Human beings are members of a whole,
In creation of one essence and soul.
If one member is afflicted with pain,
Other members uneasy will remain.
If you've no sympathy for human pain,
The name of human you cannot retain!

Saadi Shirazi (Persian Poet)

Middle Eastern Women's Health Study-Phase II

The Effect of Monthly 50,000 IU or 100,000 IU Vitamin D Supplements on Vitamin D Status in Pre-menopausal Middle Eastern Women Living in Auckland

A research report presented in partial fulfilment of the requirements for the degree of Master of Sciences in Human Nutrition

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Abstract

Background: Middle Eastern women are at increased risk of vitamin D deficiency/insufficiency due to a number of specific lifestyle risk factors. Vitamin D supplements (50,000 IU/month) are prescribed by General Practitioners to correct vitamin D deficiency in this population in New Zealand. However, no research has investigated whether this dose of vitamin D supplement is useful for vitamin D deficiency treatment in Middle Eastern women or if larger doses are needed.

Objectives: The primary objective of this study was to conduct a double-blind, randomised, placebo-controlled trial with vitamin D supplementation for 6 months. We aimed to assess the adequacy of supplementation with monthly 50,000 IU and 100,000 IU in optimising serum 25(OH)D concentrations (≥ 50 nmol/L and ≥ 75 nmol/L) in a group of Middle Eastern premenopausal women living in Auckland. The secondary objective was to identify those factors affecting serum 25(OH)D response to the given doses of vitamin D supplements. Results from this study will help medical practitioners to provide the best options for treating vitamin D deficiency in Middle Eastern women living in New Zealand.

Method: Women of Middle Eastern origin, ≥ 20 years old and in premenopausal stage, having no major illness, living in Auckland (n=62) were recruited for the study in winter 2013. All were required to take study tablets (50,000, 100,000 IU or placebo/month) for 6 months and were required to visit the Human Nutrition Research Unit at Massey University on 3 occasions (baseline, 3-months, and 6-months). Blood samples were collected to measure serum 25(OH)D concentrations and calcium levels. Participants were required to complete questionnaires about their demographics, medical history, skin colour, lifestyle change and physical activity level. Height, weight, body fat percentage (BFP) and blood pressure were measured. Participants were also required to complete four day food dairies. The primary outcomes were the changes in serum 25(OH)D concentration and serum calcium level.

Results: Mean baseline serum 25(OH)D was 46.0±15.0 nmol/L. Supplementation with 50,000 IU/month and 100,000 IU raised the mean serum 25(OH)D concentrations from a baseline of 44.0±16.0 and 48.0±11.0 nmol/L to 70.0±15.0 and 82.0±17.0 nmol/L at 6 months, respectively (P<0.001 for both treatment groups). The mean serum 25(OH)D concentration of women assigned to placebo group increased from 45.0±18.0 nmol/L at baseline to 54.0±18.0 nmol/L at 6 months (P<0.01). The mean serum 25(OH)D concentrations reached a

plateau after 3 months of supplementation. Of 62 women, 59.7% had serum 25(OH)D concentrations <50 nmol/L and only 3.3% had serum 25(OH)D ≥ 75 nmol/L. At 6 months, the proportion of subjects achieving serum 25(OH)D concentration of 75 nmol/L or more was 31.6% and 66.7% in women receiving monthly 50,000 IU and 100,000 IU, respectively (P=0.002). There were no reports of hypervitaminosis D (serum 25(OH)D >225 nmol/L) or hypercalcemia (serum calcium ≥ 2.7 mmol/L). Response to vitamin D supplementation varied widely (increasing 1.0 to 80.0 nmol/L). In a regression analysis, dose (P<0.001), baseline serum 25(OH)D concentration (P<0.001) and baseline BFP (P=0.01) were the only variables to reach statistical significance as predictors of the change in serum 25(OH)D over 6 months.

Conclusion: The prevalence of vitamin D deficiency/insufficiency was high in this study population highlighting the significance of the situation. Monthly intake of 100,000 IU vitamin D for 6 months was more effective than 50,000 IU in achieving serum 25(OH)D concentrations of 75 nmol/L, though it did not ensure a serum 25(OH)D concentration of 75 nmol/L or more in all people. Factors affecting serum 25(OH)D response to supplementation should be taken into account when an optimal dose for individuals is determined. The unexpectedly large variance in serum 25(OH)D response to a fixed dose of vitamin D highlights the importance of follow up and measurements of serum 25(OH)D when supplementation is used in clinical practice.

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- My husband for his ongoing support and encouragement

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List of abbreviations

-2LL	-2-Log Likelihood
1,25(OH)$_2$D	1, 25-dihydroxyvitamin D
24,25(OH)$_2$D	24, 25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
25(OH)D-26,23 lactone	25-hydroxyvitamin D-26,23 Lactone
AI	Adequate Intake
ANCOVA	Analysis of Covariance
AUC	Area Under the Curve
BFP	Body Fat Percentage
BIA	Bioelectrical Impedance Analyser
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BP	Blood Pressure
Ca	Calcium
CHD	Coronary Heart Disease
CI	Confidence Interval
CV	Cardiovascular
CVD	Cardiovascular Disease
CYP2R1	Cytochrome P450, Family 2, Subfamily R, Polypeptide 1
CYP24A1	Cytochrome P450, Family 24, Subfamily A, Polypeptide 1
CYP27B1	Cytochrome P450, Family 27, Subfamily B, Polypeptide 1
d	Day
D_2	Ergocalciferol
D_3	Cholecalciferol
DCs	Dendritic Cells
DHBs	District Health Boards
DHCR7	7-dihydrocholesterol Reductase
FGF23	Fibroblast Growth Factor 23
FPB	Fasting Plasma Blood
GC	Group Specific Component gene
g	Gram

List of abbreviations

GP	General Practitioner
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HOMA-ß	Homeostatic Model Assessment of Beta Cell Function
IGI	Insulinogenic Index
IL-6	Interleukin 6
IL-10	Interleukin 10
IM	Intramuscularly
IOM	Institute of Medicine
IPAQ	International Physical Activity Questionnaire
iPTH	Intact Parathyroid Hormone
IU	International Unit
kg	Kilogram
LASA study	Longitudinal Aging Study Amsterdam study
log	Logarithm
MED	Minimum Erythemal Dose
MELAA	Middle Eastern, Latin American, African
mg	Milligram
Mg/dl	Milligram per Decilitre
MI	Myocardial Infarction
ml	Millilitre
mmHg	Millimetre of Mercury
mo	Month
MS	Multiple Sclerosis
MUFA	Monounsaturated Fatty Acid
m^2	Metre Squared
n	Number
N	North
ng/ml	Nanograms per Millilitre
nmol/L	Nanomol per Litre
NZPAQ-SF	New Zealand Physical Activity Questionnaire-Short Form
NZ$	New Zealand Dollar
OFELY	Os des Femmes de Lyon
OPRA Trial	Osteoporotic Prospective Risk Assessment Trial

List of abbreviations

OR	Odds Ratio
oz	Ounce
PHARMAC	The Pharmaceutical Management Agency
Pmol/mg	Picomole per milligram
PTH	Parathyroid Hormone
PUFA	Polyunsaturated Fatty Acid
QUICKI	Quantitative Insulin Sensitivity Check Index
RCT	Randomised Controlled Trial
RDA	Recommended Daily Allowance
RR	Relative Risk
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
SPF	Sun Protection Factor
SZA	Solar Zenith Angle
TNF-α	Tumour Necrosis Factor alpha
T1DM	Type 1 Diabetes Mellitis
UAE	United Arab Emirates
UK	United Kingdom
US NHANES III	The United States National Health And Nutrition Examination Survey 3
UV	Ultraviolet
UVB	Ultraviolet Beta radiation
US$	United States dollar
VDBP	Vitamin D Binding Protein
VDR	Vitamin D Receptor
vs.	Versus
y	Year
µg	Microgram
µmol/L	Micromole per litre

Chapter 1: Preface

Introduction

Vitamin D has a well-established role in bone metabolism, and in calcium & phosphorus homeostasis, and its deficiency is associated with several adverse health outcomes. The serious clinical signs of vitamin D deficiency are rickets in children and osteomalacia in adults. Serum 25(OH)D status has been, also, associated with musculoskeletal [3] and non-musculoskeletal health outcomes [4]. The discovery of vitamin D receptors in variety of cells has provided a clue for scientists to investigate relationships between serum 25(OH)D levels and several diseases. Recent research has shown relationships between serum 25(OH)D status and cancer, cardiovascular diseases (CVD) and autoimmune diseases such as type 1 diabetes and multiple sclerosis [5].

Concurrent with these fascinating discoveries is the re-emergence of vitamin D deficiency/insufficiency as a worldwide public health issue [6]. Evidence shows that Middle Eastern women living in their home country [7-13] or in other countries as immigrants [14, 15] have low serum 25(OH)D concentrations. As a result of many lifestyle risk factors, these women are at greater risk of vitamin D deficiency regardless of where they live. Anecdotal evidence [16] and the results from our previous pilot study [17] are indicative of high prevalence of vitamin D deficiency in women of Middle Eastern origin living in Auckland, New Zealand whose population is steadily increasing.

The Middle Eastern population has been increasing in New Zealand and more specifically in Auckland. Middle Eastern is the largest of MELAA (ethnicities categorized by Auckland District Health Board; Middle Eastern, Latin American and African) populations in Auckland [16]. In 2006, the Middle Eastern population accounted for 0.4% (n=17,457) and 0.8% (n=10,692) of the total New Zealand and Auckland population, respectively, with a population growth of 5% per annum since then. Based on MELAA grouping, Iraqis (21.7%) and Iranians (20.7%) are the largest populations [16]. Unless there are major changes in political and economical status of countries in the Middle Eastern region, New Zealand will probably continue to see a flow of refugees at a similar or higher rate to that which we have seen over the past decade. In New Zealand, these populations have higher prevalence of CVD and type 2 diabetes than Europeans, and have a high rate of mental health utilization [16]. On the basis of available evidence, one can postulate that vitamin D deficiency might be a contributing factor.

A pilot study conducted at the research unit at Massey University revealed that 100% of the study population had serum 25(OH)D concentrations <50 nmol/L, with 75% being vitamin D deficient (25(OH)D <25 nmol/L). Yet, despite 21% of women using vitamin D supplements prescribed by General Practitioners (GPs) (50,000 IU/month), and 37% taking vitamin D containing supplements (200-600 IU/tablet), these women did not have optimal vitamin D concentrations. Although prescribed vitamin D supplement use was significantly associated with increased levels of serum 25(OH)D, none of the participants reached the sufficient cut-off levels of ≥ 50 nmol/L [18]. These findings agree with those from the Middle Eastern region in that vitamin D deficiency is highly prevalent in this population [8-12, 19-21], and that the majority of participants did not reach the sufficient cut of levels of 50 nmol/L or more [7, 22].

To achieve optimal serum vitamin D concentrations of at least 50 nmol/L (adequate for bone health) [23], vitamin D should be obtained either from adequate sun exposure or from greater ingestion of vitamin D [24]. Approximately 90%-100% of vitamin D requirement can come from exposure of skin to UVB radiation [24]. However, this is not the case for the Middle Eastern women who mostly maintain very conservative clothing style by which most of their body is covered when outside.

When skin synthesis of vitamin D is either insufficient or absent, oral vitamin D intake is another approach. Daily doses of 400-800 IU are recommended age-dependently, and in some populations with vitamin D deficiency/insufficiency up to 2000 IU vitamin D is required [25-27], and dosages up to 10,000 IU have been considered to be safe [26]. Dietary sources of vitamin D are very limited, and they contain very low amounts of vitamin D [23]. In addition, fortification of foods with vitamin D is not mandatory in New Zealand [28]. Hence, supplements are considered to be the most appropriate mode of action for improving vitamin D status of this population.

