatory blood pressure in patients with essential hypertension. *J Hypertens, 14*(12), 1421-1425.

Minasyan, A., Keisala, T., Lou, Y. R., Kalueff, A. V., & Tuohimaa, P. (2007). Neophobia, sensory and cognitive functions, and hedonic responses in vitamin D receptor mutant mice. J Steroid Biochem Mol Biol, 104(3-5), 274-280. doi: 10.1016/j.jsbmb.2007.03.032

Mithal, A., Wahl, D. A., Bonjour, J. P., Burckhardt, P., Dawson-Hughes, B., Eisman, J. A., . . . Group, I. O. F. C. o. S. A. N. W. (2009). Global vitamin D status and determinants of hypovitaminosis D. *Osteoporos Int, 20*(11), 1807-1820. doi: 10.1007/s00198-009-0954-6

Mizwicki MT, Menegaz D, Zhang J, Barrientos-Durán A, Tse S, Cashman JR, Griffin PR, Fiala M. Genomic and nongenomic signaling induced by 1α,25(OH)2-vitamin D3 promotes the recovery of amyloid-β phagocytosis by Alzheimer's disease macrophages (2012*). J Alzheimers Dis, 29*(1):51-62

Molinari, C., Uberti, F., Grossini, E., Vacca, G., Carda, S., Invernizzi, M., & Cisari, C. (2011). 1alpha,25-dihydroxycholecalciferol induces nitric oxide production in cultured endothelial cells. *Cell Physiol Biochem, 27*(6), 661-668. doi: 10.1159/000330075

Molinari, S., Battini, R., Ferrari, S., Pozzi, L., Killcross, A. S., Robbins, T. W., . . . Emson, P. C. (1996). Deficits in memory and hippocampal long-term potentiation in mice with reduced calbindin D28K expression. *Proc Natl Acad Sci U S A, 93*(15), 8028-8033.

Mozolowski W. Jedrzej Sniadecki (1768–1838) on the cure of rickets. Nature. . 1939;143:121–124.

Moon, M., Song, H., Hong, H. J., Nam, D. W., Cha, M. Y., Oh, M. S., . . . Mook-Jung, I. (2013). Vitamin D-binding protein interacts with Abeta and suppresses Abeta-mediated pathology. *Cell Death Differ, 20*(4), 630-638. doi: 10.1038/cdd.2012.161

Moore, C., Murphy, M. M., Keast, D. R., & Holick, M. F. (2004). Vitamin D intake in the United States. *J Am Diet Assoc, 104*(6), 980-983. doi: 10.1016/j.jada.2004.03.028

Moore, M. E., Piazza, A., McCartney, Y., & Lynch, M. A. (2005). Evidence that vitamin D3 reverses age-related inflammatory changes in the rat hippocampus. *Biochem Soc Trans, 33*(Pt 4), 573-577. doi: 10.1042/BST0330573

Morabia, A., Bernstein, M. S., & Antonini, S. (2000). Smoking, dietary calcium and vitamin D deficiency in women: a population-based study. *Eur J Clin Nutr, 54*(9), 684-689.

Morelli, S., Buitrago, C., Boland, R., & de Boland, A. R. (2001). The stimulation of MAP kinase by 1,25(OH)(2)-vitamin D(3) in skeletal muscle cells is mediated by protein kinase C and calcium. *Mol Cell Endocrinol, 173*(1-2), 41-52.

Moreno J, Krishnan AV, Feldman D. Molecular mechanisms mediating the anti-proliferative effects of Vitamin D in prostate cancer (2005). *J Steroid Biochem Mol Biol, 97*(1-2):31-6.

Morfis, L., Smerdely, P., & Howes, L. G. (1997). Relationship between serum parathyroid hormone levels in the elderly and 24 h ambulatory blood pressures. *J Hypertens, 15*(11), 1271-1276.

Morimoto, S., Imaoka, M., Kitano, S., Imanaka, S., Fukuo, K., Miyashita, Y., . . . Ogihara, T. (1991). Exaggerated natri-calci-uresis and increased circulating levels of parathyroid hormone and 1,25-dihydroxyvitamin D in patients with senile hypertension. *Contrib Nephrol, 90*, 94-98.

Mortola, J. L. (1992). Premenstrual syndrome. *West J Med, 156*(6), 651.

Motsinger, S., Lazovich, D., MacLehose, R. F., Torkelson, C. J., & Robien, K. (2012). Vitamin D intake and mental health-related quality of life in older women: the Iowa Women's Health Study. *Maturitas, 71*(3), 267-273. doi: 10.1016/j.maturitas.2011.12.005

Mowe, M., Haug, E., & Bohmer, T. (1999). Low serum calcidiol concentration in older adults with reduced muscular function. *J Am Geriatr Soc, 47*(2), 220-226.

Mozaffari-Khosravi, H., Nabizade, L., Yassini-Ardakani, S. M., Hadinedoushan, H., & Barzegar, K. (2013). The effect of 2 different single injections of high dose of vitamin D on improving the depression in depressed patients with vitamin D deficiency: a randomized clinical trial. *J Clin Psychopharmacol, 33*(3), 378-385. doi: 10.1097/JCP.0b013e31828f619a

Muir, S. W., & Montero-Odasso, M. (2011). Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. *J Am Geriatr Soc, 59*(12), 2291-2300. doi: 10.1111/j.1532-5415.2011.03733.x

Murad, M. H., Elamin, K. B., Abu Elnour, N. O., Elamin, M. B., Alkatib, A. A., Fatourechi, M. M., . . . Montori, V. M. (2011). Clinical review: The effect of vitamin D on falls: a systematic review and meta-analysis. *J Clin Endocrinol Metab, 96*(10), 2997-3006. doi: 10.1210/jc.2011-1193

Muray, S., Parisi, E., Cardus, A., Craver, L., & Fernandez, E. (2003). Influence of vitamin D receptor gene polymorphisms and 25-hydroxyvitamin D on blood pressure in apparently healthy subjects. *J Hypertens, 21*(11), 2069-2075. doi: 10.1097/01.hjh.0000098139.70956.21

Murphy, P. K., Mueller, M., Hulsey, T. C., Ebeling, M. D., & Wagner, C. L. (2010). An exploratory study of postpartum depression and vitamin d. *J Am Psychiatr Nurses Assoc, 16*(3), 170-177. doi: 10.1177/1078390310370476

Musiol, I. M., Stumpf, W. E., Bidmon, H. J., Heiss, C., Mayerhofer, A., & Bartke, A. (1992). Vitamin D nuclear binding to neurons of the septal, substriatal and amygdaloid area in the Siberian hamster (Phodopus sungorus) brain. *Neuroscience, 48*(4), 841-848.

Myrup, B., Jensen, G. F., & McNair, P. (1992). Cardiovascular risk factors during estrogen-norethindrone and cholecalciferol treatment. *Arch Intern Med, 152*(11), 2265-2268.

Nagpal J, Pande JN, Bhartia A. A double-blind, randomized, placebo-controlled trial of the short-term effect of vitamin D3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men (2009). *Diabet Med, 26*(1):19-27.

Nakamura, K., Nashimoto, M., Okuda, Y., Ota, T., & Yamamoto, M. (2002). Fish as a major source of vitamin D in the Japanese diet. *Nutrition, 18*(5), 415-416.

Nam GE, Kim DH, Cho KH, Park YG, Han KD, Kim SM, Lee SH, Ko BJ, Ji Kim M. 25-Hydroxyvitamin D insufficiency is associated with cardiometabolic risk in Korean adolescents: the 2008-2009 Korea National Health and Nutrition Examination Survey (KNHANES),(2012). *Public Health Nutr, 20*:1-9. [Epub ahead of print]

Nanri, A., Foo, L. H., Nakamura, K., Hori, A., Poudel-Tandukar, K., Matsushita, Y., & Mizoue, T. (2011). Serum 25-hydroxyvitamin d concentrations and season-specific correlates in Japanese adults. *J Epidemiol, 21*(5), 346-353. doi: 10.2188/jea.JE20100161

Nanri, A., Mizoue, T., Matsushita, Y., Poudel-Tandukar, K., Sato, M., Ohta, M., & Mishima, N. (2009). Association between serum 25-hydroxyvitamin D and depressive symptoms in Japanese: analysis by survey season. *Eur J Clin Nutr, 63*(12), 1444-1447. doi: 10.1038/ejcn.2009.96

Nathan, C., Calingasan, N., Nezezon, J., Ding, A., Lucia, M. S., La Perle, K., . . . Beal, M. F. (2005). Protection from Alzheimer's-like disease in the mouse by genetic ablation of inducible nitric oxide synthase. *J Exp Med, 202*(9), 1163-1169. doi: 10.1084/jem.20051529

Naveilhan, P., Neveu, I., Wion, D., & Brachet, P. (1996). 1,25-Dihydroxyvitamin D3, an inducer of glial cell line-derived neurotrophic factor. *Neuroreport, 7*(13), 2171-2175.

Neveu, I., Naveilhan, P., Baudet, C., Brachet, P., & Metsis, M. (1994). 1,25-dihydroxyvitamin D3 regulates NT-3, NT-4 but not BDNF mRNA in astrocytes. *Neuroreport, 6*(1), 124-126.

Neveu, I., Naveilhan, P., Jehan, F., Baudet, C., Wion, D., De Luca, H. F., & Brachet, P. (1994). 1,25-dihydroxyvitamin D3 regulates the synthesis of nerve growth factor in primary cultures of glial cells. *Brain Res Mol Brain Res, 24*(1-4), 70-76.

Neveu, I., Naveilhan, P., Menaa, C., Wion, D., Brachet, P., & Garabedian, M. (1994). Synthesis of 1,25-dihydroxyvitamin D3 by rat brain macrophages in vitro. *J Neurosci Res, 38*(2), 214-220. doi: 10.1002/jnr.490380212

Newmark, H. L., & Newmark, J. (2007). Vitamin D and Parkinson's disease--a hypothesis. *Mov Disord, 22*(4), 461-468. doi: 10.1002/mds.21317

NIH (2007). Dietary Supplement Fact Sheet: Vitamin D. Office of Dietary Supplements, Clinical Centre, National Institute of Health.

Nimitphong, H., Saetung, S., Chanprasertyotin, S., Chailurkit, L. O., & Ongphiphadhanakul, B. (2013). Changes in circulating 25-hydroxyvitamin D according to vitamin D binding protein genotypes after vitamin D(3) or D(2)supplementation. *Nutr J*, 12, 39. doi: 10.1186/1475-2891-12-39

Norman, A. W. (1972). Problems relating to the definition of an international unit for vitamin D and its metabolites. *J Nutr, 102*(10), 1243-1246.

Norman, A. W. (2006). Minireview: vitamin D receptor: new assignments for an already busy receptor. *Endocrinology, 147*(12), 5542-5548. doi: 10.1210/en.2006-0946

Norman, P. E., & Powell, J. T. (2005). Vitamin D, shedding light on the development of disease in peripheral arteries. *Arterioscler Thromb Vasc Biol, 25*(1), 39-46. doi: 10.1161/01.ATV.0000148450.56697.4a

Obeidat, B. A., Alchalabi, H. A., Abdul-Razzak, K. K., & Al-Farras, M. I. (2012). Premenstrual symptoms in dysmenorrheic college students: prevalence and relation to vitamin D and parathyroid hormone levels. *Int J Environ Res Public Health, 9*(11), 4210-4222. doi: 10.3390/ijerph9114210

Obradovic, D., Gronemeyer, H., Lutz, B., & Rein, T. (2006). Cross-talk of vitamin D and glucocorticoids in hippocampal cells. *J Neurochem, 96*(2), 500-509. doi: 10.1111/j.1471-4159.2005.03579.x

O'Donnell, S., Cranney, A., Horsley, T., Weiler, H. A., Atkinson, S. A., Hanley, D. A., . . . Yazdi, F. (2008). Efficacy of food fortification on serum 25-hydroxyvitamin D concentrations: systematic review. *Am J Clin Nutr, 88*(6), 1528-1534. doi: 10.3945/ajcn.2008.26415

Ohrvall, U., Akerstrom, G., Ljunghall, S., Lundgren, E., Juhlin, C., & Rastad, J. (1994). Surgery for sporadic primary hyperparathyroidism in the elderly. *World J Surg, 18*(4), 612-618.