Supplemental sources of vitamin D can be purchased over-the-counter as either multivitamin or single vitamin D preparations containing a daily dosage of 100 – 1000 IU vitamin D_2 or D_3 or prescribed by doctors as pharmaceutical preparations containing a monthly dosage of 50,000 IU vitamin D_3. The latter is fully funded by the New Zealand government (PHARMAC subsidised), and might be considered by GPs for those populations at risk of vitamin D deficiency with no baseline and follow-up testing and without taking into account inter-individual factors [29]. Although the number of people supplemented with prescribed

vitamin D tablets (50,000 IU/month) has more than doubled since 2007 (from 84,090 in 2007 to 174,440 in 2010) [23], the adequacy of this dose in some populations, especially in people of Middle Eastern origin, remains doubtful [22].

In their study, Saadi et al (2007) found that daily 2000 IU increased serum 25(OH)D concentrations significantly though only a small proportion (30%) reached the sufficient concentration of ≥ 50 nmol/L [7], a finding confirmed by Al-Daghri et al (2012) [22]. The authors assigned type 2 diabetic male and female Saudi subjects to receive 2000 IU vitamin D daily for 18 months. Serum 25(OH)D concentrations improved significantly but remained at suboptimal levels [22]. Based on my personal view, one explanation for such phenomena could be that these populations are chronically vitamin D deficient and they may need to have an initial loading phase with high doses of vitamin D followed by a maintenance prescription (50,000 IU/month). Further complicating the issue is that people respond differently to a given dose of vitamin D supplement as discussed below.

Response to vitamin D supplements depends on a variety of factors such as body weight/body fat percentage [30], basal 25(OH)D concentration [31, 32], dose [31, 32], type of vitamin D, D_3 vs. D_2 [33], season [31, 32], the use of hormonal contraceptives (oestrogens) [34] and dietary calcium intake [35]. Recently, Zittermann et al (2013) [30] published a systematic review concerning the importance of body weight for the dose-response relation in circulating 25(OH)D. The authors demonstrated that 34.5% of variation in circulating 25(OH)D was explained by body weight, followed by type of supplement (9.8%), age (3.7%), calcium intake (2.4%) and basal 25(OH)D concentrations (1.9%). For these reason, there is a considerable variation in circulating 25(OH)D concentrations in response to a given dose of vitamin D supplement even in similar age groups [31, 32, 36-38].

Gallagher et al (2012) [36] predicted that supplementation with 1600 IU/d would be needed to increase serum vitamin D concentrations to 75 nmol/L in 97.5% of white postmenopausal women with vitamin D insufficiency. However, Aloia et al (2008) recommended that supplementation with 4600 IU/d vitamin D would be needed to maintain most subjects (healthy black and white, men and women) within the range of 75-220 nmol/L [32]. This discrepancy was explained by Talwar et al (2007) [31] who suggested that patients with serum 25(OH)D concentrations >45 nmol/L and <45 nmol/L needed 2800 IU/d and 4000 IU/d vitamin D, respectively, to achieve concentrations >75 nmol/L.

All of these assumptions and calculations are based on studies on white or African American postmenopausal women [31, 36] and vitamin D deplete or replete subjects [7, 32, 38]. Accordingly, sufficient evidence now exists to justify investigations into the adequacy of different available and recommended dosages of vitamin D, the identification of predictors of response to vitamin D supplements and the significance of these predictors in managing vitamin D deficiency in women of Middle Eastern origin living in Auckland. If the adequate dose is determined and the predictors are identified, health care providers working with Middle Eastern populations can easily estimate the doses required for improving vitamin D status of this population and consequently ameliorate the risk of vitamin D deficiency-related diseases.

Aims

Aim 1: To investigate the adequacy of supplementation with monthly 50,000 IU and 100,000 IU cholecalciferol (vitamin D_3) for 6 months in increasing serum 25(OH)D concentrations ≥ 50 and ≥ 75 nmol/L in Middle Eastern premenopausal women living in Auckland.

Aim 2: To investigate the safety of supplementation with monthly 50,000 IU and 100,000 IU vitamin D_3 for 6 months in Middle Eastern premenopausal women living in Auckland; based on serum calcium levels and serum 25(OH)D levels to ≥ 2.7 mmol/L and >225 nmol/L, respectively.

Aim 3: To determine predictors of serum 25(OH)D response to supplementation with given doses of vitamin D in Middle Eastern premenopausal women living in Auckland.

Objectives

The Middle Eastern Women's Health study-Phase II commenced in Auckland, New Zealand in June 2013.

The primary objective of this study was to conduct a double-blind, randomised-controlled trial (RCT) with vitamin D supplementation in Middle Eastern who were apparently healthy. We aimed to assess the adequacy of supplementation with monthly 50,000 IU and 100,000 IU in optimising serum 25(OH)D concentrations (≥ 50 nmol/L & ≥ 75 nmol/L) in a group of 62 Middle Eastern premenopausal women living in Auckland. To our knowledge, this is the

first intervention study of this nature to be conducted in this vitamin D deficient migrant population in New Zealand.

Because vitamin D increases serum 25(OH)D concentrations in the circulation and increases calcium absorption, a secondary objective was developed to determine the likelihood of hypercalaemia or hypervitaminosis D in this population. Furthermore, variation in circulating 25(OH)D in response to supplementation with vitamin D is affected by several factors as such other objectives were developed to a) investigate the dietary calcium intake, body composition and hormonal contraceptive use of the study population, and to b) investigate the effect of these factors on circulating 25(OH)D in response to the given doses of vitamin D supplements.

Hypotheses

Hypothesis 1: While supplementation with monthly 50,000 IU vitamin D_3 for 6 months is inadequate for increasing serum 25(OH)D concentrations ≥ 50 and ≥ 75 nmol/L in the majority of women, supplementation with monthly 100,000 IU vitamin D_3 for 6 months is adequate for increasing serum 25(OH)D concentrations ≥ 50 and ≥ 75 nmol/L in the majority of women.

Hypothesis 2: Supplementation with monthly 50,000 IU or 100,000 IU are considered to be safe, and do not increase serum calcium levels and serum 25(OH)D levels to ≥ 2.7 mmol/L and >225 nmol/L, respectively

Hypothesis 3: Lower calcium intake, higher baseline serum 25(OH)D concentrations, larger body fat percentage or not using hormonal contraceptives are associated with lower serum 25(OH)D response to supplementation with given doses.

Structure

Following this preface chapter, Chapter 2 consists of a review of the relevant literature. This review begins with an overview of vitamin D, including definition, chemistry and synthesis, dietary sources and recommended intakes and vitamin D intake in New Zealand, measuring blood vitamin D concentration and optimal levels for blood vitamin D concentration, and health effects including bone health, muscle strength and diabetes. This is followed by an exploration of vitamin D deficiency globally, in New Zealand and in Middle Eastern

countries. Risk factors for vitamin D deficiency are then discussed, including obesity, age, covering skin, and low dietary intakes. Finally, known risk factors for vitamin D deficiency in Middle Eastern population are highlighted, followed by a summary and conclusion.

Chapter 3 includes all the details regarding the methodological procedures of the current study, from the recruitment to the statistical analysis.

Chapter 4 is the results section which includes the baseline characteristics of the study population, followed by the primary outcome findings and then the results of post hoc analyses.

Chapter 5 consists of a discussion about the findings of the current study, followed by a brief conclusion.

Chapter 6 contains a conclusion, followed by an assessment of the strengths and limitations of this research and recommendations for future research.

Appendices include the Middle Eastern Women's Health Study-Phase II participant information form, consent form, details, demographic, medical history and Fitzpatrick questionnaire, change of lifestyle questionnaire, New Zealand Physical Activity Questionnaire-Short Form and 4-day food diary.

The references are included at the end of thesis report.

Researchers' Contribution

Researchers	Contribution
Hajar Mazahery	Master of Science researcher Responsible for all aspects of the study including: • Conceptualisation and the study design • Ethics application • Recruitment • Data collection • Statistical analysis • Writing the research report
Dr Pamela R von-Hurst	The supervisor

I declare that my role in the study as indicated above is representative of my actual contribution.

Hajar Mazahery

_____

Chapter 2: Literature Review

Vitamin D deficiency

Vitamin D deficiency is defined as being when serum 25-hydroxyvitamin D (25(OH)D) concentration falls below 25 nmol/L and levels of 25 to 50 nmol/L are considered to be insufficient [29]. It should be acknowledged that there are a number of different cut-offs for vitamin D deficiency and insufficiency (<50 and 50-75 nmol/L, respectively) [39], but for the purpose of this review, otherwise stated, the New Zealand Ministry of Health levels have been chosen. Serum 25(OH)D concentrations below 50 nmol/L is associated with many musculoskeletal and non-musculoskeletal health conditions [39]. Rickets in children, osteomalacia in adults and poor bone health conditions such as osteoporosis are the clinical consequences of serum 25(OH)D concentrations <50 nmol/L [39, 40]. Skeletal health is compromised by vitamin D deficiency and insufficiency (<75 nmol/L) due to decreased bone maturation/remodelling, matrix formation/osteoclast activity, intestinal calcium absorption and renal calcium re-absorption [41, 42]. Cardiovascular diseases, autoimmune diseases, diabetes, cancer, and so many other chronic diseases are associated with low 25(OH)D levels in the circulations [4, 5, 43, 44]. The high cost of treating patients with these diseases is a serious public health problem and possibly exacerbated as the prevalence of vitamin D deficiency (as a risk factor) continues to rise.

Health significance of vitamin D deficiency

Estimated health costs: Increase in the prevalence of vitamin D deficiency/insufficiency negatively impacts the health system. Grant, Garland, and Holick (2005) estimated that the economic burden of vitamin D insufficiency in the United States of America (USA) is US$40-56 billion per year taking into account rickets, osteomalacia and other musculoskeletal and non-musculoskeletal diseases [45]. It has been assumed that if vitamin D and calcium intake is adequate, the risk of osteoporotic fracture could be reduced by 50 -70% equating to a saving of US$19-26 billion per year [45]. In New Zealand, the total direct cost of osteoporosis was estimated to be NZ$330 million in 2007 [46]. These costs, including treatment and management, are expected to increase to over NZ$391 million and NZ$458 million in 2013 and 2020, respectively. These costs comprise only a small proportion of the costs associated with the many adverse health outcomes of vitamin D deficiency, and the nationwide and worldwide economic costs related to vitamin D deficiency/insufficiency may be phenomenal.

Mortality rate: Vitamin D deficiency/insufficiency, independently or as a risk factor for other diseases, is associated with increased mortality and morbidity rates [45, 47]. Mathews et al (2012) investigated the impact of vitamin D deficiency on surgical intensive care unit patients. The authors found that increased length of stay, surgical intensive care unit cost and mortality rate among these patients was associated with the severity of vitamin D deficiency [47]. Grant et al (2005) predicted that 50,000 – 63,000 Americans and 19,000 – 25,000 British die prematurely from cancer annually due to vitamin D insufficiency (50-75 nmol/L) [45]. In Canada, the 2004 mortality rate from vitamin D sensitive diseases was 154,100 deaths with cancer, cardiovascular diseases, and falls and fractures accounting for 67,300, 14,000, and 1600 deaths, respectively [48]. It has been estimated that the number of osteoporotic fractures in New Zealand was 84,354 in 2007 and will increase by 30% by 2020 [46]. Hip fractures account for 5% of the total fractures [46]. These fractures are associated with serious disabilities and carry a risk of 20-25% mortality in the first 12 months [49].

Prevalence of vitamin D deficiency

Rickets and osteomalcia have been believed to be eradicated from the general population in North America and Europe [50]. However, the recent reviews highlight the resurgence of rickets in the United Kingdom (UK), the Netherlands, Denmark, and Australia [50] and in New Zealand [51]. Yet, the prevalence of rickets was and has remained high in some regions including Africa, Asia and Middle East [50].

Vitamin D insufficiency (serum 25(OH)D concentrations of 50 to 75 nmol/L) has been estimated to affect one billion people around the world [52]. Van Schoor (2011) compiled a large number of studies and data from different continents and countries including Asia, Europe, Latin America, Middle East/Africa, North America and Oceania to provide a global perspective [53]. While vitamin D insufficiency has been reported to be common in every region including New Zealand [18], vitamin D deficiency (<50 nmol/L) is highly prevalent in South Asia and Middle East [53].