Okuno J, Tomura S, Yanagi H (2007). Correlation between vitamin D and functional capacity, physical function among Japanese frail elderly living in the community.*Nihon Ronen Igakkai Zasshi,* 44(5):634-40

Onder, G., Capoluongo, E., Danese, P., Settanni, S., Russo, A., Concolino, P., . . . Landi, F. (2008). Vitamin D receptor polymorphisms and falls among older adults living in the community: results from the ilSIRENTE study. *J Bone Miner Res, 23*(7), 1031-1036. doi: 10.1359/jbmr.080225

Orwoll, E. S., & Oviatt, S. (1990). Relationship of mineral metabolism and long-term calcium and cholecalciferol supplementation to blood pressure in normotensive men. *Am J Clin Nutr, 52*(4), 717-721.

Oudshoorn, C., Mattace-Raso, F. U., van der Velde, N., Colin, E. M., & van der Cammen, T. J. (2008). Higher serum vitamin D3 levels are associated with better cognitive test performance in patients with Alzheimer's disease. *Dement Geriatr Cogn Disord, 25*(6), 539-543. doi: 10.1159/000134382

Ozkan, B., Hatun, S., & Bereket, A. (2012). Vitamin D intoxication. *Turk J Pediatr, 54*(2), 93-98.

Palm T. A. (1890). The geographical distribution and aetiology of rickets. Practitioner. 1;270

Pan, W. H., Wang, C. Y., Li, L. A., Kao, L. S., & Yeh, S. H. (1993). No significant effect of calcium and vitamin D supplementation on blood pressure and calcium metabolism in elderly Chinese. *Chin J Physiol, 36*(2), 85-94.

Pan A, Lu L, Franco OH, Yu Z, Li H, Lin X.Association between depressive symptoms and 25-hydroxyvitamin D in middle aged and elderly Chinese (2009). *J Affective Disord.,118*(1-3):240-243.

Pang, P. K., & Lewanczuk, R. Z. (1989). Parathyroid origin of a new circulating hypertensive factor in spontaneously hypertensive rats. *Am J Hypertens, 2*(12 Pt 1), 898-902.

Papassotiropoulos, A., Heun, R., & Maier, W. (1999). The impact of dementia on the detection of depression in elderly subjects from the general population. *Psychol Med, 29*(1), 113-120.

Park EA (1940): The therapy of rickets. *JAMA; 115*:370-379.

Parker, G., & Brotchie, H. (2011). 'D' for depression: any role for vitamin D? 'Food for Thought' II. *Acta Psychiatr Scand, 124*(4), 243-249. doi: 10.1111/j.1600-0447.2011.01705.x

Partonen, T. (1998). Vitamin D and serotonin in winter. *Med Hypotheses, 51*(3), 267-268.

Partonen T, Vakkuri O, Lamberg-Allardt C, Lonnqvist J (1996). Effects of bright light on sleepiness, melatonin, and 25-hydroxyvitamin D(3) in winter seasonal affective disorder. *Biol Psychiatry, 39*(10):865-72.

Pasco, J. A., Henry, M. J., Nicholson, G. C., Brennan, S. L., & Kotowicz, M. A. (2009). Behavioural and physical characteristics associated with vitamin D status in women. *Bone, 44*(6), 1085-1091. doi: 10.1016/j.bone.2009.02.020

Patten, B. M., Bilezikian, J. P., Mallette, L. E., Prince, A., Engel, W. K., & Aurbach, G. D. (1974). Neuromuscular disease in primary hyperparathyroidism. *Ann Intern Med, 80*(2), 182-193.

Paulson, D., & Lichtenberg, P. A. (2013). Vascular depression: an early warning sign of frailty. *Aging Ment Health, 17*(1), 85-93. doi: 10.1080/13607863.2012.692767

Peiris, A. N., Bailey, B. A., Guha, B. N., Copeland, R., & Manning, T. (2011). Can a model predictive of vitamin D status be developed from common laboratory tests and demographic parameters? *South Med J, 104*(9), 636-639. doi: 10.1097/SMJ.0b013e3182297169

Perkovic, V., Hewitson, T. D., Kelynack, K. J., Martic, M., Tait, M. G., & Becker, G. J. (2003). Parathyroid hormone has a prosclerotic effect on vascular smooth muscle cells. *Kidney Blood Press Res, 26*(1), 27-33. doi: 69761

Peters, B. S., Roque, J. P., Fisberg, M., & Martini, L. A. (2009). [There are no association between vitamin D metabolites and blood pressure in adolescents]. *Arq Bras Endocrinol Metabol, 53*(4), 416-424.

Peters, S., & Adams, A. (2010). Vitamin D supplementation to reduce the risk of falls and fractures: the dosing dilemma. *Orthopedics, 33*(10), 748-751. doi: 10.3928/01477447-20100826-19

Peterson, A., Mattek, N., Clemons, A., Bowman, G. L., Buracchio, T., Kaye, J., & Quinn, J. (2012). Serum vitamin D concentrations are associated with falling and cognitive function in older adults. *J Nutr Health Aging, 16*(10), 898-901. doi: 10.1007/s12603-012-0378-4

Pfeifer, M., Begerow, B., Minne, H. W., Abrams, C., Nachtigall, D., & Hansen, C. (2000). Effects of a short-term vitamin D and calcium supplementation on body sway and secondary hyperparathyroidism in elderly women. *J Bone Miner Res, 15*(6), 1113-1118. doi: 10.1359/jbmr.2000.15.6.1113

Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C (2001). 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, 86*(4):1633-7.

Pfeifer, M., Begerow, B., Minne, H. W., Schlotthauer, T., Pospeschill, M., Scholz, M., . . . Pollahne, W. (2001). Vitamin D status, trunk muscle strength, body sway, falls, and fractures among 237 postmenopausal women with osteoporosis. *Exp Clin Endocrinol Diabetes, 109*(2), 87-92. doi: 10.1055/s-2001-14831

Pfeifer, M., Begerow, B., & Minne, H. W. (2002). Vitamin D and muscle function. *Osteoporos Int, 13*(3), 187-194.

Pfeifer M, Begerow B, Minne HW, Suppan K, Fahrleitner-Pammer A, Dobnig H. Effects of a long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals (2008). *Osteoporos Int, 20*(2):315-22

Pfeifer, M., Begerow, B., Minne, H. W., Suppan, K., Fahrleitner-Pammer, A., & Dobnig, H. (2009). Effects of a long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals. *Osteoporos Int, 20*(2), 315-322. doi: 10.1007/s00198-008-0662-7

Pilz, S., & Tomaschitz, A. (2010). Role of vitamin D in arterial hypertension. *Expert Rev Cardiovasc Ther, 8*(11), 1599-1608. doi: 10.1586/erc.10.142

Pilz, S., Tomaschitz, A., Ritz, E., & Pieber, T. R. (2009). Vitamin D status and arterial hypertension: a systematic review. *Nat Rev Cardiol, 6*(10), 621-630. doi: 10.1038/nrcardio.2009.135

Pittas, A. G., Chung, M., Trikalinos, T., Mitri, J., Brendel, M., Patel, K., . . . Balk, E. M. (2010). Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med, 152*(5), 307-314. doi: 10.7326/0003-4819-152-5-201003020-00009

Plum LA & De Luca HF (2010). The Functional Metabolism and Molecular Biology of Vitamin D Action. In: Holick M.F. (eds*). Vitamin D :Physiology, Molecular Biology and Clinical Applications*. 2nd Edition, Humana Press, New York.

Poduslo, S. E., & Yin, X. (2001). A new locus on chromosome 19 linked with late-onset Alzheimer's disease. *Neuroreport, 12*(17), 3759-3761.

Pourdjabbar, A., Dwivedi, G., & Haddad, H. (2013). The role of vitamin D in chronic heart failure. *Curr Opin Cardiol, 28*(2), 216-222. doi: 10.1097/HCO.0b013e32835bd480

Pramyothin, P., Techasurungkul, S., Lin, J., Wang, H., Shah, A., Ross, P. D., . . . Wasnich, R. D. (2009). Vitamin D status and falls, frailty, and fractures among postmenopausal Japanese women living in Hawaii. *Osteoporos Int, 20*(11), 1955-1962. doi: 10.1007/s00198-009-0910-5

Pramyothin P, Holick M.F (2012) . Vitamin D supplementation: guidelines and evidence for subclinical deficiency. *Curr Opin Gastroenterol, 28*(2):139-50.

Prince, M. J., Harwood, R. H., Blizard, R. A., Thomas, A., & Mann, A. H. (1997). Impairment, disability and handicap as risk factors for depression in old age. The Gospel Oak Project V. *Psychol Med, 27*(2), 311-321.

Prineas, J. W., Mason, A. S., & Henson, R. A. (1965). Myopathy in Metabolic Bone Disease. *Br Med J, 1*(5441), 1034-1036.

Prosser, D. E., & Jones, G. (2004). Enzymes involved in the activation and inactivation of vitamin D. *Trends Biochem Sci, 29*(12), 664-673. doi: 10.1016/j.tibs.2004.10.005

Prufer, K., Veenstra, T. D., Jirikowski, G. F., & Kumar, R. (1999). Distribution of 1,25-dihydroxyvitamin D3 receptor immunoreactivity in the rat brain and spinal cord. *J Chem Neuroanat, 16*(2), 135-145.

Przybelski, R., Agrawal, S., Krueger, D., Engelke, J. A., Walbrun, F., & Binkley, N. (2008). Rapid correction of low vitamin D status in nursing home residents. *Osteoporos Int, 19*(11), 1621-1628. doi: 10.1007/s00198-008-0619-x

Przybelski, R. J., & Binkley, N. C. (2007). Is vitamin D important for preserving cognition? A positive correlation of serum 25-hydroxyvitamin D concentration with cognitive function. *Arch Biochem Biophys, 460*(2), 202-205. doi: 10.1016/j.abb.2006.12.018

Puchacz E, Stumpf WE, Stachowiak EK, Stachowiak MK (1996). Vitamin D increases expression of the tyrosine hydroxylase gene in adrenal medullary cells. *Brain Res Mol Brain Res, 36*(1):193.

Puepet, F. H., Agaba, E. I., Chuhwak, E. K., & Ugoya, S. O. (2008). Primary hyperparathyroidism presenting with severe hypertension in a middle aged Nigerian--a case report. *Niger Postgrad Med J, 15*(1), 58-60.

Rachez, C., Gamble, M., Chang, C. P., Atkins, G. B., Lazar, M. A., & Freedman, L. P. (2000). The DRIP complex and SRC-1/p160 coactivators share similar nuclear receptor binding determinants but constitute functionally distinct complexes. *Mol Cell Biol, 20*(8), 2718-2726.

Radloff LS. (1977) .The CES-D Scale A Self-Report Depression Scale for Research in the General Population.Applied Psychological Measurement.

Randolph, C., Tierney, M. C., Mohr, E., & Chase, T. N. (1998). The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): preliminary clinical validity. *J Clin Exp Neuropsychol, 20*(3), 310-319. doi: 10.1076/jcen.20.3.310.823

Rauschkolb, E. W., Davis, H. W., Fenimore, D. C., Black, H. S., & Fabre, L. F., Jr. (1969). Identification of vitamin D3 in human skin. *J Invest Dermatol, 53*(4), 289-294.

Reichel, H., Liebethal, R., Hense, H. W., Schmidt-Gayk, H., & Ritz, E. (1992). Disturbed calcium metabolism in subjects with elevated diastolic blood pressure. *Clin Investig, 70*(9), 748-751.

Reis, J. P., von Muhlen, D., Miller, E. R., 3rd, Michos, E. D., & Appel, L. J. (2009). Vitamin D status and cardiometabolic risk factors in the United States adolescent population. *Pediatrics, 124*(3), e371-379. doi: 10.1542/peds.2009-0213

Resnick, L. M. (1990). Calciotropic hormones in human and experimental hypertension. *Am J Hypertens, 3*(8 Pt 2), 171S-178S.

Resnick, L. M. (1990). The effects of sodium and calcium in clinical hypertension: mediating role of vitamin D metabolism. *Adv Second Messenger Phosphoprotein Res, 24*, 535-541.

Resnick, L. M. (1999). The role of dietary calcium in hypertension: a hierarchical overview. *Am J Hypertens, 12*(1 Pt 1), 99-112.

Resnick, L. M., Lewanczuk, R. Z., Laragh, J. H., & Pang, P. K. (1993). Parathyroid hypertensive factor-like activity in human essential hypertension: relationship to plasma renin activity and dietary salt sensitivity. *J Hypertens, 11*(11), 1235-1241.

Resnick, L. M., Muller, F. B., & Laragh, J. H. (1986). Calcium-regulating hormones in essential hypertension. Relation to plasma renin activity and sodium metabolism. *Ann Intern Med, 105*(5), 649-654.

Resnick, L. M., Nicholson, J. P., & Laragh, J. H. (1986). Calcium metabolism in essential hypertension: relationship to altered renin system activity. *Fed Proc, 45*(12), 2739-2745.

Richy, F., Dukas, L., & Schacht, E. (2008). Differential effects of D-hormone analogs and native vitamin D on the risk of falls: a comparative meta-analysis. *Calcif Tissue Int, 82*(2), 102-107. doi: 10.1007/s00223-008-9102-0

Ringe, J. D. (2012). The effect of Vitamin D on falls and fractures. *Scand J Clin Lab Invest Suppl, 243*, 73-78. doi: 10.3109/00365513.2012.681965

Rizzoli, R., Boonen, S., Brandi, M. L., Bruyere, O., Cooper, C., Kanis, J. A., . . . Reginster, J. Y. (2013). Vitamin D supplementation in elderly or postmenopausal women: a 2013 update of the 2008 recommendations from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Curr Med Res Opin, 29*(4), 305-313. doi: 10.1185/03007995.2013.766162

Robinson-Cohen C, Hoofnagle A.N, Ix J.H, Sachs M.C, Tracy R.P, Siscovick DS, Kestenbaum BR, de Boer IH (2013). Racial differences in the association of serum 25- hydroxyvitamin D concentration with coronary heart disease events. JAMA, 310(2):179-88

Robbins J, Hirsch C, Whitmer R, Cauley J, Harris T(2001). The association of bone mineral density and depression in an older population). *J Am Geriatr Soc (6):*732-6.

Rockell, J. E., Skeaff, C. M., Williams, S. M., & Green, T. J. (2006). Serum 25-hydroxyvitamin D concentrations of New Zealanders aged 15 years and older. *Osteoporos Int, 17*(9), 1382-1389. doi: 10.1007/s00198-006-0118-x

Roman SA, Sosa JA, Mayes L, Desmond E, Boudourakis L, Lin R, Snyder PJ, Holt E, Udelsman R. Parathyroidectomy improves neurocognitive deficits in patients with primary hyperparathyroidism (2005). *Surgery, 138*(6):1121-8.

Romero-Ortuno R, Cogan L, Browne J, Healy M, Casey MC, Cunningham C, Walsh JB, Kenny RA (2011). Seasonal variation of serum vitamin D and the effect of vitamin D supplementation in Irish community-dwelling older people. *Age Ageing, 40*(2):168-74

Rondanelli, M., Trotti, R., Opizzi, A., & Solerte, S. B. (2007). Relationship among nutritional status, pro/antioxidant balance and cognitive performance in a group of free-living healthy elderly. *Minerva Med, 98*(6), 639-645.

Rosen, C. J., Adams, J. S., Bikle, D. D., Black, D. M., Demay, M. B., Manson, J. E., . . . Kovacs, C. S. (2012). The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. *Endocr Rev, 33*(3), 456-492. doi: 10.1210/er.2012-1000

Rosenthal, N. E., Sack, D. A., Skwerer, R. G., Jacobsen, F. M., & Wehr, T. A. (1988). Phototherapy for seasonal affective disorder. *J Biol Rhythms, 3*(2), 101-120.

Rosenthal, T. (2004). Seasonal variations in blood pressure. *Am J Geriatr Cardiol, 13*(5), 267-272.

Ross, A. C., Manson, J. E., Abrams, S. A., Aloia, J. F., Brannon, P. M., Clinton, S. K., . . . Shapses, S. A. (2011). The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab, 96*(1), 53-58. doi: 10.1210/jc.2010-2704

Rossom, R. C., Espeland, M. A., Manson, J. E., Dysken, M. W., Johnson, K. C., Lane, D. S., . . . Margolis, K. L. (2012). Calcium and vitamin D supplementation and cognitive impairment in the women's health initiative. *J Am Geriatr Soc, 60*(12), 2197-2205. doi: 10.1111/jgs.12032

Rostand, S. G. (1997). Ultraviolet light may contribute to geographic and racial blood pressure differences. *Hypertension, 30*(2 Pt 1), 150-156.

Roth, S. M., Zmuda, J. M., Cauley, J. A., Shea, P. R., & Ferrell, R. E. (2004). Vitamin D receptor genotype is associated with fat-free mass and sarcopenia in elderly men. *J Gerontol A Biol Sci Med Sci, 59*(1), 10-15.

Rubin, M. R., Maurer, M. S., McMahon, D. J., Bilezikian, J. P., & Silverberg, S. J. (2005). Arterial stiffness in mild primary hyperparathyroidism. *J Clin Endocrinol Metab, 90*(6), 3326-3330. doi: 10.1210/jc.2004-1400

Rydberg, E., Birgander, M., Bondeson, A. G., Bondeson, L., & Willenheimer, R. (2010). Effect of successful parathyroidectomy on 24-hour ambulatory blood pressure in patients with primary hyperparathyroidism. *Int J Cardiol, 142*(1), 15-21. doi: 10.1016/j.ijcard.2008.12.027

Sabanayagam, C., Shankar, A., & Somasundaram, S. (2012). Serum vitamin D level and prehypertension among subjects free of hypertension. *Kidney Blood Press Res, 35*(2), 106-113. doi: 10.1159/000330716

Saglikes, Y., Massry, S. G., Iseki, K., Nadler, J. L., & Campese, V. M. (1985). Effect of PTH on blood pressure and response to vasoconstrictor agonists. *Am J Physiol, 248*(5 Pt 2), F674-681.

Sakamoto, R., Jaceldo-Siegl, K., Haddad, E., Oda, K., Fraser, G. E., & Tonstad, S. (2013). Relationship of vitamin D levels to blood pressure in a biethnic population. *Nutr Metab Cardiovasc Dis, 23*(8), 776-784. doi: 10.1016/j.numecd.2012.04.014

Saliba, W., Barnett, O., Rennert, H. S., & Rennert, G. (2012). The risk of all-cause mortality is inversely related to serum 25(OH)D levels. *J Clin Endocrinol Metab, 97*(8), 2792-2798. doi: 10.1210/jc.2012-1747.

Salawu, F. K., Umar, J. T., & Olokoba, A. B. (2011). Alzheimer's disease: a review of recent developments. *Ann Afr Med, 10*(2), 73-79. doi: 10.4103/1596-3519.82057

Sambrook, P. N., Chen, J. S., March, L. M., Cameron, I. D., Cumming, R. G., Lord, S. R., . . . Seibel, M. J. (2004). Serum parathyroid hormone predicts time to fall independent of vitamin D status in a frail elderly population. *J Clin Endocrinol Metab, 89*(4), 1572-1576.

Sancho, J. J., Rouco, J., Riera-Vidal, R., & Sitges-Serra, A. (1992). Long-term effects of parathyroidectomy for primary hyperparathyroidism on arterial hypertension. *World J Surg, 16*(4), 732-735; discussion 736.

Sanders, K. M., Stuart, A. L., Williamson, E. J., Jacka, F. N., Dodd, S., Nicholson, G., & Berk, M. (2011). Annual high-dose vitamin D3 and mental well-being: randomised controlled trial. *Br J Psychiatry, 198*(5), 357-364. doi: 10.1192/bjp.bp.110.087544

Saneei, P., Salehi-Abargouei, A., & Esmaillzadeh, A. (2013). Serum 25-hydroxy vitamin D levels in relation to body mass index: a systematic review and meta-analysis. *Obes Rev, 14*(5), 393-404. doi: 10.1111/obr.12016

Sato, Y., Iwamoto, J., Kanoko, T., & Satoh, K. (2005). Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. *Cerebrovasc Dis, 20*(3), 187-192. doi: 10.1159/000087203

Savaskan, E., Hock, C., Olivieri, G., Bruttel, S., Rosenberg, C., Hulette, C., & Muller-Spahn, F. (2001). Cortical alterations of angiotensin converting enzyme, angiotensin II and AT1 receptor in Alzheimer's dementia. *Neurobiol Aging, 22*(4), 541-546.

Schachter D. Vitamin D and the active transport of calcium by the small intestine. In Wasserman RD (ed). The Transfer of calcium and strontium across biological membranes. Acadamic Press, New York,1963: 197-210

Schleithoff, S. S., Zittermann, A., Tenderich, G., Berthold, H. K., Stehle, P., & Koerfer, R. (2006). Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr, 83*(4), 754-759.

Schmitz, K. J., Skinner, H. G., Bautista, L. E., Fingerlin, T. E., Langefeld, C. D., Hicks, P. J., . . . Engelman, C. D. (2009). Association of 25-hydroxyvitamin D with blood pressure in predominantly 25-hydroxyvitamin D deficient Hispanic and African

Schmorl G. (1909). Die pathologigische anatomie de rachitischen knockenerkrankung mit besonderer ber ucksichtigung imer histologic und pathogenese. Ergeb d Inn Med Kinderheilkd IV 403. *Am J Hypertens, 22*(8), 867-870. doi: 10.1038/ajh.2009.88

Schneider, B., Weber, B., Frensch, A., Stein, J., & Fritz, J. (2000). Vitamin D in schizophrenia, major depression and alcoholism. *J Neural Transm, 107*(7), 839-842.

Schott, G. D., & Wills, M. R. (1976). Muscle weakness in osteomalacia. *Lancet, 1*(7960), 626-629.

Schottker, B., Ball, D., Gellert, C., & Brenner, H. (2013). Serum 25-hydroxyvitamin D levels and overall mortality. A systematic review and meta-analysis of prospective cohort studies. *Ageing Res Rev, 12*(2), 708-718. doi: 10.1016/j.arr.2012.02.004

Scragg, R., Holdaway, I., Jackson, R., & Lim, T. (1992). Plasma 25-hydroxyvitamin D3 and its relation to physical activity and other heart disease risk factors in the general population. *Ann Epidemiol, 2*(5), 697-703.

Scragg, R., Holdaway, I., Singh, V., Metcalf, P., Baker, J., & Dryson, E. (1995). Serum 25-hydroxycholecalciferol concentration in newly detected hypertension. *Am J Hypertens, 8*(4 Pt 1), 429-432.

Scragg R, Khaw KT, Murphy S. Effect of winter oral vitamin D3 supplementation on cardiovascular risk factors in elderly adults (1995). *Eur J Clin Nutr, 49*(9):640-6.

Scragg, R., Holdaway, I., Singh, V., Metcalf, P., Baker, J., & Dryson, E. (1995). Serum 25-hydroxyvitamin D3 is related to physical activity and ethnicity but not obesity in a multicultural workforce. *Aust N Z J Med, 25*(3), 218-223.