Middle East: The Middle East is a sunny region with a hot and arid climate and the latitudes spanning from 12 to 42° N **(Figure 2.1)** [54]. The definition of Middle East varies from one source to another due to its imprecisely defined borders. For the purpose of this review, the definition used by Statistics New Zealand has been adopted [16]. Ethnicities considered in this review include: Algerian, Arab, Assyrian, Egyptian, Iranian/Persian, Iraqi,

Jewish/Hebrew, Jordanian, Kurd, Lebanese, Libyan, Moroccan, Omani, Palestinian, Syrian, Tunisian, Turkish, Yemeni, and other countries from Arab Gulf (Persian Gulf).

Figure 2.1: Map of Middle East

Based on the available evidence, prevalence of vitamin D deficiency/insufficiency in Middle East is a significant public health problem. A large proportion of adolescents, up to 84% in Iran [55] and 65% in the United Arab emirates [56] and up to 97.2% of college students in Qatar [57] had serum 25(OH)D concentrations below 50 nmol/L. Similarly, vitamin D status of people across other age groups and geographic locations is poor: The mean serum 25(OH)D concentrations for women of child bearing age from the UAE [58], for pregnant women from Turkey [59], for men and women from Iran [60], and for elderly women and men from Lebanon [61] were 20.9±14.9 nmol/L, 28.75±13.5 nmol/L, 13.41±13 nmol/L, 36.75±16 nmol/L, respectively.

Evidence is suggestive of poor vitamin D status of these populations living in countries other than their home countries including Norway [14], the Netherlands [15] the US [62], and New Zealand [17]. Iranian women living in Oslo, Norway, had a median serum 25(OH)D concentration of 27 (20 – 36) nmol/L. Comparable to Turkish women, 45% of Iranians had serum levels below 20 nmol/L (8 ng/ml) [14].

New Zealand: Vitamin D deficiency and insufficiency are highly prevalent in New Zealand [18, 63, 64]. Grant et al (2009) in their study of 6-23 months children residing in Auckland (n=353) found that 10.0% of children had serum 25(OH)D concentrations <27.5 nmol/L [63].

Of 5 -14 years old children, 3.0% and 31.0% had serum 25(OH)D concentrations below 17.5 and 37.0 nmol/L, respectively [64]. Based on recent findings from the 2008/09 New Zealand Adult Nutrition Survey, 0.2%, 4.9%, and 27.1% of adults aged ≥ 15 had severe vitamin D deficiency (serum 25(OH)D <12.5 nmol/L), vitamin deficiency, and suboptimal levels of vitamin D, respectively [18]. Pacific adults had the highest prevalence of vitamin D deficiency, and unfortunately no data was available for Asian and other ethnic subgroups such as people of Middle Eastern origin.

Based on the available data across the region, vitamin D deficiency and insufficiency are more pronounced in some ethnic groups including Pacific, Maori, South Eastern ethnicities [18, 65] and people of Middle Eastern origin [17].

Middle Eastern Population in New Zealand: The Middle Eastern population has been increasing in New Zealand and more specifically in Auckland. Middle Eastern population is the largest of MELAA (ethnicities categorized by Auckland District Health Board; Middle Eastern, Latin American and African) in Auckland [16]. In 2006, the Middle Eastern population accounted for 0.4% (n=17,457) and 0.8% (n=10,692) of the total New Zealand and Auckland population, respectively, with a population growth of 5% per annum since then. Based on MELAA grouping, Iraqis (21.7%) and Iranians (20.7%) are the largest populations [16]. The population of this ethnic group is growing steadily in New Zealand, and therefore it is important to determine the prevalence of diseases and deficiencies (vitamin D deficiency) in Middle Eastern population (**Table 2.1**).

Anecdotal evidence [16] and the results from our previous pilot study [17] are indicative of high prevalence of vitamin D deficiency in Middle Eastern women in New Zealand. In our study, we recruited 43 women of Middle Eastern origin living in Auckland. None of the participants were vitamin D sufficient (serum 25(OH)D ≥ 50 nmol/L) [17]. Vitamin D deficiency (serum 25(OH)D <25 nmol/L) was observed in 74.4% of subjects, with 27.9% having severe deficiency (serum-25(OH)D <12.5 nmol/L).

Table 2.1: Estimated national ethnic population of New Zealand at June 30 1996, 2001 and 2006

Year at 30 June	1996	2001	2006
Ethnicity			
Total People, Ethnicity	3,732,000	3,880,500	4,184,600
European or Other Ethnicity (including New Zealander)	3,074,600	3,074,000	3,213,300
Mäori	573,200	585,900	624,300
Pacific Peoples	229,300	261,800	301,600
Asian	194,800	272,500	404,400
Middle Eastern/Latin American/African	18,450	27,600	38,600

Retrieved from http://wdmzpub01.stats.govt.nz/wds/TableViewer/tableView.aspx on 20th March 2013

Metabolism of vitamin D

Vitamin D, a fat soluble vitamin, is a general name for a collection of steroid like substances including ergocalciferol (D_2) and cholecalciferol (D_3) [66]. The former is found in plants, and the latter is synthesised in the skin of animals and humans upon exposure to Ultraviolet-B-Radiation (UVB) [66]. Vitamin D exerts its function on specific target tissues via the vitamin D receptor (VDR) [52, 66]. Accordingly, it has been classified as a secosteroid hormone [66] with the major role in bone metabolism and calcium homeostasis.

During sun exposure, 7-dihydroxycholesterol (pro-vitamin D) which is mainly found in the layers of epidermis and to a lesser extent in dermis [67] absorbs UVB radiation (290–315 nm) and is converted to pre-vitamin D_3, a thermodynamically unstable metabolite [66]. Then pre-vitamin D_3 undergoes heat-induced isomerisation and forms vitamin D_3 within one to three days. Carried by vitamin D binding protein (VDBP), vitamin D is mainly transported to the liver, but in a smaller amounts it is deposited in the adipose tissue for storage [68, 69].

Vitamin D metabolites circulate mainly bound to transport proteins; 85%-90% bound to VDBP, 10-15% bound to albumin and less than 1% is in a free form [70]. It is postulated that free hormones, unbound or liberated from binding proteins, enter cells and are biologically active (free hormone hypothesis; [71]). Hence, VDBP acts as a carrier and may act as a reservoir of vitamin D, as well as a regulator of its bioavailability to other tissues [72]. VDBP is synthesised in many tissues including the liver, kidneys and adipose tissue [73] and it is encoded by a group specific component gene (GC). GC variants (rs7041, rs4588, rs2282679

and rs1155563) have been shown to be associated with serum 25(OH)D concentration [74-78]. Furthermore, evidence shows that oestrogen and therefore oral contraceptive use is associated with increased production of VDBP [79].

Excessive sun exposure, as is expected, does not cause vitamin D intoxication. Interestingly, excessive sun exposure degrades both pre-vitamin D_3 and vitamin D_3 and converts them into inactive photoproducts including lumisterol, tachysterol, and 7-dihydroxy cholesterol [52, 80].

Dietary vitamin D intake is incorporated into chylomicrons in the intestinal cells. In the bloodstream, chylomicrons containing vitamin D are converted to chylomicron remnants which are taken up by the liver [52]. While vitamin D ingested from the diet is rapidly taken up by hepatocyte membrane receptors, vitamin D synthesised in the skin is gradually released to the bloodstream. The former results in a rapid increase in 25(OH)D concentrations but the latter leads to a prolonged production of 25(OH)D [81].

In the liver, vitamin D undergoes enzymatic hydroxylation and forms 25(OH)D [66]. Vitamin D 25-hydroxylase which is encoded by CYP2R1 hydroxylates both vitamin D_2 and vitamin D_3. It has been shown that a mutation in CYP2R1 results in vitamin D resistant rickets and is a significant determinant of serum 25(OH)D concentrations [82].

This metabolite, 25(OH)D, is the major circulating form of vitamin D, and is metabolically inactive until it is converted to 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. That is when vitamin D can exert its role in calcium homoestasis, in bone, muscle, and other organs in the body. Cytochrome 450, CYP27B1 which encodes 25-hydroxyvitamin D-1α-hydroxylase is responsible for the hydroxylation process in the kidneys [52]. Mutations in CYP27B1 have been shown to cause vitamin D-deficiency rickets and to correlate with the clinical symptoms of 1α-hydroxylase deficiency [83]. While CYP27B1 is down-regulated in phosphate loop by fibroblast growth factor 23 (FGF23), it is up-regulated by parathyroid hormone (PTH) in the calcium homoestasis loop. A decrease in serum calcium level stimulates the parathyroid gland to produce more PTH. PTH then up-regulates CYP27B1 and stimulates conversion of 25(OH)D to 1,25$(0H)_2$D in the kidneys. 1,25$(OH)_2$D acts either synergistically with PTH or alone to increase serum calcium concentrations by increasing mobilisation of calcium from skeleton and renal calcium re-absorption [66], and by increasing active calcium absorption from intestine, respectively [84].

In contrast, high concentrations of $1,25(OH)_2D$ decrease the production and excretion of PTH [52] through a negative feedback loop, decreases the half-life of 25(OH)D [85, 86], inhibits CYP27B1 activity and increases CYP24A1 (24-hydroxylase) activity [52]. CYP24A1 is responsible for the degradation of both 25(OH)D and $1,25(OH)_2D$ and is in a well-balanced equilibrium with CYP27B1 to maintain adequate levels of $1,25(OH)_2D$. Mutations in CYP24A1 are associated with severe hypercalciuric nephrolithiasis and nephrocalcinosis [87] and increased sensitivity to vitamin D and risk of hypercalcaemia in patients with idiopathic infantile hypercalcaemia [2]. The catabolic products of $1,25(OH)_2D$ and 25(OH)D are $24,25(OH)_2D$ and 25(OH)D–26,23-lactone which are excreted in faeces and urine [52]. For a schematic representation of vitamin D metabolism refer to **Figure 2.2**.

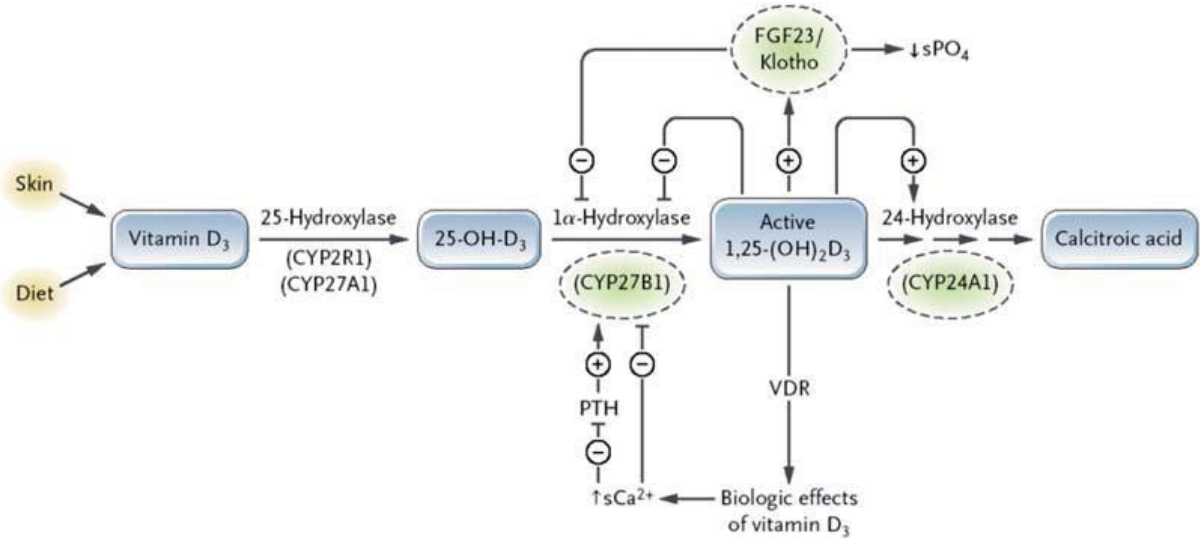

Figure 2.2: A schematic representation of vitamin D metabolism and some physiological actions. Reproduced with permission from Schlingmann et al (2011) [2], copyright Massachusetts Medical Society.

Blood Biomarkers of vitamin D; 25(OH)D vs. 1, $25(OH)_2D$

Over the past 25 years, more than 50 different vitamin D metabolites have been identified. Of these metabolites, vitamin D, $1,25(OH)_2D$ and 25(OH)D have been the focus of vitamin D assay methods [88]. Evidence shows that 25(OH)D is a robust and more reliable marker of vitamin D status compared to vitamin D and $1,25(OH)_2D$. Vitamin D [88] and $1,25(OH)_2D$ [86] have a short half-life of 24 hours and 4 to 6 hours, respectively. Furthermore, serum concentration of $1,25(OH)_2D$ compared to 25(OH)D is very low (about thousand fold less) [86] and its production is tightly regulated by a person's calcium requirements [88].

In contrast, the half-life of 25(OH)D is much longer, though the definite time is controversial (ranging from 3 weeks to 3 months) [86, 89]. Thus its concentration reflects body stores of

both vitamin D synthesised in the skin and the vitamin D ingested from a diet or supplement. Furthermore, the production of 25(OH)D in the liver is not significantly regulated and depends on the substrate availability [88]. Accordingly, quantifying serum 25(OH)D concentrations is the most reliable index of a person's vitamin D status.

Optimal Serum 25(OH)D Levels

Historically, rickets/osteomalacia was used as a surrogate for determining optimal levels of serum 25(OH)D. It is well documented that the rickets/osteomalacia threshold is 20 nmol/L and any range up to the lower reference range is defined as vitamin D insufficiency [90]. However, as our knowledge about health benefits of vitamin D has been increasing, the debates about defining optimal levels for serum 25(OH)D have increased. The use of other clinical endpoints and health outcomes including maximal suppression of PTH, highest calcium absorption, greatest bone mineral density (BMD), lowest incidence of factures and falls, and non-musculoskeletal health outcomes for determining optimal levels have recently been the focus of research in this field.

The optimal level of serum 25(OH)D is associated with maximal suppression of PTH. As a physiological response, PTH level, a biomarker of bone remodelling, decreases as serum 25(OH)D increases [90-92]. This inverse relationship has been reported in many studies [93-95]. Maximal suppression of serum PTH levels has been reported at serum 25(OH)D concentrations of 30-99 nmol/L, with a range of cluster at 75-80 nmol/L [96]. In a study of osteoporotic women from 25 countries, Lips et al (2001) reported that women with serum 25(OH)D concentrations <25 nmol/L and 25-50 nmol/L had 30% and 15% higher levels of PTH, respectively, compared to those with serum 25(OH)D concentrations > 50nmol/L [97].

Intestinal absorption of calcium is modulated by serum 25(OH)D. Compared to persons with serum 25(OH)D concentrations of 50 nmol/L, calcium absorption has been shown to be greater in subjects with serum 25(OH)D concentrations of 86.5 nmol/L by 45%-65% [98]. The authors concluded that the current lower reference level of 50 nmol/L might be insufficient for the greatest calcium absorption in some individuals. Furthermore, a decrease in median serum 25(OH)D concentrations from 122 nmol/L in summer to 75 nmol/L in winter did not significantly change calcium absorption fraction and urinary calcium/creatinine ratio [99], indicating that a minimum serum 25(OH)D concentrations of 75 nmol/L might be required for optimal calcium absorption, and any concentrations below this threshold might result in decreased BMD and in increased risk of falls and fractures.

Serum 25(OH)D concentration has been shown to correlate positively with BMD, muscle strength and physical performance [97, 100, 101], and negatively with the incidence of falls and fractures [102]. In the United States National Health And Nutrition Examination Survey 3 (US NHANES III) data, Bischoff-Ferrari et al (2009) found a significant positive correlation between BMD and serum 25(OH)D concentrations (<50, 50-74, and ≥ 75 nmol/L) [100]. The increase was stepwise and proportional.

Furthermore, supplementation with vitamin D increased muscle strength and physical performance [101] and decreased the incidence of falls by 49% [91] and fracture risk by 22% [102] in line with an increase in serum 25(OH)D concentration to 55-70 nmol/L, 70-80 nmol/L, 65 nmol/L and 74 nmol/L, accordingly. However, a meta-analysis by Reid et al (2014) failed to show an effect of vitamin D supplementation on bone density [103].

Further evidence comes from studies investigating the 25(OH)D thresholds for non-musculoskeletal health endpoints. During 10 years follow-up, higher prevalence of myocardial infarction (MI) was reported in men with serum 25(OH)D concentrations ≤ 37.5 nmol/L compared to ≥ 75 nmol/L [44]. The mean serum 25(OH)D concentration was also significantly lower in Diabetic subjects than healthy subjects, 69 nmol/L vs. 76 nmol/L, respectively [104]. Interpretation of these outcomes should be done with caution as they are cross-sectional data and causality cannot be confirmed.

The above evidence suggests that minimum serum 25(OH)D concentrations for a better health outcome are at least >50 nmol/L, but concentrations of ≥ 75 nmol/L are optimal for multiple clinical outcomes. The 2012 consensus statement on vitamin D and sun exposure in New Zealand selected 50 nmol/L as the lower end of the optimal serum 25(OH)D range and recommended that individuals should aim for annual mean serum 25(OH)D concentrations above this level [29].

Actions of vitamin D

Vitamin D has musculoskeletal and non-musculoskeletal health benefits. Its role in the prevention of rickets in children [105, 106] and osteomalacia, osteoporotic fractures and falls in adults [20, 40, 107, 108] as well as improvement of muscle strength [109] has been widely examined. Vitamin D has also been shown to have protective effects on other diseases such as autoimmune diseases including type 1 diabetes mellitis (T1DM) [5] and multiple sclerosis (MS), cardiovascular diseases (CVD) [44], and cancer (breast, prostate, and colorectal

cancer) [43]. For the purpose of this literature review, the well-established and more studied roles of vitamin D (bone health, muscle health, diabetes and CVD) will be discussed.

Musculoskeletal Health Benefits

Musculoskeletal disorders affect one fourth of adults in New Zealand [110]. It had been thought that rickets had been eradicated in developed countries. However, case studies of rickets in some countries like New Zealand and Australia has been reported over the past decade [51, 111]. An Australian paediatric surveillance unit study reported that the annual estimated incidence of rickets in children ≤ 15 years of age between 2006 and 2007 was 4.9/100,000 in Australia [111]. Rickets was more prevalent in refugees and immigrants from developing countries such as Sudanese. The annual incidence for patients of Sudanese background was 350 times greater than the national estimated incidence [111, 112]. Rickets in developing countries ranks amongst the five most common diseases in children and adolescents, and the Middle Eastern region has the highest incidence of both rickets [113] and osteomalacia [54] worldwide.

El-Hajj Fuleihan (2009) has suggested that in those regions where rickets is prevalent, osteomalacia is likely to be present [54]. Unfortunately, information about worldwide prevalence of osteomalacia is lacking because it is an asymptomatic disease and usually goes undetected or misdiagnosed as fibromyalgia [54], and in the long term, if left untreated, may increase the risk of osteoporosis [114].

The 2006/07 New Zealand Health Survey reports 123,624 people affected by osteoporosis [110]. The burden of this disease is expected to increase in the Middle East. In Iran, it has been reported that two million people are at risk of fracture with approximately 8,000 hip fractures occurring annually [115]. Vitamin D deficiency/insufficiency is highly prevalent in Middle Eastern women [8, 9, 13, 19, 20, 116], and has been reported to be associated with increased risk of osteoporotic fractures and other bone disorders [20, 40, 108].

Mechanisms of Action

The primary role of vitamin D in bone health is to facilitate the absorption of calcium from small intestine and to prevent an increase in PTH. When serum 25(OH)D concentration is inadequate, renal calcium re-absorption and intestinal calcium absorption decreases [41, 42] and serum calcium drops. While 30%-40% of dietary calcium is absorbed when serum vitamin D is adequate, it drops to 10%-15% at the presence of vitamin D deficiency [42]. In

response to a drop in circulating calcium level, the production and secretion of PTH by the parathyroid gland is triggered resulting in secondary hyperparathyroidism [117]. Elevated PTH, in turn, stimulates bone remodelling, primarily affecting the number and activity of osteoclasts in order to maintain serum calcium level and to increase serum $1,25(OH)_2D$ concentration [41, 42]. As a consequence, bone loss increases and bone mass decreases [118].

Both the enzyme required for converting the 25(OH)D to $1,25(OH)_2D$ and vitamin D receptors have been found in the parathyroid gland [119] and skeletal cells such as chondrocytes, osteoblasts, and osteoclasts [120], and in muscle tissues [121]. The role of vitamin D starts when $1,25(OH)_2D$ binds to a VDR in these cells and tissues. VDR, in turn, forms a complex with DNA which suppresses the transcription of genes involved in the expression of PTH [119]. Furthermore, this complex induces the expression of some proteins such as calbindin 9K, osteocalcin and osteopontin, type I collagen and osteoclast [122] as well as type II muscle fibres in muscle tissues [121]. These proteins facilitate the absorption of calcium, the binding of calcium to bone matrix during bone modelling, the formation of the primary component of the bone matrix which is type 1 collagen and its maturation, resorption of bone and the formation of type II muscle fibres which are needed for fast reactions [122]. It is well documented that type II muscle fibres decrease with aging [123]. Clinically shown, vitamin D has selective effect on type II muscle fibres, and vitamin D supplementation results in an increase in type II muscle fibre [121].

Supporting Evidence

Maternal vitamin D intake and breast milk are the main sources of vitamin D during foetal, neonatal and infancy (if exclusively breast-fed) [124]. Judkins and Eagleton (2006) in their prospective clinical study in 90 pregnant women from a Wellington (New Zealand) general practice where cases of rickets in children had been diagnosed, reported that 87.0% and 61.2% of subjects had 25(OH)D serum level <50nmol/l, and <25nmol/l, respectively [51]. The authors and others [125, 126] stated that maternal vitamin D deficiency may contribute to the resurgence of rickets in infants.

Low vitamin D levels have been consistently shown to be associated with, and to be a risk factor for nutritional rickets. A retrospective study of 34 Saudi Arabian adolescents with rickets revealed that the majority of cases were associated with vitamin D deficiency, followed by calcium deficiency and genetics (58.8%, 11.8% and 8.8%, respectively) [127], a finding confirmed by another study from Qatar [126]. Low intake of vitamin D and calcium,

lack of exposure to sun and prolonged breast feeding were the most important risk factors for nutritional rickets. A significantly larger proportion of rachitic children had a family history of vitamin D deficiency (P=0.001), had vitamin D deficiency themselves (P<0.001) and spent shorter period of time under the sun than non-rachitic children (P<0.001).

Further evidence comes from intervention trials; in a randomised placebo controlled trial, 42 infants aged 6-30 months with rickets were randomised to receive either high dose vitamin D [300,000 international unit (IU), intravenously], calcium lactate (3 g/day), or both for four weeks [106]. Better improvements were observed in response to treatment with vitamin D only or vitamin D in combination with calcium than calcium only. Furthermore, vitamin D supplementation has been shown to improve bone strength [128] and bone mineralisation [129] in infants. These findings may have important implications in reducing the risk of osteoporosis in adult life.

Vitamin D deficiency is highly prevalent in osteoporotic subjects [107]. Based on an international epidemiological investigation of the prevalence of vitamin D deficiency among osteoporotic women, 30.8% of women had serum 25(OH)D concentrations <50 nmol/L, with Middle Eastern women having the highest prevalence of vitamin D deficiency (57.9%) [107].