Scragg, R., Sowers, M., & Bell, C. (2007). Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. *Am J Hypertens, 20*(7), 713-719. doi: 10.1016/j.amjhyper.2007.01.017

Scragg, R., & Camargo, C. A., Jr. (2008). Frequency of leisure-time physical activity and serum 25-hydroxyvitamin D levels in the US population: results from the Third National Health and Nutrition Examination Survey. *Am J Epidemiol, 168*(6), 577-586; discussion 587-591. doi: 10.1093/aje/kwn163

Scragg RK, Camargo CA Jr, Simpson RU. Relation of serum 25-hydroxyvitamin D to heart rate and cardiac work (from the National Health and Nutrition Examination Surveys),(2010). Am J Cardiol, 1;105(1):122-8.

Scragg, R. (2012). Do we need to take calcium with vitamin D supplements to prevent falls, fractures, and death? *Curr Opin Clin Nutr Metab Care, 15*(6), 614-624. doi: 10.1097/MCO.0b013e328359ef21

Seamans, K. M., Hill, T. R., Scully, L., Meunier, N., Andrillo-Sanchez, M., Polito, A., . . . Cashman, K. D. (2010). Vitamin D status and measures of cognitive function in healthy older European adults. *Eur J Clin Nutr, 64*(10), 1172-1178. doi: 10.1038/ejcn.2010.117

Seiki, S., Chonchol, M., Cheung, A. K., Kaufman, J. S., Greene, T., Roberts, W. L., . . . Investigators, H. (2012). 25-hydroxyvitamin D deficiency is associated with an increased risk of metabolic syndrome in patients with non-diabetic chronic kidney disease. *Clin Nephrol, 78*(6), 432-441. doi: 10.5414/CN107498

Shahar, D., Levi, M., Kurtz, I., Shany, S., Zvili, I., Mualleme, E., . . . Melzer, I. (2009). Nutritional status in relation to balance and falls in the elderly: a preliminary look at serum folate. *Ann Nutr Metab, 54*(1), 59-66. doi: 10.1159/000207356

Shea, M. K., Houston, D. K., Tooze, J. A., Davis, C. C., Johnson, M. A., Hausman, D. B., . . . Body Composition, S. (2011). Correlates and prevalence of insufficient 25-hydroxyvitamin D status in black and white older adults: the health, aging and body composition study. *J Am Geriatr Soc, 59*(7), 1165-1174. doi: 10.1111/j.1532-5415.2011.03476.x

Shipowick CD, Moore CB, Corbett C, Bindler R (2009). Vitamin D and depressive symptoms in women during the winter: a pilot study. *Appl Nurs Res, 22*(3):221-5.

Shroff, R., Wan, M., & Rees, L. (2012). Can vitamin D slow down the progression of chronic kidney disease? *Pediatr Nephrol, 27*(12), 2167-2173. doi: 10.1007/s00467-011-2071-

Signorello, L. B., Shi, J., Cai, Q., Zheng, W., Williams, S. M., Long, J., . . . Blot, W. J. (2011). Common variation in vitamin D pathway genes predicts circulating 25-hydroxyvitamin D Levels among African Americans. *PLoS One, 6*(12), e28623. doi: 10.1371/journal.pone.0028623

Silverberg, S. J. (2007). Vitamin D deficiency and primary hyperparathyroidism. *J Bone Miner Res, 22 Suppl 2*, V100-104. doi: 10.1359/jbmr.07s202

Silverman, S. L., Shen, W., Minshall, M. E., Xie, S., & Moses, K. H. (2007). Prevalence of depressive symptoms in postmenopausal women with low bone mineral density and/or prevalent vertebral fracture: results from the Multiple Outcomes of Raloxifene Evaluation (MORE) study. *J Rheumatol, 34*(1), 140-144.

Simon, K. C., Munger, K. L., & Ascherio, A. (2012). Vitamin D and multiple sclerosis: epidemiology, immunology, and genetics. *Curr Opin Neurol, 25*(3), 246-251. doi: 10.1097/WCO.0b013e3283533a7e

Skaaby T, Husemoen LL, Pisinger C, Jørgensen T, Thuesen BH, Fenger M, Linneberg A (2012).Vitamin D status and changes in cardiovascular risk factors: a prospective study of a general population. *Cardiology, 123*(1):62-70.

Skalska, A., Galas, A., & Grodzicki, T. (2012). 25-hydroxyvitamin D and physical and cognitive performance in older people with chronic conditions. *Pol Arch Med Wewn, 122*(4), 162-169.

Slinin, Y., Paudel, M., Taylor, B. C., Ishani, A., Rossom, R., Yaffe, K., . . . Study of Osteoporotic Fractures Research, G. (2012). Association between serum 25(OH) vitamin D and the risk of cognitive decline in older women. *J Gerontol A Biol Sci Med Sci, 67*(10), 1092-1098. doi: 10.1093/gerona/gls075

Slinin, Y., Paudel, M. L., Taylor, B. C., Fink, H. A., Ishani, A., Canales, M. T., . . . Osteoporotic Fractures in Men Study Research, G. (2010). 25-Hydroxyvitamin D levels and cognitive performance and decline in elderly men. *Neurology, 74*(1), 33-41. doi: 10.1212/WNL.0b013e3181c7197b

Smerdon, G.T. Daniel Whistler and the English Disease (2007). Journal of the History of Medicine 5:397-415.

Smith, H., Anderson, F., Raphael, H., Maslin, P., Crozier, S., & Cooper, C. (2007). Effect of annual intramuscular vitamin D on fracture risk in elderly men and women--a population-based, randomized, double-blind, placebo-controlled trial. *Rheumatology (Oxford), 46*(12), 1852-1857. doi: 10.1093/rheumatology/kem240

Smith, R., & Stern, G. (1967). Myopathy, osteomalacia and hyperparathyroidism. *Brain, 90*(3), 593-602.

Smogorzewski, M., Koureta, P., Fadda, G. Z., Perna, A. F., & Massry, S. G. (1991). Chronic parathyroid hormone excess in vivo increases resting levels of cytosolic calcium in brain synaptosomes: studies in the presence and absence of chronic renal failure. *J Am Soc Nephrol, 1*(10), 1162-1168.

Smogorzewski, M., Piskorska, G., Borum, P. R., & Massry, S. G. (1988). Chronic renal failure, parathyroid hormone and fatty acids oxidation in skeletal muscle. *Kidney Int, 33*(2), 555-560.

Sniadecki J (1939). Cited by W Mozolowski "Jedrzej Sniadecki: 91768-1883) on the cure of rickets." Nature 143:121

Snijder, M. B., Lips, P., Seidell, J. C., Visser, M., Deeg, D. J., Dekker, J. M., & van Dam, R. M. (2007). Vitamin D status and parathyroid hormone levels in relation to blood pressure: a population-based study in older men and women. *J Intern Med, 261*(6), 558-565. doi: 10.1111/j.1365-2796.2007.01778.x

Snijder, M. B., van Schoor, N. M., Pluijm, S. M., van Dam, R. M., Visser, M., & Lips, P. (2006). Vitamin D status in relation to one-year risk of recurrent falling in older men and women. *J Clin Endocrinol Metab, 91*(8), 2980-2985. doi: 10.1210/jc.2006-0510

Sokol, S. I., Srinivas, V., Crandall, J. P., Kim, M., Tellides, G., Lebastchi, A. H., . . . Alderman, M. H. (2012). The effects of vitamin D repletion on endothelial function and inflammation in patients with coronary artery disease. *Vasc Med, 17*(6), 394-404. doi: 10.1177/1358863X12466709

Sohl, E., de Jongh, R. T., Heijboer, A. C., Swart, K. M., Brouwer-Brolsma, E. M., Enneman, A. W., . . . van Schoor, N. M. (2013). Vitamin D status is associated with physical performance: the results of three independent cohorts. *Osteoporos Int, 24*(1), 187-196. doi: 10.1007/s00198-012-2124-5

Somjen, D., Weisman, Y., Kohen, F., Gayer, B., Limor, R., Sharon, O., . . . Stern, N. (2005). 25-hydroxyvitamin D3-1alpha-hydroxylase is expressed in human vascular smooth muscle cells and is upregulated by parathyroid hormone and estrogenic compounds. *Circulation, 111*(13), 1666-1671. doi: 10.1161/01.CIR.0000160353.27927.70

Song, C., & Wang, H. (2011). Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. *Prog Neuropsychopharmacol Biol Psychiatry, 35*(3), 760-768. doi: 10.1016/j.pnpbp.2010.06.020

Soni, M., Kos, K., Lang, I. A., Jones, K., Melzer, D., & Llewellyn, D. J. (2012). Vitamin D and cognitive function. *Scand J Clin Lab Invest Suppl, 243*, 79-82. doi: 10.3109/00365513.2012.681969

Sorensen, O. H., Lund, B., Saltin, B., Lund, B., Andersen, R. B., Hjorth, L., . . . Mosekilde, L. (1979). Myopathy in bone loss of ageing: improvement by treatment with 1 alpha-hydroxycholecalciferol and calcium. *Clin Sci (Lond), 56*(2), 157-161.

Souberbielle, J. C., Body, J. J., Lappe, J. M., Plebani, M., Shoenfeld, Y., Wang, T. J., . . . Zittermann, A. (2010). Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: Recommendations for clinical practice. Autoimmun Rev, 9(11), 709-715. doi: 10.1016/j.autrev.2010.06.009

Sowers, M. R., Wallace, R. B., & Lemke, J. H. (1985). The association of intakes of vitamin D and calcium with blood pressure among women. *Am J Clin Nutr, 42*(1), 135-142.

Sowers, M. F., Wallace, R. B., Hollis, B. W., & Lemke, J. H. (1988). Relationship between 1,25-dihydroxyvitamin D and blood pressure in a geographically defined population. *Am J Clin Nutr, 48*(4), 1053-1056.

Sowers, J. R., Zemel, M. B., Standley, P. R., & Zemel, P. C. (1989). Calcium and hypertension. *J Lab Clin Med, 114*(4), 338-348.

St John, A., Dick, I., Hoad, K., Retallack, R., Welborn, T., & Prince, R. (1994). Relationship between calcitrophic hormones and blood pressure in elderly subjects. *Eur J Endocrinol, 130*(5), 446-450.

Steenbock, H. (1924). The Induction of Growth Promoting and Calcifying Properties in a Ration by Exposure to Light. *Science, 60*(1549), 224-225. doi: 10.1126/science.60.1549.224

Stefenelli, T., Mayr, H., Bergler-Klein, J., Globits, S., Woloszczuk, W., & Niederle, B. (1993). Primary hyperparathyroidism: incidence of cardiac abnormalities and partial reversibility after successful parathyroidectomy. *Am J Med, 95*(2), 197-202.

Stein, M. S., Wark, J. D., Scherer, S. C., Walton, S. L., Chick, P., Di Carlantonio, M., . . . Flicker, L. (1999). Falls relate to vitamin D and parathyroid hormone in an Australian nursing home and hostel. *J Am Geriatr Soc, 47*(10), 1195-1201.

Stein, M. S., Scherer, S. C., Ladd, K. S., & Harrison, L. C. (2011). A randomized controlled trial of high-dose vitamin D2 followed by intranasal insulin in Alzheimer's disease. *J Alzheimers Dis, 26*(3), 477-484. doi: 10.3233/JAD-2011-110149

Stewart, R., & Hirani, V. (2010). Relationship between vitamin D levels and depressive symptoms in older residents from a national survey population. *Psychosom Med, 72*(7), 608-612. doi: 10.1097/PSY.0b013e3181e9bf15

Stockton, K. A., Mengersen, K., Paratz, J. D., Kandiah, D., & Bennell, K. L. (2011). Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. *Osteoporos Int, 22*(3), 859-871. doi: 10.1007/s00198-010-1407-y

Stumpf, W. E., Sar, M., Clark, S. A., & DeLuca, H. F. (1982). Brain target sites for 1,25-dihydroxyvitamin D3. Science, 215(4538), 1403-1405.