Being the surrogate measures of osteoporosis, BMD, bone turnover markers, bone biomarkers and fractures have been used in other studies. Several observational studies, but not all [94, 130, 131], have shown that serum 25(OH)D concentrations positively correlate with BMD, and negatively with fracture risk and bone turn over markers [20, 40, 108, 132, 133]. In a cross-sectional study among Saudi nationals, Sadat-Ali and co-workers (2011) [132] found that serum 25(OH)D concentration was positively correlated with BMD and negatively with PTH levels. Low bone mass was more prevalent in subjects (25-35 years old) with vitamin D deficiency, followed by vitamin D insufficiency and then sufficiency. While low bone mass was observed in all subjects with vitamin D deficiency (<50 nmol/L), it was seen in 84.0% and 89.0% of women and men with vitamin D insufficiency (50-75 nmol/L) and 50.0% and 7.0% of women and men with vitamin D sufficiency (≥ 75 nmol/L), respectively. The same pattern was observed in subjects aged 50 years and older. This study had a large sample size (n=400) and included different age groups and gender.

Conversely, Powe et al (2011) [72] found no association between total 25(OH)D concentrations and BMD. However, free and bio-available 25(OH)D concentrations significantly correlated with BMD, indicating a role of VDBP in vitamin D-BMD relationship. Based on the free hormone hypothesis, a hormone is biologically active only if it is liberated from binding proteins [71]. Although these studies have confirmed the relationship between serum 25(OH)D and BMD in different age, gender and ethnic groups, the causality cannot be determined due to their cross-sectional nature.

Further support comes from intervention trials where vitamin D has been shown to improve BMD of osteoporotic and healthy subjects [132, 134-137]. Heckman et al (2002) reported that supplementation with daily 1000 IU vitamin D improved BMD at lumbar spine in patients with osteoporosis who were not responding to treatment with bisphosphonates [136]. In another study, a combination of calcium and vitamin D (1200 mg calcium + 1000 IU vitamin D_2) preserved hip BMD of community dwelling elderly and maintained it after three and five years, but had no extra beneficial effect over calcium only (1200 mg) on bone structure, bone chemistry and bone markers [138]. A limitation of this study is that the individual effect of vitamin D on bone markers cannot be determined.

An effect of vitamin D supplementation on BMD has also been shown in healthy Finish girls with the mean age of 11.2 years (n=212) [135]. These girls were randomly assigned to receive either 200 IU or 400 IU vitamin D_3 or placebo for one year. The bone mineral content (BMC) increased in proportion to supplemental dose although no difference in bone turn over markers was detected indicating an effect of vitamin D supplementation on decreasing bone re-sorption.

Evidence shows that low BMD is able to predict, and is an important risk factor, for fracture risk in the short- and long-term [139-142]. It has been postulated that a 5% increase in total body BMD and hip and lumbar BMD is associated with a reduction in the risk of relative childhood fractures by 17.0% and 9.0%, respectively [141]. Yet, this effect is more pronounced in adult life; a 10% increase in BMD in the peak bone mass period can reduce risk of osteoporotic fractures in later life by 50.0% [142].

Using fracture risk as a surrogate for bone health, researchers studying 1311 community dwelling men and women in the Longitudinal Aging Study Amsterdam (LASA study) found a significant inverse relationship between serum 25(OH)D concentration and fracture risk in

persons aged 65-75 years, but not in persons aged 75-89 years. Levels below 30 nmol/L were significantly associated with increased risk of fractures [143]. These findings are in agreement with those of the Osteoporotic Prospective Risk Assessment trial (OPRA) [144]. However, the serum vitamin D threshold for an effect was 50 nmol/L.

The observational Os des Femmes de Lyon (OFELY) study, however, did not find such relationship [145]. This discrepancy could be due to the differences in the mean age and the ethnicity of participants in these studies. While the participants in the latter study were relatively younger (healthy postmenopausal women with the mean age of 62.2 years old), they were ambulatory elderly women in OPRA [144] and elderly men and women ≥ 65 years old in LASA trials [143]. Regarding fracture risk, age is a significant confounder [143]. Furthermore, race/ethnicity may affect the relationship between serum 25(OH)D concentrations and fracture risk [146]. Cauley et al (2011) found that higher concentrations of 25(OH)D were associated with increased fracture risk among Hispanic or Indian American women, but were associated with decreased fracture risk among white women [146]. The authors suggested that different ethnic groups might have different thresholds for optimal 25(OH)D.

Larson et al, however, demonstrated that supplementation with 1000 mg calcium + 400 IU vitamin D for three years reduced the fracture risk by 16% [147]. In agreement with these findings, Trivedi et al (2003) assigned community dwelling aged ≥ 65 years (n=2686) to receive 100,000 IU oral vitamin D_3 or placebo every four months for five years. The risk for primary fracture in the supplemental group decreased by 22% compared to control group [148]. However, in a study by Lyons et al, four-monthly mega dose vitamin D_2 supplementation had no effect on incidence of fracture risk [149]. To note, vitamin D_2 is less bio-available than vitamin D_3 [150].

The meta-analyses of randomised controlled trials (RCTs) have also yielded mixed results. While one meta-analysis did not find any beneficial effects of vitamin D supplementation alone on fracture risk (hip fracture: relative risk (RR), 1.15; 95% CI, 0.99-1.33; vertebral fracture: RR, 0.90; 95% CI, 0.42-1.92; new fracture: RR, 1.01; 95% CI, 0.93-1.09), other studies showed that vitamin D + calcium or vitamin D alone were beneficial [151, 152]. Bischoff-Ferrari et al (2005) found significant associations between 700-800/day IU, but not 400 IU/day, vitamin D supplementation and risk of hip and any non-vertebral fracture [152]. The relative risk of hip and non-vertebral fracture reduced by 26%, pooled RR, 0.74; 95% CI,

0.61-0.88, and by 22%, pooled RR, 0.77; 95% CI, 0.68-0.87, respectively. Based on a pooled analysis of 11 double-blind, randomised, controlled trials of oral vitamin D supplementation, it has been suggested that doses ≥ 800 IU are needed for the prevention of hip fracture and any non-vertebral fracture in persons aged ≥ 65 years [153].

The observed decreased fracture risk in some populations could be due to the potential beneficial effect of vitamin D supplementation on muscle strength. Improved muscle strength in the elderly may increase body balance and consequently improve physical performance [101] and decrease the risk of falling and the associated health complications [144].

Gerdhem et al found a significant positive correlation between serum 25(OH)D concentration and thigh muscle strength (r=0.08, *P*=0.02), gait speed (r=0.17, *P*<0.001), and Romberg balance test (r=0.14, *P*<0.001) in 986 ambulatory elderly women [144], comparable to the results from a study in patients with previous fracture [154].

This positive relationship was also seen over a broader age range [108, 109, 155, 156]. Serum 25(OH)D concentration correlated significantly positively with isometric and isokinetic arm strength and isometric leg strength in healthy men and women [155], with jump velocity, jump height, power, Esslinger fitness index, and force in post-menarchal females [109], and significantly inversely with exercise-induced muscular weakness [156]. It should be noted that the data from these studies are cross-sectional, and, therefore, the causality cannot be established.

Further support for such relationship comes from intervention trials [157, 158]. Carrillo et al (2013) could show that supplementation with 4000 IU vitamin D/day for 12 weeks significantly increased the peak power in overweight and obese persons during a resistance training intervention [158]. The majority of participants had basal serum 25(OH)D concentrations <75 nmol/L. A systematic review and meta-analysis of RCTs showed that supplementation with 800-1000 IU/day improved muscle strength and balance in older adults (≥ 60 years) [159]. In another meta-analysis, this beneficial effect was evident in proximal muscle strength in people with serum 25(OH)D concentrations ≤ 25 nmol/L [160].

If vitamin D supplementation results in improved muscle health in the elderly, the risk of falling should be reduced as a consequence. To establish the efficacy of vitamin D supplementation, Bischoff-Ferrari et al included five RCTs of vitamin D supplement in their meta-analysis. Vitamin D supplementation was associated with reduced risk of falling (-22%,

with an effect size independent of calcium supplementation and other confounding factors) [161]. This finding was confirmed by Dawson-Hughes who reported improved body balance and muscle health, and reduced falling risk by 20% with vitamin D supplementation. The effective dose for lowering risk of falls was 700-1000 IU vitamin D/day and people with serum 25(OH)D concentrations ≤ 60 nmol/L were more likely to benefit from supplementation [162].

Conclusion

In conclusion, while cross-sectional trials provide strong evidence for an effect of vitamin D on musculoskeletal health, interventional trials provide mixed results. The discrepancies could be attributed to the genetic background, race/ethnicity and characteristics of study populations, factors modifying the vitamin D-musculoskeletal relationship and study design. Overall, people with vitamin D deficiency, elderly, and adolescents are more likely to benefit from vitamin D supplementation. If it is confirmed that vitamin D has beneficial effect on skeletal health and muscle strength, it might have very important implications for our aging population's health and sportsmen's/sportswomen's performance [163].

Non-musculoskeletal Health Benefits

Vitamin D can alter the expression of several genes and consequently can alter the function of different systems and organs where these genes are involved [164]. In a genome wide trial, Hossein-Nezhad et al (2013) [164] revealed that the expression of 66 genes was significantly different between those having serum 25(OH)D concentrations of <50 nmol/L and >50 nmol/L (P<0.01) at baseline. These differences disappeared after supplementation, and improvement in serum 25(OH)D was associated with alteration in the expression of 291 genes (1.5 fold inhibition of 82 genes and 1.5 fold induction of 209 genes). Some of the these genes are involved in the immune system function, transcriptional regulation, cell cycle, DNA replication and stress; all of which are involved in the biological pathways of non-musculoskeletal diseases.

Furthermore, vitamin D receptors and enzymes involved in vitamin D metabolism have been identified in different organs and tissues indicating a role for vitamin D in these organs [66]. The proposed roles of low 25(OH)D in the circulation as an independent risk factor for chronic diseases such as autoimmune diseases (MS and type 1 diabetes) [5], cancer (colorectal cancer) [43], CVD [4, 5] and type 2 diabetes [165] have been documented.

There is sufficient evidence to suggest that vitamin D deficiency is a real problem in Middle Eastern women living in Auckland [17], and at the same time, these women have higher prevalence of CVD and diabetes compared to their European counterparts [16]. Accordingly, the role of vitamin D in CVD and diabetes will be reviewed here.

Cardiovascular Diseases (CVD)

Cardiovascular disease is the leading cause of death worldwide accounting for 17.3 million deaths in 2008 which is expected to increase to more than 23.3 million by 2030 [166]. In New Zealand, 30% of deaths are due to CVDs [167]. CVD mortality increasingly affects low to middle income countries and in minorities of different race/ethnic groups [166]. Approximately one third of total deaths (31%-35%) in the Middle East from 1987-1992 were due to CVDs [168]. A review of 27,065 reported deaths from 1999-2003 revealed that circulatory diseases accounted for 45% of deaths [169]. Projection for coronary heart disease (CHD) and stroke mortality suggest that the mortality will triple in this region [168]. CVD is also more prevalent in immigrants from this region living in Australia and New Zealand than their European counterparts [16, 170]. In New Zealand, with the exception of Maori, the age-standardised prevalence of CVD in 2007/2008 was more pronounced in Middle Eastern than all other ethnicities [16]. There are many risk factors for CVD including behavioural/environmental and metabolic/physiologic risk factors [168]. Although Middle Eastern populations may share CVD risk factors and causal pathways with populations in other parts of the world, certain risk factors, such as vitamin D deficiency, are more prevalent in this population. Accordingly, the role of vitamin D deficiency as a risk factor for CVDs will be discussed in the next section.

Mechanisms of Action

The presence of VDR and the enzymes involved in vitamin D metabolism in the cardiovascular (CV) system indicates a role of vitamin D in the pathogenesis of CVDs (**Figure 2.3**) [5, 171]. Vitamin D can modulate the mechanisms of CV system mainly through genomic pathways [171]. These genomic actions are mediated by $1,25(OH)_2D$ through VDRs [171]. Vitamin D is believed to modulate key processes of CVD pathogenesis including vascular inflammation [172, 173], rennin-angiotensin system [174], vascular smooth muscle cell, cardiomyocyte and myocardial proliferation and differentiation [175], platelet aggregation [176] and vascular calcification [177].

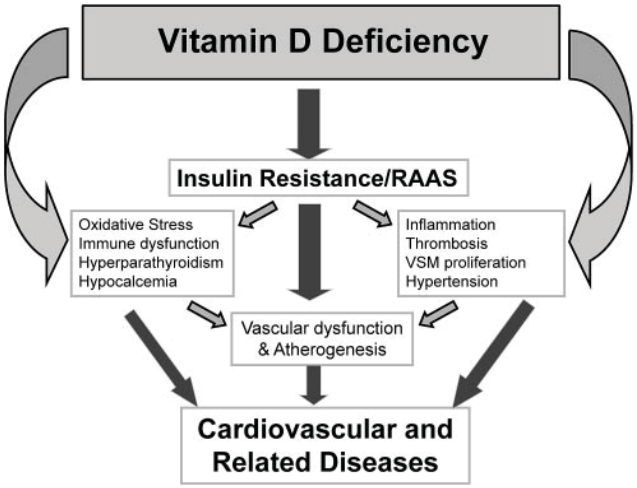

Figure 2.3: Conceptual model of major pathways through which vitamin D deficiency may lead to CVD. Reproduced from Artaza, Mehrotra, & Norris (2009) [1]

Supporting Evidence

Observational and clinical studies support the evidence from animal and cell culture studies investigating the role of vitamin D in CV system. Low serum 25(OH)D and $1,25(OH)_2D$ concentrations and vitamin D supplementation have been shown to be independently associated with all-cause mortality, CV mortality and CVDs [44, 178-182].

During a median follow up of 7.7 years in the following studies, the multivariate adjusted hazard ratios for all-cause mortality, CV mortality [178], deaths due to heart failure and sudden cardiac death [179] were higher for patients in the lowest 25(OH)D quartiles than patients in the highest 25(OH)D quartiles (>70 nmol/L) [2.08; 95% CI, 1.60-2.70; 2.22; 95% CI, 1.57-3.13; 2.84; 95% CI, 1.20-6.74; and 5.05; 95% CI, 2.13-11.97, respectively]. The multivariate-adjusted risk for ischemic heart disease increased by 40% (95% CI, 14%-72%), for MI by 64% (95% CI, 25%-114%), for early death by 57% (95% CI, 38%-78%) and for fatal ischemic heart disease/MI by 81% (95% CI, 40%-135%) in a Danish general population (n=10,170) with plasma 25(OH)D concentrations at the 1^{st}-4^{th} percentiles compared with those at 50^{th}-100^{th} percentiles [181]. In a community based case-control study from New Zealand, the relative risk of MI was lower for subjects with plasma 25(OH)D concentrations of 32 nmol/L or above than those with <32 nmol/L [0.43 (95% CI, 0.27-0.69) vs. 1.0, respectively] [182]. While vitamin D deficiency has been shown to be a strong independent predictor of all cause death and decreased survival, vitamin D supplementation has been shown to confer beneficial effects [180].

Further support comes from studies investigating the relationship between levels of 25(OH)D in circulation and CVD risk factors [180, 183]. Vacek et al (2012) conducted an observational retrospective study using a cohort of patients (n=10,899) followed up by a cardiovascular practice (approximately 6 years) [180]. Serum 25(OH)D concentrations of less than 75 nmol/L was associated with increased risk of diabetes (2.31; 95% CI, 2.02–2.63, $P<0.001$) and hypertension (1.40; 95% CI, 1.29–1.54, $P<0.0001$); a finding confirmed by others [183].

Using the data from the Third National Health Nutrition Examination Survey (NHANES III), the authors showed that participants with obesity, diabetes, hypertriglyceridemia and hypertension had lower serum 25(OH)D concentrations, and the odds ratio for having these risk factors were significantly higher in the first than in the fourth quartile of serum 25(OH)D concentrations (2.29, 1.98, 1.47, 1.30, respectively) [183].

Confirming the findings of these cross-sectional trials, supplementation with an oral daily dose of 2000 IU vitamin D for 18 months was shown to decrease LDL and total cholesterol and to improve ß cell function assessed by homeostasis model assessment (HOMA-ß) ($P=0.002$) in diabetic Saudi Arabian men and women (mostly vitamin D deficient), with more pronounced improvements observed in women [22]. A limitation to this study is that it lacked placebo arm.

In a double-blind, parallel group, placebo-controlled randomised trial, patients (n=34) with type 2 diabetes and low serum 25(OH)D concentrations (<50 nmol/L) were assigned to receive a single oral dose of 100,000 IU of vitamin D_2 or placebo and their blood pressure was measured 8 weeks after the supplementation. Vitamin D supplement improved systolic blood pressure (-7.3±11.8 vs. 6.6±9.7, $P=0.001$) and diastolic blood pressure (-2.2±8.6 vs. 2.3±5.7, $P=0.08$) in treatment group compared with the placebo group, respectively. Flow-mediated vasodilation, a marker of endothelial function, was also significantly improved (2.35%±3.12% in treatment group vs. 0.06%±3.39 in control group; $P=0.048$) [184]. The improvement in endothelial function was independent of the change in systolic blood pressure, a finding confirmed by other trial in stroke patients [185].

The support for a role for vitamin D in CVD prevention was opposed by a randomised double-blind, placebo-controlled trial conducted among healthy post-menopausal women without severe vitamin D deficiency [186]. Women were randomised to receive daily vitamin D (2400 IU/d) (n=55) or placebo (n=54) for four months. After four months, although serum

25(OH)D concentrations improved significantly ($P<0.001$), the change in endothelial function, arterial stiffness or inflammation was not significantly different across treatment groups ($P>0.05$). The inclusion of healthy and not vitamin D deficient (mean serum 25(OH)D concentration of 78.3 nmol/L) subjects may drive the results toward the null. Participants with higher vitamin D levels are less likely to benefit from extra load of vitamin D. In contrary, where subjects had medical conditions (diabetes and stroke) or were vitamin D deficient the results showed beneficial effects.

Conclusion

In summary, observational data consistently show an inverse relationship between levels of 25(OH)D in circulation and CVD [181], though intervention trials are less satisfactory, a finding confirmed by meta-analysis While a meta-analyses showed that the risk of ischemic heart disease (18 trials) and early death (17 trials) was increased by 39.0% (95% CI, 25.0%-54.0%) and 46.0% (95% CI, 31.0%-64.0%), respectively, in the lowest vs. the highest quartiles of serum 25(OH)D concentrations, a more recent meta-analysis failed to show any beneficial effects [187]. Worth noting, these outcomes were secondary outcomes in most studies. Accordingly, more studies with CVD as their primary outcomes are warranted.

Diabetes

Diabetes is a global health problem and is associated with serious health complications. It is expected that the prevalence of people particularly with type 2 diabetes increases significantly in the next few decades; from 366 million in 2011 to 552 million by 2030 [188]. From a regional perspective, the total number of people diagnosed with diabetes exceeds 200,000 people, plus another 100,000 undiagnosed cases in New Zealand alone[1]. In the Middle East and North Africa together, 26.6 million cases of diabetes have been reported[2]. Based on the data from New Zealand Health Tracker [16], within the MELAA group, the prevalence of type 2 diabetes was higher in Middle Eastern population than European counterparts. There are several environmental and genetic risk factors for diabetes. One such environmental risk factor for both type 1 and type 2 diabetes is believed to be vitamin D deficiency [189].

[1] Retrieved from http://www.health.govt.nz/our-work/diseases-and-conditions/diabetes/about-diabetes on 2end of July 2012

[2] Retrieved from http://www.nytimes.com/2011/01/13/world/middleeast/13iht-M13CDIABET.html on 2end of July 2012

Mechanisms of Action

The existence of a VDR on the plasma membrane [190] as well as the expression of genes related to VDRs, VDBPs and 24-hydroxylase in pancreatic islets are suggestive of rapid non-genomic and genomic effects of vitamin D on pancreatic beta cells and insulin receptor signalling pathway, respectively [191-193].

Furthermore, vitamin D may affect the secretion and synthesis of insulin [194]. This rapid non-genomic effect of vitamin D in ß cells is believed to be through voltage-gated calcium channels which in turn, stimulate the secretion of insulin [195]. Vitamin D may also have an indirect role in insulin metabolism [165]. Vitamin D deficiency/insufficiency results in an increase in the concentrations of PTH. The ratio of PTH to serum 25(OH)D and PTH concentration have been shown to be associated negatively with Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and positively with Quantitative Insulin Sensitivity Check Index (QUICKI) suggesting an independent role for PTH in insulin resistance and sensitivity [165].

Vitamin D is also postulated to affect the immune system and to have anti-inflammatory effects [196]. 25(OH)D is believed to decrease the secretion of inflammatory cytokines such as interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α) [196], and to enhance the expression of anti-inflammatory cytokines such as interleukin 10 (IL-10) from activated *B* cells [197], as well as to direct dendritic cells (DCs) toward a more tolerogenic state [198]. These observations are of utmost importance in the context of type 1 and 2 diabetes where impaired autoimmune system and failure of tolerance and inflammation are central features, respectively [192].

Vitamin D, in *in-vivo* studies, has been shown to increase insulin receptor gene expression, insulin receptor numbers and up regulate insulin related glucose transport genes [199]. Stimulation of insulin receptor signalling pathway activity increases insulin sensitivity and modulates glucose tolerance [192].

Supporting Evidence

Vitamin D deficiency/insufficiency has been reported to be highly prevalent in patients with type 1 and type 2 diabetes [189, 200-202]. In a 5 year prospective study of non diabetic Korean subjects with one or more risk factors for type 2 diabetes (n=1080), Lim et al (2013) showed that 10.5%, 51.6% and 38.0% of participants had serum 25(OH)D concentrations <25 (deficiency), <50 (insufficiency) and ≥ 50 nmol/L (sufficiency), respectively [203]. At

the follow-up, the incidence of diabetes was significantly higher in subjects with deficiency followed by insufficiency and then sufficiency; 15.9%, 10.2% and 5.4%, respectively (P<0.001). Compared with those having vitamin D sufficiency, insufficient and sufficient subjects had hazard ratios of 2.06 (95% CI: 1.22, 3.49) and 3.23 (95% CI: 1.66, 6.30), respectively. Interestingly, the relationship was not dependent on known risk factors for type 2 diabetes such as body mass index (BMI), HOMA2-IR and insulinogenic index (IGI). Furthermore, this relationship, in another study [204], was independent of lifestyle interventions such as weight loss and physical activity indicating a direct role of vitamin D in the pathogenesis of type 2 diabetes.

Intervention trials, particularly those from Middle East and Southeast Asia, provide supportive evidence for a role of vitamin D in glucose metabolism in patients with type 1 diabetes [205], in non-diabetic insulin resistant subjects [206], in patients with type 2 diabetes [22, 207, 208] and in women with gestational diabetes [209]. To assess the efficacy of vitamin D supplementation on glycemic control in patients with type 1 diabetes, Aljabri et al (2010) recruited 80 patients aged > 12 years old [205]. Patients with serum 25(OH)D concentration <50 nmol/L received 4000 IU/day vitamin D + 1200 mg calcium/day for 12 weeks. Those achieving serum 25(OH)D concentration of >75 nmol/L had significantly lower mean glycosylated haemoglobin than those achieving <75 nmol/L (7.1±1.5 vs. 9.1±2.4, *P*=0.02). This study had several limitations; it was not a randomised blinded trial and it lacked the placebo arm. Furthermore, the individual significance of vitamin D cannot be determined. Calcium supplementation may have confounded the results because appropriate cytosolic calcium concentration is needed for glucose transport in muscle cells [210].