Stumpf, W. E., & O'Brien, L. P. (1987). 1,25 (OH)2 vitamin D3 sites of action in the brain. An autoradiographic study. *Histochemistry, 87*(5), 393-406.

Stumpf, W. E., & Privette, T. H. (1989). Light, vitamin D and psychiatry. Role of 1,25 dihydroxyvitamin D3 (soltriol) in etiology and therapy of seasonal affective disorder and other mental processes. *Psychopharmacology (Berl), 97*(3), 285-294.

Sugden, J. A., Davies, J. I., Witham, M. D., Morris, A. D., & Struthers, A. D. (2008). Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. *Diabet Med, 25*(3), 320-325. doi: 10.1111/j.1464-5491.2007.02360.x

Sutherland, M. K., Somerville, M. J., Yoong, L. K., Bergeron, C., Haussler, M. R., & McLachlan, D. R. (1992). Reduction of vitamin D hormone receptor mRNA levels in Alzheimer as compared to Huntington hippocampus: correlation with calbindin-28k mRNA levels. *Brain Res Mol Brain Res, 13*(3), 239-250.

Suzuki, T., Kwon, J., Kim, H., Shimada, H., Yoshida, Y., Iwasa, H., & Yoshida, H. (2008). Low serum 25-hydroxyvitamin D levels associated with falls among Japanese community-dwelling elderly. *J Bone Miner Res, 23*(8), 1309-1317. doi: 10.1359/jbmr.080328

Swapna, N., Vamsi, U. M., Usha, G., & Padma, T. (2011). Risk conferred by FokI polymorphism of vitamin D receptor (VDR) gene for essential hypertension. *Indian J Hum Genet, 17*(3), 201-206. doi: 10.4103/0971-6866.92104

Styren SD, Civin WH, Rogers J. Molecular, cellular, and pathologic characterization of HLA-DR immunoreactivity in normal elderly and Alzheimer's disease brain (1990). *Exp Neurol, 110*(1):93-104

Szekely, J. I., & Pataki, A. (2012). Effects of vitamin D on immune disorders with special regard to asthma, COPD and autoimmune diseases: a short review. *Expert Rev Respir Med, 6*(6), 683-704. doi: 10.1586/ers.12.57

Taghizadeh, M., Djazayery, A., Salami, M., Eshraghian, M. R., & Zavareh, S. A. (2011). Vitamin-D-free regimen intensifies the spatial learning deficit in Alzheimer's disease. *Int J Neurosci, 121*(1), 16-24. doi: 10.3109/00207454.2010.523132

Taghizadeh, M., Talaei, S. A., & Salami, M. (2013). Vitamin D deficiency impairs spatial learning in adult rats. *Iran Biomed J, 17*(1), 42-48.

Talmor, Y., Bernheim, J., Klein, O., Green, J., & Rashid, G. (2008). Calcitriol blunts pro-atherosclerotic parameters through NFkappaB and p38 in vitro. *Eur J Clin Invest, 38*(8), 548-554. doi: 10.1111/j.1365-2362.2008.01977.x

Tamez, H., & Thadhani, R. I. (2012). Vitamin D and hypertension: an update and review. *Curr Opin Nephrol Hypertens, 21*(5), 492-499. doi: 10.1097/MNH.0b013e3283557bf0

Tangpricha, V., Turner, A., Spina, C., Decastro, S., Chen, T. C., & Holick, M. F. (2004). Tanning is associated with optimal vitamin D status (serum 25-hydroxyvitamin D concentration) and higher bone mineral density. *Am J Clin Nutr, 80*(6), 1645-1649.

Taniura H, Ito M, Sanada N, Kuramoto N, Ohno Y, Nakamichi N, Yoneda Y (2006). Chronic vitamin D3 treatment protects against neurotoxicity by glutamate in association with upregulation of vitamin D receptor mRNA expression in cultured rat cortical neurons. *J Neurosci Res, 15*;83(7):1179-89.

Tarcin, O., Yavuz, D. G., Ozben, B., Telli, A., Ogunc, A. V., Yuksel, M., . . . Akalin, S. (2009). Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. *J Clin Endocrinol Metab, 94*(10), 4023-4030. doi: 10.1210/jc.2008-1212

Tare, M., Emmett, S. J., Coleman, H. A., Skordilis, C., Eyles, D. W., Morley, R., & Parkington, H. C. (2011). Vitamin D insufficiency is associated with impaired vascular endothelial and smooth muscle function and hypertension in young rats. *J Physiol, 589*(Pt 19), 4777-4786. doi: 10.1113/jphysiol.2011.214726

Targher, G., Bertolini, L., Padovani, R., Zenari, L., Scala, L., Cigolini, M., & Arcaro, G. (2006). Serum 25-hydroxyvitamin D3 concentrations and carotid artery intima-media thickness among type 2 diabetic patients. *Clin Endocrinol (Oxf), 65*(5), 593-597. doi: 10.1111/j.1365-2265.2006.02633.x

Thibault O, Hadley R, Landfield PW. Elevated postsynaptic [Ca2+]i and L-type calcium channel activity in aged hippocampal neurons: relationship to impaired synaptic plasticity (2001). *J Neurosci, 15*;21(24):9744-56.

Thomas, M. K., Lloyd-Jones, D. M., Thadhani, R. I., Shaw, A. C., Deraska, D. J., Kitch, B. T., . . . Finkelstein, J. S. (1998). Hypovitaminosis D in medical inpatients. *N Engl J Med, 338*(12), 777-783. doi: 10.1056/NEJM199803193381201

Thuesen, B., Husemoen, L., Fenger, M., Jakobsen, J., Schwarz, P., Toft, U., . . . Linneberg, A. (2012). Determinants of vitamin D status in a general population of Danish adults. *Bone, 50*(3), 605-610. doi: 10.1016/j.bone.2011.12.016

Thys-Jacobs, S. (2000). Micronutrients and the premenstrual syndrome: the case for calcium. *J Am Coll Nutr, 19*(2), 220-227.

Thys-Jacobs S, McMahon D, Bilezikian JP (2007). Cyclical changes in calcium metabolism across the menstrual cycle in women with premenstrual dysphoric disorder (2007).*J Clin Endocrinol Metab, 92*(8):2952-9.

Tieland, M., Brouwer-Brolsma, E. M., Nienaber-Rousseau, C., van Loon, L. J., & De Groot, L. C. (2013). Low vitamin D status is associated with reduced muscle mass and impaired physical performance in frail elderly people. *Eur J Clin Nutr*. doi: 10.1038/ejcn.2013.144

Timms, P. M., Mannan, N., Hitman, G. A., Noonan, K., Mills, P. G., Syndercombe-Court, D., . . . Boucher, B. J. (2002). Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? *QJM, 95*(12), 787-796.

TILDA: Fifty Plus in Ireland in 2011: Results form the Irish Longitudinal Study on Ageing (eds); Barrett A, Savv G, Timonen V, Kenny RA.

Tinetti, M. E., & Williams, C. S. (1998). The effect of falls and fall injuries on functioning in community-dwelling older persons. *J Gerontol A Biol Sci Med Sci, 53*(2), M112-119.

Tokuda, N., & Levy, R. B. (1996). 1,25-dihydroxyvitamin D3 stimulates phagocytosis but suppresses HLA-DR and CD13 antigen expression in human mononuclear phagocytes. *Proc Soc Exp Biol Med, 211*(3), 244-250.

Tolppanen, A. M., Williams, D. M., & Lawlor, D. A. (2011). The association of serum ionized calcium and vitamin D with adult cognitive performance. *Epidemiology, 22*(1), 113-117. doi: 10.1097/EDE.0b013e3181f74683

Tolppanen, A. M., Williams, D., & Lawlor, D. A. (2011). The association of circulating 25-hydroxyvitamin D and calcium with cognitive performance in adolescents: cross-sectional study using data from the third National Health and Nutrition Examination Survey. *Paediatr Perinat Epidemiol, 25*(1), 67-74. doi: 10.1111/j.1365-3016.2010.01171.x

Tolppanen, A. M., Sayers, A., Fraser, W. D., Lewis, G., Zammit, S., & Lawlor, D. A. (2012). The association of serum 25-hydroxyvitamin D3 and D2 with depressive symptoms in childhood--a prospective cohort study. *J Child Psychol Psychiatry, 53*(7), 757-766. doi: 10.1111/j.1469-7610.2011.02518.x

Tomac, A., Lindqvist, E., Lin, L. F., Ogren, S. O., Young, D., Hoffer, B. J., & Olson, L. (1995). Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo. Nature, 373(6512), 335-339. doi: 10.1038/373335a0

Trang, H. M., Cole, D. E., Rubin, L. A., Pierratos, A., Siu, S., & Vieth, R. (1998). Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2. *Am J Clin Nutr, 68*(4), 854-858.

Tripkovic, L., Lambert, H., Hart, K., Smith, C. P., Bucca, G., Penson, S., . . . Lanham-New, S. (2012). Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. *Am J Clin Nutr, 95*(6), 1357-1364. doi: 10.3945/ajcn.111.031070

Tuohimaa, P., Keisala, T., Minasyan, A., Cachat, J., & Kalueff, A. (2009). Vitamin D, nervous system and aging. Psychoneuroendocrinology, 34 Suppl 1, S278-286. doi: 10.1016/j.psyneuen.2009.07.003

Turner, R. T., Puzas, J. E., Forte, M. D., Lester, G. E., Gray, T. K., Howard, G. A., & Baylink, D. J. (1980). In vitro synthesis of 1 alpha,25-dihydroxycholecalciferol and 24,25-dihydroxycholecalciferol by isolated calvarial cells. *Proc Natl Acad Sci U S A, 77*(10), 5720-5724.

Ullah, M. I., Uwaifo, G. I., Nicholas, W. C., & Koch, C. A. (2010). Does vitamin d deficiency cause hypertension? Current evidence from clinical studies and potential mechanisms. *Int J Endocrinol, 2010*, 579640. doi: 10.1155/2010/579640

Ulu, S. M., Ulasli, A., Yaman, F., Yaman, G., Ozkececi, G., & Yuksel, S. (2013). The relationship between vitamin D and PTH levels and cardiovascular risk in the elderly hypertensives. *Clin Exp Hypertens*. doi: 10.3109/10641963.2013.783054

Umhau, J. C., George, D. T., Heaney, R. P., Lewis, M. D., Ursano, R. J., Heilig, M., . . . Schwandt, M. L. (2013). Low vitamin D status and suicide: a case-control study of active duty military service members. *PLoS One, 8*(1), e51543. doi: 10.1371/journal.pone.0051543

Usdin, T. B., Gruber, C., & Bonner, T. I. (1995). Identification and functional expression of a receptor selectively recognizing parathyroid hormone, the PTH2 receptor. *J Biol Chem, 270*(26), 15455-15458.

Usdin, T. B., Bonner, T. I., Harta, G., & Mezey, E. (1996). Distribution of parathyroid hormone-2 receptor messenger ribonucleic acid in rat. *Endocrinology, 137*(10), 4285-4297.

Vaidya, A., Forman, J. P., & Williams, J. S. (2011). Vitamin D and the vascular sensitivity to angiotensin II in obese Caucasians with hypertension. *J Hum Hypertens, 25*(11), 672-678. doi: 10.1038/jhh.2010.110

Vaidya, A., Pojoga, L., Underwood, P. C., Forman, J. P., Hopkins, P. N., Williams, G. H., & Williams, J. S. (2011). The association of plasma resistin with dietary sodium manipulation, the renin-angiotensin-aldosterone system, and 25-hydroxyvitamin D3 in human hypertension. *Clin Endocrinol (Oxf), 74*(3), 294-299. doi: 10.1111/j.1365-2265.2010.03922.x

Vaidya, A., Sun, B., Forman, J. P., Hopkins, P. N., Brown, N. J., Kolatkar, N. S., . . . Williams, J. S. (2011). The Fok1 vitamin D receptor gene polymorphism is associated with plasma renin activity in Caucasians. *Clin Endocrinol (Oxf), 74*(6), 783-790. doi: 10.1111/j.1365-2265.2011.03991.x

Vallianou, N., Bountziouka, V., Akalestos, T., Evangelopoulos, A., Vogiatzakis, E., Bonou, M., . . . Panagiotakos, D. B. (2012). Vitamin D status and health correlates among apparently healthy participants in an urban, sunny region. *Cent Eur J Public Health, 20*(4), 262-269.

van Dam, R. M., Snijder, M. B., Dekker, J. M., Stehouwer, C. D., Bouter, L. M., Heine, R. J., & Lips, P. (2007). Potentially modifiable determinants of vitamin D status in an older population in the Netherlands: the Hoorn Study. *Am J Clin Nutr, 85*(3), 755-761.

van der Meer, I. M., Boeke, A. J., Lips, P., Grootjans-Geerts, I., Wuister, J. D., Deville, W. L., . . . Middelkoop, B. J. (2008). Fatty fish and supplements are the greatest modifiable contributors to the serum 25-hydroxyvitamin D concentration in a multiethnic population. *Clin Endocrinol (Oxf), 68*(3), 466-472. doi: 10.1111/j.1365-2265.2007.03066.x

van der Mei, I. A., Ponsonby, A. L., Engelsen, O., Pasco, J. A., McGrath, J. J., Eyles, D. W., . . . Jones, G. (2007). The high prevalence of vitamin D insufficiency across Australian populations is only partly explained by season and latitude. *Environ Health Perspect, 115*(8), 1132-1139. doi: 10.1289/ehp.9937

van der Schaft, J., Koek, H. L., Dijkstra, E., Verhaar, H. J., van der Schouw, Y. T., & Emmelot-Vonk, M. H. (2013). The association between vitamin D and cognition: A systematic review. *Ageing Res Rev*. doi: 10.1016/j.arr.2013.05.004

van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts (2005). *J Steroid Biochem Mol Biol, 97*(1-2):93-101.

van Etten, E., Stoffels, K., Gysemans, C., Mathieu, C., & Overbergh, L. (2008). Regulation of vitamin D homeostasis: implications for the immune system. *Nutr Rev, 66*(10 Suppl 2), S125-134. doi: 10.1111/j.1753-4887.2008.00096.x

van Mierlo, L. A., Arends, L. R., Streppel, M. T., Zeegers, M. P., Kok, F. J., Grobbee, D. E., & Geleijnse, J. M. (2006). Blood pressure response to calcium supplementation: a meta-analysis of randomized controlled trials. *J Hum Hypertens, 20*(8), 571-580. doi: 10.1038/sj.jhh.1002038

Vazquez, G., de Boland, A. R., & Boland, R. L. (2000). Involvement of calmodulin in 1alpha,25-dihydroxyvitamin D3 stimulation of store-operated Ca2+ influx in skeletal muscle cells. *J Biol Chem, 275*(21), 16134-16138. doi: 10.1074/jbc.C901008199

Veng, L. M., Mesches, M. H., & Browning, M. D. (2003). Age-related working memory impairment is correlated with increases in the L-type calcium channel protein alpha1D (Cav1.3) in area CA1 of the hippocampus and both are ameliorated by chronic nimodipine treatment. *Brain Res Mol Brain Res, 110*(2), 193-202.

Verhoeven V, Vanpuyenbroeck K, Lopez-Hartmann M, Wens J, Remmen R(2012). Walk on the sunny side of life--epidemiology of hypovitaminosis D and mental health in elderly nursing home *Nutr Health Aging, 16*(4):417-20

Vierucci, F., Del Pistoia, M., Fanos, M., Gori, M., Carlone, G., Erba, P., . . . Saggese, G. (2013). Vitamin D status and predictors of hypovitaminosis D in Italian children and adolescents: a cross-sectional study. *Eur J Pediatr*. doi: 10.1007/s00431-013-2119-z

Vieth, R. (1999). Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr, 69*(5), 842-856.

Vieth, R., Pinto, T. R., Reen, B. S., & Wong, M. M. (2002). Vitamin D poisoning by table sugar. *Lancet, 359*(9307), 672. doi: 10.1016/S0140-6736(02)07814-5

Vieth R, Kimball S, Hu A, Walfish PG (2004). Randomised comparison of the effects of the vitamin D3 adequate intake versus 4000 IU per day on biochemical responses and the wellbeing of patients. *Nutr J, 19*;3:8.

Vieth, R. (2004). Why the optimal requirement for Vitamin D3 is probably much higher than what is officially recommended for adults. *J Steroid Biochem Mol Biol, 89-90*(1-5), 575-579. doi: 10.1016/j.jsbmb.2004.03.038

vinh quoc Lu'o'ng, K., & Nguyen, L. T. (2013). The beneficial role of vitamin D in obesity: possible genetic and cell signaling mechanisms. *Nutr J, 12*, 89. doi: 10.1186/1475-2891-12-89

Wakasugi, M., Noguchi, T., Inoue, M., Kazama, Y., Tawata, M., Kanemaru, Y., & Onaya, T. (1991). Vitamin D3 stimulates the production of prostacyclin by vascular smooth muscle cells. *Prostaglandins, 42*(2), 127-136.

Walbert, T., Jirikowski, G. F., & Prufer, K. (2001). Distribution of 1,25-dihydroxyvitamin D3 receptor immunoreactivity in the limbic system of the rat. *Horm Metab Res, 33*(9), 525-531. doi: 10.1055/s-2001-17210

Walker, M. D., Fleischer, J., Rundek, T., McMahon, D. J., Homma, S., Sacco, R., & Silverberg, S. J. (2009). Carotid vascular abnormalities in primary hyperparathyroidism. *J Clin Endocrinol Metab, 94*(10), 3849-3856. doi: 10.1210/jc.2009-1086

Walker, M. D., McMahon, D. J., Inabnet, W. B., Lazar, R. M., Brown, I., Vardy, S., . . . Silverberg, S. J. (2009). Neuropsychological features in primary hyperparathyroidism: a prospective study. *J Clin Endocrinol Metab, 94*(6), 1951-1958. doi: 10.1210/jc.2008-2574

Walling, M. W. (1977). Intestinal Ca and phosphate transport: differential responses to vitamin D3 metabolites. *Am J Physiol, 233*(6), E488-494.

Walling, M. W., Hartenbower, D. L., Coburn, J. W., & Norman, A. W. (1977). Effects of 1 alpha,25-, 24R,25-, and 1 alpha,24R,25-hydroxylated metabolites of vitamin D3 on calcium and phosphate absorption by duodenum from intact and nephrectomized rats. *Arch Biochem Biophys, 182*(1), 251-257.

Wallingford, S. C., Jones, G., Kobayashi, L. C., Grundy, A., Miao, Q., Tranmer, J., & Aronson, K. J. (2013). UV and dietary predictors of serum 25-hydroxyvitamin D concentrations among young shift-working nurses and implications for bone density and skin cancer. *Public Health Nutr*, 1-8. doi: 10.1017/S1368980013001754

Walters, M. R., Ilenchuk, T. T., & Claycomb, W. C. (1987). 1,25-Dihydroxyvitamin D3 stimulates 45Ca2+ uptake by cultured adult rat ventricular cardiac muscle cells. *J Biol Chem, 262*(6), 2536-2541.

Wamberg, L., Pedersen, S. B., Richelsen, B., & Rejnmark, L. (2013). The effect of high-dose vitamin d supplementation on calciotropic hormones and bone mineral density in obese subjects with low levels of circulating 25-hydroxyvitamin d: results from a randomized controlled study. *Calcif Tissue Int, 93*(1), 69-77. doi: 10.1007/s00223-013-9729-3

Wang J.Y, Wu J.N, Cherng T.L, Hoffer B.J, Chen H.H, Borlongan C.V, Wang Y (2001). Vitamin D(3) attenuates 6-hydroxydopamine-induced neurotoxicity in rats. *Brain Res, 15*;904(1):67-75.

Wang, L., Manson, J. E., Buring, J. E., Lee, I. M., & Sesso, H. D. (2008). Dietary intake of dairy products, calcium, and vitamin D and the risk of hypertension in middle-aged and older women. *Hypertension, 51*(4), 1073-1079. doi: 10.1161/HYPERTENSIONAHA.107.107821

Wang, L., Hara, K., Van Baaren, J. M., Price, J. C., Beecham, G. W., Gallins, P. J., . . . Gilbert, J. R. (2012). Vitamin D receptor and Alzheimer's disease: a genetic and functional study. *Neurobiol Aging, 33*(8), 1844 e1841-1849. doi: 10.1016/j.neurobiolaging.2011.12.038

Wang, L., Ma, J., Manson, J. E., Buring, J. E., Gaziano, J. M., & Sesso, H. D. (2012). A prospective study of plasma vitamin D metabolites, vitamin D receptor gene polymorphisms, and risk of hypertension in men. *Eur J Nutr*. doi: 10.1007/s00394-012-0480-8

Wang, L., Song, Y., Manson, J. E., Pilz, S., Marz, W., Michaelsson, K., . . . Sesso, H. D. (2012). Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease: a meta-analysis of prospective studies. *Circ Cardiovasc Qual Outcomes, 5*(6), 819-829. doi: 10.1161/CIRCOUTCOMES.112.967604

Wang, T. T., Tavera-Mendoza, L. E., Laperriere, D., Libby, E., MacLeod, N. B., Nagai, Y., . . . White, J. H. (2005). Large-scale in silico and microarray-based identification of direct 1,25-dihydroxyvitamin D3 target genes. *Mol Endocrinol, 19*(11), 2685-2695. doi: 10.1210/me.2005-0106

Wang, Y., Chiang, Y. H., Su, T. P., Hayashi, T., Morales, M., Hoffer, B. J., & Lin, S. Z. (2000). Vitamin D(3) attenuates cortical infarction induced by middle cerebral arterial ligation in rats. *Neuropharmacology, 39*(5), 873-880.

Wang, Y., Liu, X. J., Robitaille, L., Eintracht, S., Macnamara, E., & Hoffer, L. J. (2013). Effects of vitamin C and vitamin D administration on mood and distress in acutely hospitalized patients. *Am J Clin Nutr*. doi: 10.3945/ajcn.112.056366

Ward, K. A., Das, G., Berry, J. L., Roberts, S. A., Rawer, R., Adams, J. E., & Mughal, Z. (2009). Vitamin D status and muscle function in post-menarchal adolescent girls. *J Clin Endocrinol Metab, 94*(2), 559-563. doi: 10.1210/jc.2008-1284

Watson, K. E., Abrolat, M. L., Malone, L. L., Hoeg, J. M., Doherty, T., Detrano, R., & Demer, L. L. (1997). Active serum vitamin D levels are inversely correlated with coronary calcification. *Circulation, 96*(6), 1755-1760.

Weaver, D. R., Deeds, J. D., Lee, K., & Segre, G. V. (1995). Localization of parathyroid hormone-related peptide (PTHrP) and PTH/PTHrP receptor mRNAs in rat brain. *Brain Res Mol Brain Res, 28*(2), 296-310.

Webb, A. R., Kline, L., & Holick, M. F. (1988). Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab, 67*(2), 373-378.

Webb, A. R., DeCosta, B. R., & Holick, M. F. (1989). Sunlight regulates the cutaneous production of vitamin D3 by causing its photodegradation. *J Clin Endocrinol Metab, 68*(5), 882-887.

Wehr, T. A., & Rosenthal, N. E. (1989). Seasonality and affective illness. *Am J Psychiatry, 146*(7), 829-839.