In a pre-post study, Talaei et al (2013) assigned all subjects with type 2 diabetes (n=90) to receive weekly 50,000 IU vitamin D for eight weeks [208]. Vitamin D supplementation significantly decreased mean fasting plasma blood (FPB), insulin and HOMA-IR [from 138.48±36.74 at baseline to 131.02±39 mg/dl at the follow up (P=0.05) for FPB, from 10.76±9.46 to 8.6±8.25 μIu/ml (P=0.028) for insulin and from 3.57±3.18 to 2.89±3.28 (P=0.008) for HOMA-IR]. Unexpectedly, the mean serum 25(OH)D concentration was high in this population (107.5±48.2 nmol/L). Yet, significant improvements in glucose homeostasis were observed. There are some limitations to this study; firstly, it lacked a placebo arm. Furthermore, the results could be confounded by the type of treatment used;

patients were treated with either a hypoglycaemic agent or a combination of hypoglycaemic agents and diet modification.

In another study from Iran, 73.3% and 38.9% of study population, who had diabetes, were vitamin D deficient and severely deficient, respectively [207]. The authors assigned patients (n=90) to receive either a plain yogurt drink (150 mg calcium per 250 ml) or vitamin D fortified yogurt drink (500 IU vitamin D + 150 mg calcium per 250 ml) or a vitamin D + calcium fortified yogurt drink (500 IU vitamin D + 250 mg calcium per 250 ml) twice a day for 12 weeks. Daily consumption of vitamin D or vitamin D + calcium improved glycosylated haemoglobin (P<0.001 for both), HOMA-IR (P=0.001 for both), fasting serum glucose (P<0.05 for both), waist circumference (P<0.001 for both) and BMI (P<0.001 for both), all of which were significantly different from the plain yogurt group after intervention. Overall, the authors found an inverse relationship between changes in serum 25(OH)D concentration and the mentioned parameters. The compliance rate was very high in this study (100%).

Vitamin D deficiency was also highly prevalent (80.0%) in women with first time gestational diabetes (n=45) in another study from Iran [209]. A single intracellular injection of 300,000 IU vitamin D immediately after delivery inhibited a significant increase in indices of insulin resistance and a significant decrease in Quantitative Insulin Sensitivity Check Index (QUICKI) in these women. In contrast, in the control group, glucose tolerance and insulin resistance indices deteriorated 3 months after intervention. The indices of HOMA-IR and ß cell function increased significantly (P=0.01 for both), and the insulin sensitivity and QUICK decreased significantly (P=0.002 and 0.008, respectively).

However, a study among patients with type 2 diabetes from United Kingdom (UK) failed to show any improvements in insulin resistance and glycosylated haemoglobin in response to different doses of vitamin D supplementation (single oral dose of 100,000, 200,000 IU or placebo) for 16 weeks [211]. This study was underpowered due to small sample size in each arm (19, 20, and 22). It has also been reported that single oral mega-dose of vitamin D is not as effective as more frequent daily doses [212].

Conclusion

In conclusion, while observational studies repeatedly show an inverse association between serum vitamin D and the incidence of both types of diabetes, reviewed by several authors

[192, 202], the therapeutic effect of vitamin D on glycaemia measures is less promising [213]. To note, interpreting such results should be done with caution as carrying certain genes involved in vitamin D metabolism can increase the susceptibility of person to develop type I diabetes [189], and polymorphisms in VDR can modify the susceptibility to develop diabetes type 2 [214]. Well-designed randomised controlled trials with prolonged supplementation (at least 6 months [22, 206]) while controlling for confounders such as genetic polymorphisms and ethnicity is warranted. The positive effects are consistently observed in populations at risk of chronic vitamin D deficiency such as Iranians and Saudi Arabians. One can conclude that long-term exposure to low serum 25(OH)D may increase susceptibility to diabetes, and as such may increase the responsiveness to treatments with vitamin D supplementation.

Risk factors for vitamin D deficiency

Inadequate Cutaneous Vitamin D Synthesis

Ultraviolet radiation, a component of solar radiation, comprises of a wide range of ultraviolet (UV) wavebands. UVA and -B have wavebands of 320-400 nm spectrum and 290-320 nm, respectively, and are the only UV wavelengths reaching the earth's surface [215]. UVB contributes to the development of skin cancers and photo aging [215] in one hand, and to the cutaneous vitamin D synthesis [39] in the other hand. Exposure to UVB can produce up to 90% of human's vitamin D requirement [24]. In some circumstances when dietary intake of vitamin D is minimal, sun exposure would account for up to 100% of the requirement [24]. But making a broad uniform recommendation for daily sun exposure by which vitamin D requirement is met is very complicated.

To make such predictions, the level of sun exposure that is required for adequate vitamin D synthesis, but does not result in increased risk of skin cancer and other health complications should be considered [29]. McKenzie et al (2009) attempted to shed light on this issue with a study design measuring UV spectrum reaching the ground at Lauder, NIWA's Central Otago site in different seasons [216]. The authors found that the peak sun-burning and vitamin D producing UV in winter is 10% and 5% of its summer value, respectively. In winter; the window of optimum UV exposure times decreases due to a decrease in fraction of skin exposed. Complicating the issues further are those factors affecting cutaneous vitamin D synthesis such as skin pigmentation, aging, sunscreen use, time of the day, time of the year, latitude, zenith angle and pollution [217].

Skin Colour

Concentration of melanin in epidermis determines levels of UVB which reach the skin site for vitamin D synthesis [218]. Melanin absorbs electromagnetic radiation, and therefore competes with 7-dehydroxycholesterol for UVB photons [218]. Migration to countries with higher latitudes exacerbates the risk of vitamin D deficiency in migrants with darker skin pigmentation [42]. This is because they require greater amount of sun exposure than those with moderately fair skin (2-5 times more) to make adequate vitamin D [216]. Darker skin pigmentation has been associated with lower 25(OH)D concentration [64, 95, 219, 220]. In a study of 14-18 years old black and white adolescent subjects in Georgia, vitamin D deficiency prevalence was 73.8% and 46.9% in black girls and boys, and 2.6% and 3.9% in white girls and boys, respectively [219]. These findings were replicated by other studies from New Zealand [18, 64, 220, 221]. Rockell et al (2005) and New Zealand Ministry of Health (2012) reported higher prevalence of vitamin D deficiency in children of Pacific and Maori origin compared to their European and other ethnicities [18, 64]. People of these ethnicities are known to have light to medium brown skin compared to fair skin of Caucasians. Furthermore, quantitative measures of skin colour have been shown to correlate with 25(OH)D levels [222, 223]. It has been recommended that people with fair, dark and black skin need 6-9, 12-18 and 30-45 minutes of sun exposure before 10 am or after 2 pm during summer (December and January) in different regions in New Zealand (**Table 2.2**). It should be noted that the needed time in winter is not similar across different regions in New Zealand (Diamond, et al., 2005; Ministry of Health and Cancer Society of New Zealand, 2012a) due to the different latitude and solar zenith angles (SZA).

Latitude, Time of Day, Season and Pollution

Latitude, time of day and season affect UVR levels [224]. In comparison with areas closer to the equator, the UVR must travel farther to reach areas nearer to the north or south poles. This is because of the tilt of the earth on its axis [224]. Also, the position of the sun on the horizon and the zenith angle of UVB radiation affect UVB photons reaching the earth's surface [216]. In winter, the SZA is larger than summer. Larger angles are associated with lower UVB levels [224]. Accordingly, serum 25(OH)D concentrations fall during winter compared with summer [64, 99, 220, 225].

Table 2.2: Recommended sun exposure times (minutes) which result in 1/3 MED for people with moderately fair skin* at different times of day

Region	December-January	July-August	
	Before 10 am or after 2 pm	At 10 am or 2 pm	At 12 noon
Auckland	6-8	30-47	24
Christchurch	6-9	49-97	40

MED: minimal erythmal dose
*Exposure time for people with darker skin would be 2-5 times greater [216]. However, people should always avoid sunburn and refer to the daily Sun Protection Alert or daily Ultraviolet index regional forecast services for more details of ultraviolet radiation. Reproduced and adapted from [23, 226]

In 26 men with extended outdoor activity, serum 25(OH)D concentrations in late summer were statistically significantly higher than in winter (a median [25^{th} – 75^{th} percentile] difference of 49 [29-67] nmol/L, $P<0.0001$). In a study of 1585 children (both gender and different ethnicities) aged 5-14 years old, Rockell et al. (2005) found higher prevalence (unadjusted) of vitamin D deficiency and insufficiency in winter versus summer, 5.0% & 43.0% vs. 2.0% & 16.0%, respectively [64]. The Ministry of Health reported that the prevalence of vitamin D deficiency was higher from August to October which are the late winter months in New Zealand compared to summer months [18].

However, the trials from the Middle Eastern region show contradicting results. While season was a significant predictor of serum vitamin D levels in a study by Saadi et al. (2006) from United Arab of Emirates (UAE) [20], Rassouli et al. (2001) did not find any correlation between these two variables in Iran [12]. Clothing style in the UAE is more variable, but it is more uniform in Iran (completely covered clothing style) undermining the importance of cutaneous vitamin D synthesis. Furthermore, this discrepancy can be due to the ample sun shine all year round in the Middle East as well as pollution in some countries like Iran.

Man-made air pollution in New Zealand is associated with hundreds of cases of morbidity and premature mortality [227]. Yet, this phenomenon (due to either natural or anthropogenic components) is posing a bigger threat to the health and wellbeing of people living in the Middle East. In some cities like Tehran, the capital city of Iran, air pollution rises to such a dangerous level that public holidays are declared. Air pollution (aerosol) is reported to

obstruct sunrays [228]. Lower exposure of skin to sunlight, in turn, leads to inadequate cutaneous vitamin D synthesis [229].

Comparing Tehran with Ghazvin two large cities in Iran with two different levels of pollution and ground UVB but similar latitudes, Hosseinpanah et al (2010) [228] demonstrated that the ground level of UVB in Ghazvin, where pollution is less was higher than Tehran. Ghazvinian women had significantly higher median serum 25(OH)D concentration than Tehranian women ($P<0.001$) despite having the same covered dressing style. The prevalence of vitamin D deficiency and insufficiency was higher in Tehran than Ghazvin (36.0% and 54.0% in Tehran vs. 31.0% and 32.0% in Ghazvin, respectively) coupled with higher secondary hyperparathyroidism [229]. The OR for living in Tehran and having serum 25(OH)D concentration < 50 nmol/L was 5.22 (95% CI, 2.2 – 12.2). This adverse effect of pollution on cutaneous vitamin D synthesis has also been observed in infants, toddlers and postmenopausal women [230, 231].

Clothing Style

Middle Eastern women, in particular Muslim women who are more likely to cover themselves (totally or partially) due to religious and cultural reasons, are at higher risk of vitamin D deficiency [19, 131, 225, 232]. Mishal showed that vitamin D insufficiency was more prevalent in women with total covering dress (niqab), followed by partially covered (hijab) women, than western dressed women in summer and winter, 83.3%, 54.8% and 30.8% in summer, and 81.8%, 77.6% and 75% in winter respectively [225].

Conversely, Saadi et al (2006) and Hashemipour et al (2004) did not find any statistically significant relationship between serum 25(OH)D concentration and clothing style [11, 20]. The lack of association observed in these studies is because of the public dressing norm and the regulatory dressing code in The UAE and Iran, respectively where all women with no exception cover their body except for face and hands. This was elucidated by a study examining the effect of dressing style on serum 25(OH)D concentration among Emirati women and comparing them within their ethnicity (fully covered vs. face and hand uncovered dressing style) and with non-Arabic Caucasian women who have western clothing style. Women with western clothing style had significantly higher serum 25(OH)D concentrations than Emirati women who have conservative clothing style [20]. While the former comparison showed no relationship between serum 25(OH)D concentration and dressing style, the latter showed a significant relationship. As a consequence, the authors concluded that the darker

skin colour of Emirati women could be the reason. However, it should be noted that people should expose themselves to the sun for longer hours to get enough UVB if they want to have bare hands and face only [233], and this is less likely to happen due to the hot weather in The UAE.