Weick, M. T. (1967). A history of rickets in the United States. *Am J Clin Nutr, 20*(11), 1234-1241.

Weishaar, R. E., & Simpson, R. U. (1987). Vitamin D3 and cardiovascular function in rats. *J Clin Invest, 79*(6), 1706-1712. doi: 10.1172/JCI113010

Weisman, Y., Harell, A., Edelstein, S., David, M., Spirer, Z., & Golander, A. (1979). 1 alpha, 25-Dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 in vitro synthesis by human decidua and placenta. *Nature, 281*(5729), 317-319.

Wellington, K., & Mulla, Z. D. (2012). Seasonal trend in the occurrence of preeclampsia and eclampsia in Texas. *Am J Hypertens, 25*(1), 115-119. doi: 10.1038/ajh.2011.173

Weng, S., Sprague, J. E., Oh, J., Riek, A. E., Chin, K., Garcia, M., & Bernal-Mizrachi, C. (2013). Vitamin D deficiency induces high blood pressure and accelerates atherosclerosis in mice. *PLoS One, 8*(1), e54625. doi: 10.1371/journal.pone.0054625

Walker DG, Link J, Lue LF, sing-Hernandez JE, Boyes BE. Gene expression changes by amyloid beta peptide-stimulated human postmortem brain microglia identify activation of multiple inflammatory processes (2006). *J Leukoc Biol 79*:596–610

Whitehouse, A. J., Holt, B. J., Serralha, M., Holt, P. G., Kusel, M. M., & Hart, P. H. (2012). Maternal serum vitamin D levels during pregnancy and offspring neurocognitive development. *Pediatrics, 129*(3), 485-493. doi: 10.1542/peds.2011-2644

Whiting SJ, Green TJ, Calvo MS (2007). Vitamin D intakes in North America and Asia-Pacific countries are not sufficient to prevent vitamin D insufficiency. J Steroid Biochem Mol Biol. ,103(3-5):626-30.

Wicherts, I. S., van Schoor, N. M., Boeke, A. J., Visser, M., Deeg, D. J., Smit, J., . . . Lips, P. (2007). Vitamin D status predicts physical performance and its decline in older persons. *J Clin Endocrinol Metab, 92*(6), 2058-2065. doi: 10.1210/jc.2006-1525

Wilhelm-Leen, E. R., Hall, Y. N., Deboer, I. H., & Chertow, G. M. (2010). Vitamin D deficiency and frailty in older Americans. *J Intern Med, 268*(2), 171-180. doi: 10.1111/j.1365-2796.2010.02248.x

Wilkins, C. H., Sheline, Y. I., Roe, C. M., Birge, S. J., & Morris, J. C. (2006). Vitamin D deficiency is associated with low mood and worse cognitive performance in older adults. *Am J Geriatr Psychiatry, 14*(12), 1032-1040. doi: 10.1097/01.JGP.0000240986.74642.7c

Williams, D. M., Fraser, A., & Lawlor, D. A. (2011). Associations of vitamin D, parathyroid hormone and calcium with cardiovascular risk factors in US adolescents. *Heart, 97*(4), 315-320. doi: 10.1136/hrt.2010.203224

Windaus A, Bock F (1937). Uber das provitamin aus dem sterin der schweineschwarte. Hoppe-Seylers Z Physiol Chem 245:168.

Witham, M. D., Dove, F. J., Dryburgh, M., Sugden, J. A., Morris, A. D., & Struthers, A. D. (2010). The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. *Diabetologia, 53*(10), 2112-2119. doi: 10.1007/s00125-010-1838-1

Witham, M. D., Nadir, M. A., & Struthers, A. D. (2009). Effect of vitamin D on blood pressure: a systematic review and meta-analysis. *J Hypertens, 27*(10), 1948-1954. doi: 10.1097/HJH.0b013e32832f075b

Witham, M. D., Nadir, M. A., & Struthers, A. D. (2009). Effect of vitamin D on blood pressure: a systematic review and meta-analysis. *J Hypertens, 27*(10), 1948-1954. doi: 10.1097/HJH.0b013e32832f075b

Witham, M. D., Dove, F. J., Sugden, J. A., Doney, A. S., & Struthers, A. D. (2012). The effect of vitamin D replacement on markers of vascular health in stroke patients - a randomised controlled trial. *Nutr Metab Cardiovasc Dis, 22*(10), 864-870. doi: 10.1016/j.numecd.2010.11.001

Witham, M. D., Price, R. J., Struthers, A. D., Donnan, P. T., Messow, C. M., Ford, I., & McMurdo, M. E. (2013). Cholecalciferol Treatment to Reduce Blood Pressure in Older Patients With Isolated Systolic Hypertension: The VitDISH Randomized Controlled Trial. *JAMA Intern Med.* doi: 10.1001/jamainternmed.2013.9043

Wolf, G. (2004). The discovery of vitamin D: the contribution of Adolf Windaus. *J Nutr, 134*(6), 1299-1302.

Wolfson, L., Judge, J., Whipple, R., & King, M. (1995). Strength is a major factor in balance, gait, and the occurrence of falls. *J Gerontol A Biol Sci Med Sci, 50 Spec No*, 64-67.

Wong, M. S., Delansorne, R., Man, R. Y., Svenningsen, P., & Vanhoutte, P. M. (2010). Chronic treatment with vitamin D lowers arterial blood pressure and reduces endothelium-dependent contractions in the aorta of the spontaneously hypertensive rat. *Am J Physiol Heart Circ Physiol, 299*(4), H1226-1234. doi: 10.1152/ajpheart.00288.2010

Wong, Y. Y., McCaul, K. A., Yeap, B. B., Hankey, G. J., & Flicker, L. (2013). Low vitamin D status is an independent predictor of increased frailty and all-cause mortality in older men: the Health In Men Study. *J Clin Endocrinol Metab*. doi: 10.1210/jc.2013-1702

Wood, A. D., Secombes, K. R., Thies, F., Aucott, L., Black, A. J., Mavroeidi, A., . . . Macdonald, H. M. (2012). Vitamin D3 supplementation has no effect on conventional cardiovascular risk factors: a parallel-group, double-blind, placebo-controlled RCT. *J Clin Endocrinol Metab, 97*(10), 3557-3568. doi: 10.1210/jc.2012-2126

Wortsman, J., Matsuoka, L. Y., Chen, T. C., Lu, Z., & Holick, M. F. (2000). Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr, 72*(3), 690-693.

Wu, S. H., Ho, S. C., & Zhong, L. (2010). Effects of vitamin D supplementation on blood pressure. *South Med J, 103*(8), 729-737. doi: 10.1097/SMJ.0b013e3181e6d389

Wu, X., Vieth, R., Milojevic, S., Sonnenberg, H., & Melo, L. G. (2000). Regulation of sodium, calcium and vitamin D metabolism in Dahl rats on a high-salt/low-potassium diet: genetic and neural influences. *Clin Exp Pharmacol Physiol, 27*(5-6), 378-383.

Xiang, Z., Ho, L., Yemul, S., Zhao, Z., Qing, W., Pompl, P., . . . Pasinetti, G. M. (2002). Cyclooxygenase-2 promotes amyloid plaque deposition in a mouse model of Alzheimer's disease neuropathology. *Gene Expr, 10*(5-6), 271-278.

Yalamanchili, V., & Gallagher, J. C. (2012). Treatment with hormone therapy and calcitriol did not affect depression in older postmenopausal women: no interaction with estrogen and vitamin D receptor genotype polymorphisms. *Menopause, 19*(6), 697-703. doi: 10.1097/gme.0b013e31823bcec5

Yiu, Y. F., Chan, Y. H., Yiu, K. H., Siu, C. W., Li, S. W., Wong, L. Y., . . . Tse, H. F. (2011). Vitamin D deficiency is associated with depletion of circulating endothelial progenitor cells and endothelial dysfunction in patients with type 2 diabetes. *J Clin Endocrinol Metab, 96*(5), E830-835. doi: 10.1210/jc.2010-2212

Yoshikawa, S., Nakamura, T., Tanabe, H., & Imamura, T. (1979). Osteomalacic myopathy. *Endocrinol Jpn, 26*(Suppl), 65-72.

Yoshimura, N., Muraki, S., Oka, H., Morita, M., Yamada, H., Tanaka, S., . . . Akune, T. (2013). Profiles of vitamin D insufficiency and deficiency in Japanese men and women: association with biological, environmental, and nutritional factors and coexisting disorders: the ROAD study. *Osteoporos Int.* doi: 10.1007/s00198-013-2372-z

Young, E. W., McCarron, D. A., & Morris, C. D. (1990). Calcium regulating hormones in essential hypertension. Importance of gender. *Am J Hypertens, 3*(8 Pt 2), 161S-166S.

Yu, J., Gattoni-Celli, M., Zhu, H., Bhat, N. R., Sambamurti, K., Gattoni-Celli, S., & Kindy, M. S. (2011). Vitamin D3-enriched diet correlates with a decrease of amyloid plaques in the brain of AbetaPP transgenic mice. *J Alzheimers Dis, 25*(2), 295-307. doi: 10.3233/JAD-2011-101986

Zagura, M., Serg, M., Kampus, P., Zilmer, M., Eha, J., Unt, E., . . . Kals, J. (2011). Aortic stiffness and vitamin D are independent markers of aortic calcification in patients with peripheral arterial disease and in healthy subjects. *Eur J Vasc Endovasc Surg, 42*(5), 689-695. doi: 10.1016/j.ejvs.2011.07.027

Zanello, S. B., Boland, R. L., & Norman, A. W. (1995). cDNA sequence identity of a vitamin D-dependent calcium-binding protein in the chick to calbindin D-9K. *Endocrinology, 136*(6), 2784-2787.

Zehnder, D., Bland, R., Williams, M. C., McNinch, R. W., Howie, A. J., Stewart, P. M., & Hewison, M. (2001). Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. *J Clin Endocrinol Metab, 86*(2), 888-894.

Zemel, M. B. (2001). Calcium modulation of hypertension and obesity: mechanisms and implications. *J Am Coll Nutr, 20*(5 Suppl), 428S-435S; discussion 440S-442S.

Zerwekh, J. E. (2008). Blood biomarkers of vitamin D status. *Am J Clin Nutr, 87*(4), 1087S-1091S.

Zhao, G., Ford, E. S., Li, C., & Balluz, L. S. (2010). No associations between serum concentrations of 25-hydroxyvitamin D and parathyroid hormone and depression among US adults. *Br J Nutr, 104*(11), 1696-1702. doi: 10.1017/S0007114510002588

Zhao, G., Ford, E. S., Li, C., Kris-Etherton, P. M., Etherton, T. D., & Balluz, L. S. (2010). Independent associations of serum concentrations of 25-hydroxyvitamin D and parathyroid hormone with blood pressure among US adults. *J Hypertens, 28*(9), 1821-1828. doi: 10.1097/HJH.0b013e32833bc5b4

Zhou, C., Lu, F., Cao, K., Xu, D., Goltzman, D., & Miao, D. (2008). Calcium-independent and 1,25(OH)2D3-dependent regulation of the renin-angiotensin system in 1alpha-hydroxylase knockout mice. *Kidney Int, 74*(2), 170-179. doi: 10.1038/ki.2008.101

Zhu, W., Cai, D., Wang, Y., Lin, N., Hu, Q., Qi, Y., . . . Amarasekara, S. (2013). Calcium plus vitamin D3 supplementation facilitated fat loss in overweight and obese college students with very-low calcium consumption: a randomized controlled trial. *Nutr J, 12*, 8. doi: 10.1186/1475-2891-12-8

Zhu, Y., Mahon, B. D., Froicu, M., & Cantorna, M. T. (2005). Calcium and 1 alpha,25-dihydroxyvitamin D3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease. *Eur J Immunol, 35*(1), 217-224. doi: 10.1002/eji.200425491

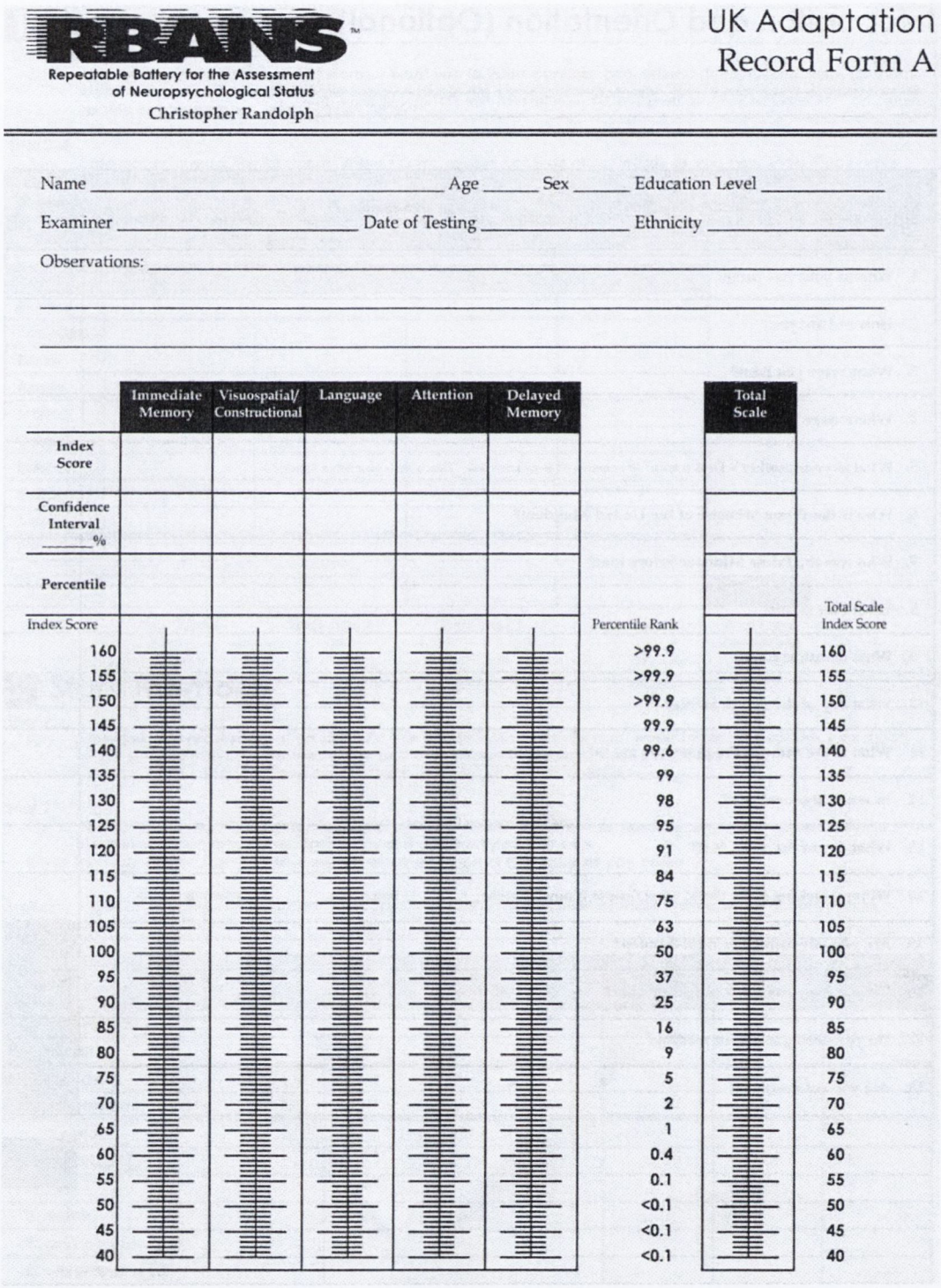
Zhu, Y., Zhou, R., Yang, R., Zhang, Z., Bai, Y., Chang, F., . . . Chen, L. (2012). Abnormal neurogenesis in the dentate gyrus of adult mice lacking 1,25-dihydroxy vitamin D3 (1,25-(OH)2 D3). *Hippocampus, 22*(3), 421-433. doi: 10.1002/hipo.20908

Zhao Y, Sun Y, Ji HF, Shen L (2013). Vitamin D levels in Alzheimer's and Parkinson's diseases: a meta-analysis. *Nutrition, 29*(6):828-32.

Zittermann, A., Frisch, S., Berthold, H. K., Gotting, C., Kuhn, J., Kleesiek, K., . . . Koerfer, R. (2009). Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. *Am J Clin Nutr, 89*(5), 1321-1327. doi: 10.3945/ajcn.2008.27004

APPENDIX

Repeatable Battery for the Assessment of Neuropsychological Status.


RBANS™
Repeatable Battery for the Assessment of Neuropsychological Status
Christopher Randolph

UK Adaptation
Record Form A

Name _____ Age _____ Sex _____ Education Level _____

Examiner _____ Date of Testing _____ Ethnicity _____

Observations: _____

	Immediate Memory	Visuospatial/ Constructional	Language	Attention	Delayed Memory		Total Scale	
Index Score								
Confidence Interval ____%								
Percentile								
Index Score						Percentile Rank		Total Scale Index Score
160						>99.9		160
155						>99.9		155
150						>99.9		150
145						99.9		145
140						99.6		140
135						99		135
130						98		130
125						95		125
120						91		120
115						84		115
110						75		110
105						63		105
100						50		100
95						37		95
90						25		90
85						16		85
80						9		80
75						5		75
70						2		70
65						1		65
60						0.4		60
55						0.1		55
50						<0.1		50
45						<0.1		45
40						<0.1		40

Manual not shown due to copyright.

Frontal Assessment Battery

1. Similarities (conceptualization)
"In what way are they alike?"

- A banana and an orange

(In the event of total failure: "they are not alike" or partial failure: "both have peel," help the patient by saying: "both a banana and an orange are fruit"; but credit 0 for the item; do not help the patient for the two following items)

- A table and a chair
- A tulip, a rose and a daisy

Score (only category responses [fruits, furniture, flowers] are considered correct)

Three correct: 3 Two correct: 2 One correct: 1 None correct: 0

2. Lexical fluency (mental flexibility)
"Say as many words as you can beginning with the letter 'S,' any words except surnames or proper nouns."

If the patient gives no response during the first 5 seconds, say: "for instance, snake." If the patient pauses 10 seconds, stimulate him by saying: "any word beginning with the letter 'S.' The time allowed is 60 seconds.

Score (word repetitions or variations [shoe, shoemaker], surnames, or proper nouns are not counted as correct responses)

> 9 words: 3 6 -9 words: 2 3 -5 words: 1 < 3 words: 0

3. Motor series "Luria" test (programming)
"Look carefully at what I'm doing."

The examiner, seated in front of the patient, performs alone three times with his left hand the series of "fist–edge–palm."
"Now, with your right hand do the same series, first with me, then alone."
The examiner performs the series three times with the patient, then says to him/her:
"Now, do it on your own."

Score

Patient performs six correct consecutive series alone: 3
Patient performs at least three correct consecutive series alone: 2
Patient fails alone, but performs three correct consecutive series with the examiner: 1
Patient cannot perform three correct consecutive series even with the examiner: 0

4. Conflicting instructions (sensitivity to interference)
"Tap twice when I tap once."
To ensure that the patient has understood the instruction, a series of 3 trials is run: 1-1-1.

"Tap once when I tap twice."
To ensure that the patient has understood the instruction, a series of 3 trials is run: 2-2-2.

The examiner then performs the following series: 1-1-2-1-2-2-2-1-1-2.

Score No errors: 3 1 -2 errors: 2 > 2 errors: 1
Patient taps like the examiner at least four consecutive times: 0

5. Go–No Go (inhibitory control)
"Tap once when I tap once."
To ensure that the patient has understood the instruction, a series of 3 trials is run: 1-1-1.

"Do not tap when I tap twice."
To ensure that the patient has understood the instruction, a series of 3 trials is run: 2-2-2.

The examiner then performs the following series: 1-1-2-1-2-2-2-1-1-2.

Score No errors: 3 1 -2 errors: 2 > 2 errors: 1
Patient taps like the examiner at least four consecutive times: 0

6. Prehension behaviour (environmental autonomy)

"Do not take my hands."

The examiner is seated in front of the patient. Place the patient's hands palm up on his knees. Without saying anything or looking at the patient, the examiner brings his own hands close to the patient's hands and touches the palms of both the patient's hands, to see if he will spontaneously take them. If the patient takes the examiner's hands, try again after asking the patient: "Now, do not take my hands."

Score Patient does not take the examiner's hands: 3
Patient hesitates and asks what he/she has to do: 2
Patient takes the hands without hesitation: 1
Patient takes the examiner's hand even after he/she has been told not to do so: 0

DEPRESSION (CES-D)

Below is a list of the ways you might have felt or behaved. Please respond on how often you have felt this way during the PAST WEEK, by ticking the most appropriate box.

I was bothered by things that usually don't bother me	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I did not feel like eating; my appetite was poor	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I felt that I could not shake off the blues even with help from my family or friends	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I felt that I was just as good as other people	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I had trouble keeping my mind on what I was doing	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I felt depressed	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I felt that everything I did was an effort	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I felt hopeful about the future	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I thought my life had been a failure	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I felt fearful	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*

My sleep was restless	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I was happy	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I talked less than usual	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I felt lonely	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
People were unfriendly	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I enjoyed life	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I had crying spells	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I felt sad	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I felt that people disliked me	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 day)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*
I could not get "going"	☐ *Never or Rarely (less than 1 day)* ☐ *Some of the time (1-2 days)* ☐ *Occasionally (3-4 days)* ☐ *Most of the time (5-7 days)*

PHYSICAL SELF MAINTENANCE SCALE (PSMS)

1) Do you eat:

3 Without any help
2 With some help (e.g. cutting food, etc.)
1 Someone must feed me

2) Do you dress and undress yourself:

3 Without help (able to pick out clothes & dress)
2 With some help
1 No, someone must dress and undress me

3) Do you take care of your own appearance? (e.g. combing your hair, or for *men*, shaving)

3 Yes, without help
2 Yes, with some help
1 No, someone must help me with this type of thing

4) Are you able to get around your house/apartment without any help?

3 Yes, without help of any kind (except a cane)
2 Yes, with some help (from a person, walker, crutches, or chair)
1 No, I cannot get around my home unless someone moves me

5) Are you able to get in and out of bed yourself?

3 Yes, without help or aid
2 Yes, with some help (from a person or device)
1 No, I cannot get out of bed unless someone lifts me

6) Are you able to bathe, --that is, take a bath, shower, or sponge bath by yourself?

3 Yes, without help
2 Yes, with some help (from a person or device)
1 No, someone must bathe me and lift me in and out of the bath

7) a. Do you ever have trouble getting to the bathroom on time?

1 Yes
2 No

b. About how often would you wet or soil yourself during the day or night?

4 Never
3 Less than once a week
2 Once or twice a week
1 Three times a week or more

INSTRUMENTAL ACTIVITIES OF DAILY LIVING

1) Can you the use the telephone?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to use the telephone

2) Can you get to places out of walking distance?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to travel unless special arrangements are made

3) Can you go shopping for groceries?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to do any shopping

4) Can you prepare your own meals?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to prepare meals

5) Can you do your own housework?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to do my own housework

6) Can you do your own handyman work?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to do my own handyman work

7) Can you do your own laundry?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to do any laundry at all

8) a. Do you take or use any medications?

1 Yes
2 No

b. Do you take your own medicine?

c. If you had to take medicine, can you do it:

3 Without help, taking the right dose at the right time
2 With some help (e.g. someone prepares it for you, or reminds you)
1 I am completely unable to take my own medicines

9) Can you manage your own money?

3 Yes, without help
2 Yes, with some help
1 No, I am completely unable to handle money