Fear of Skin Cancer

While sun exposure is the main source of vitamin D, excessive sun exposure is associated with increased risk of skin carcinoma. People are advised to limit sun exposure and to have sensible sun exposure to prevent skin cancer. This health message has led people to expose their skin less to sun, by either wearing sunscreen or wearing clothing that covers the body. Wearing a sunscreen with sun protection factor (SPF) of 30 compromises more than 95% of cutaneous vitamin D synthesis [217].

As the skin cancer awareness increases, the attitudes and beliefs toward sun exposure are shifting toward UV protection behaviour which is becoming a determining factor of serum 25(OH)D concentration. At least, exposure of arms and legs with no barrier is required for adequate synthesis of vitamin D [233]. Some ethnic groups such as Asian women in Australia [234] and South Asian women in New Zealand have been reported to be at higher risk of vitamin D deficiency possibly due to deliberate sun exposure avoidance [221]. Von Hurst et al. (2010) reported that 84.0% of South Asian women aged >20 had inadequate level of 25(OH)D (≤ 50 nmol/L), and 65.0% of the study population had sun exposure avoidance attitude due to concerns about skin cancer and the strength of New Zealand sun [221].

Aging

The aging population in New Zealand and the Middle East is increasing. The capacity of the epidermis to synthesise vitamin D [235] and the expression of VDRs [121] are reported to be compromised by aging. These findings are supported by several human trials [236, 237]. In a multicentre study of 43 osteoporosis centres in Italy, Isaia el al. (2003) studied 60-80 year-old women who were referred for osteoporosis risk assessment for the first time [237]. They found that 27.0% and 76.0% of women had serum 25(OH)D concentrations <12.5 nmol/L (< 5 ng/ml, severe vitamin D deficiency) and <30 nmol/L (<12 ng/ml, vitamin D deficiency), respectively. A limitation of this study is that the study population did not represent the whole elderly population. Type of dwelling may affect vitamin D status of elderly; it has been reported that institutionalised elderly are at higher risk of vitamin D deficiency [4]. However, age-related decline in serum 25(OH)D concentration was also seen in a nation-wide study of

non-institutionalised adolescents and adults 15 years and over in New Zealand [238]. Compared with women aged 15-18, mean serum 25(OH)D concentration declined by 9.0 and 13.0 nmol/L in women aged 45-64 and ≥ 65, respectively. These findings were confirmed by Saadi et al (2006) who reported an inverse correlation between age and serum 25(OH)D concentrations [20].

However, recent findings from the New Zealand Adult Nutrition Survey showed no difference in vitamin D deficiency prevalence across all age groups [18] as was also seen in studies from the Middle East [12]. Conversely, a few studies from the Middle Eastern region showed that vitamin D deficiency was more prevalent in younger than older populations [11, 232], a finding confirmed by our pilot study among Middle Eastern women in Auckland [17]. Older women in our study were more likely to take vitamin D supplements than the younger women.

The role of aging as an independent risk factor for vitamin D deficiency is unclear because low blood levels of 25(OH)D were improved when individuals were exposed to ultra violet radiation [239]. This finding contradicts the age-related impairment of cutaneous vitamin D synthesis hypothesis. Furthermore, aging is a less important risk factor for vitamin D deficiency than diseases such as renal dysfunction [240], low dietary vitamin D intake, smoking, ethnicity [95], sun avoidance behaviour [221], and limited outdoor physical activity [241].

Sedentary Lifestyle

Sedentary lifestyle and lack of outdoor physical activity has been associated with lower concentrations of 25(OH)D in circulation [144, 219, 234, 241-243]. Physical activity has been reported to maintain serum 25(OH)D concentration on one hand, and to increase exposure to sunlight, if it is outdoor activity, on the other hand [242]. In a study among 2621 middle aged men and women (55-74 years old) in the United States, Brock et al. (2010) found twelve risk factors for low levels of vitamin D and one modifying factor which was vigorous physical activity [242].

Inadequate dietary vitamin D intake

When adequate sun exposure is compromised by physiological, environmental, cultural and religious circumstances, dietary vitamin D intake is the main alternative. A combination of sensible sun exposure and increased intake of vitamin D rich foods is recommended during

winter. Consistent with the approach adopted by Institute of Medicine (IOM), the New Zealand guideline for daily vitamin D intake for people at all age groups is based on serum 25(OH)D concentration (the dosage corresponding to a serum 25(OH)D level of at least 50 nmol/L) rather than the median dietary intake of the population, due to the lack of data [28]. The recommended daily allowances (RDAs) for vitamin D by IOM were based on conditions of minimal sun exposure [25]. The IOM recommendations for vitamin D are more than those recommended by New Zealand Ministry of Health. Most countries in the Middle East have adopted IOM recommendations. The IOM and New Zealand recommended daily intake for people at all age groups are presented in **Table 2.3**.

Based on studies assessing the effect of vitamin D supplements on serum calcium in humans, the upper level of vitamin D of 25 µg/day or 1000 IU/day and 80 µg/day or 3200 IU/day for 0-12 month infants and all other age and gender groups, respectively, have been adopted. For adolescents, the recommendations for adults have been adopted because evidence is lacking [28].

It is worth noting that our body's response to vitamin D ingested from diet or supplements is physiologically different from that of cutaneous vitamin D synthesis. While vitamin D produced from exposure of skin to UVB stays longer in the circulation, ingested vitamin D results in a rapid but less sustained increase in vitamin D in the blood stream [244]. Furthermore, vitamin D provided by our diet is too low to maintain adequate levels of 25(OH)D in circulation [245].

Based on the data from 3-days food diary questionnaire completed by 924 female and male subjects aged >20 years old, residing in Qazvin, Iran, the mean intake of vitamin D was 168±440 IU/day. The majority of study population (90-95%) had less than the minimum daily recommended amount of 400 IU/day [246]. In the UAE, Al Ain, 37.0% of Emirate female students aged 19-23 years old had intake of vitamin D less than 200 IU/day, despite that 87.0% of milk and milk products and 100% of palm oil and hydrogenated vegetable oils in Al Ain supermarkets are fortified with vitamin D [245].

Table 2.3: Recommended vitamin D dietary reference intakes by life stage

Age groups	IOM[1]		New Zealand[2]	
	RDA (IU/day)	UL	AI (IU/day)	UL
0-6 months[3]	400	1000	200	1000
6-12 months[3]	400	1500	200	1000
1-4 years old	600	2500	200	3200
4-9 years old	600	3000	200	3200
9-51 years old	600	4000	200	3200
51-71 years old	600	4000	400	3200
≥ 71 years old	800	4000	600	3200

IOM, Institute of Medicine; RDA, Recommended Daily Allowance; UL, Upper Level of intake; AI, Adequate Intake
[1] Reproduced from Ross et al (2011) [25]
[2] Reproduced from Ministry of Health and NHMRC (2006) [28]
[3] Reflects Adequate Intake (AI) rather than RDA (RDAs have not established for infants)

In line with these studies from the Middle East, our pilot study showed that all participants taking vitamin D supplements and multivitamin containing supplements had serum 25(OH)D concentrations <50 nmol/L though using vitamin D supplements was associated with higher serum 25(OH)D concentrations [17]. Similarly, intake of supplemental sources of vitamin D and oily fish has repeatedly been reported to be associated with serum 25(OH)D concentrations in some populations [14, 17, 20]. In a cross-sectional population based study of five immigrant groups (Iran, Pakistan, Sri Lanka, Turkey, and Vietnam) living in Oslo, Norway, the use of cod liver oil supplements and oily fish intake were important predictors of vitamin D status [14].

In New Zealand, unfortunately, due to the unavailability of a complete database for vitamin D, there is no recent reliable population based data for dietary vitamin D intake [247]. But, the twenty four hour recall nutrient analysis of the 1997 New Zealand Adult Nutrition Survey data showed that the main sources of dietary vitamin D were margarine, eggs, milk and oily fish [23].

Food Sources

The dietary sources of vitamin D are very limited (**Table 2.4**). Oily fish such as salmon, mackerel, herring, tuna and fish liver oils are the richest sources of vitamin D, and other sources contain very low amounts of vitamin D (egg, beef, liver, milk and dairy products) so that they are often not acknowledged unless they are fortified with vitamin D.

Fortification of foods with vitamin D is not mandatory in New Zealand or Middle East, but food manufacturers in both regions are allowed to add vitamin D voluntarily to some foods such as milk, milk based products (yogurt, butter), some beverages, legumes, some breakfast cereals and margarines and fat products [28, 248].

Supplemental sources of vitamin D can be purchased over-the-counter as either multivitamin or single vitamin D preparations containing 100 – 1000 IU vitamin D_2 or D_3, or prescribed by doctors as pharmaceutical preparations containing 50,000 IU vitamin D_3. The latter might be considered for those populations at risk of vitamin D deficiency in New Zealand and is fully funded [subsidised by The Pharmaceutical Management Agency (PHARMAC)]. The number of people supplemented with prescribed vitamin D tablets (50,000 IU) has more than doubled since 2007 (from 84,090 in 2007 to 174,440 in 2010) [23].

Other risk factors

Obesity

Obesity is a worldwide health concern. In New Zealand, the prevalence of obesity has been increasing (from 9.0% and 11.0% in 1977 to 27.7% and 27.8% in 2007/08 in male and females, respectively) [249]. The same or maybe worse scenario has also been seen in the Middle East [250].

Vitamin D is a fat soluble vitamin and is stored in body fat stores for later use [52]. The larger the adipose tissue is, the more likely vitamin D is trapped [251]. Furthermore, obese people are more likely to have less outdoor and physical activity and consequently to be less exposed to sunlight.

Convincingly, higher BMI [64, 207, 242, 252-254] and higher adiposity [252, 253, 255] have been reported to be associated with lower levels of serum 25(OH)D, and higher concentrations of PTH [252]. Snijder and co-workers (2005) in their study on elderly men and women aged ≥ 65 found that serum 25(OH)D concentration had significant inverse relationship with body fat, but weaker relationship with BMI, waist circumference and sum of skin folds. Several New Zealand and Middle East based studies confirmed such relationship even after adjusting for several potential confounders among different age groups [18, 19, 64, 207, 238]. A recent report published by the New Zealand Ministry of Health reported that obese people had significantly lower annual mean levels of vitamin D compared with underweight people and people with normal weight (Ministry of Health, 2012b). The

differences remained significant even after adjusting for age, sex and ethnicity. It is plausible to say that the re-emergence of vitamin D deficiency may echo the increasing trend of obesity worldwide.

Contraceptive Use

Oral contraceptives are mainly prescribed to women for preventing pregnancy. In some circumstances, they are prescribed to women for controlling acne [256] and menses [257]. Oral contraceptives are hormonal and may contain progesterone (mainly in a synthetic form called progestin) or a combination of progesterone and oestrogen. These pills, depending on the prescription, provide a wide range of oestrogen dosages (15-50 μg/day) [34]. Other hormonal contraceptives such as skin patch and vaginal rings also provide a steady dose of oestrogen, though the dose is very low <15 μg/day [34].

Several studies have shown a positive relationship between contraceptive use and serum/plasma 25(OH)D concentrations [258-261]. In a study among Swedish women of child bearing age living in northern latitude [258], a trend between serum 25(OH)D concentrations and oestrogen contraceptive use was observed (73.3 nmol/L in contraceptive users vs. 62.9 nmol/L in non-users, P=0.07), and oestrogen use was a significant predictor of serum 25(OH)D concentrations (P=0.02). Further support comes from a study among 66 Caucasian women aged 20-40 years old living in Boston. After adjustment for dietary vitamin D intake and age at baseline, oral contraceptive users had 39% higher plasma 25(OH)D concentrations than non-users (P=0.003) [259]. Interestingly, those women who discontinued using oral contraceptives (n=5) saw a mean decrease of 25.5±17.7 nmol/L in their plasma 25(OH)D concentrations. Although, all measurements were done in winter, the sample size was too small to provide strong evidence. Analysis of data collected cross-sectionally in Toronto found a higher proportion of oral contraceptive users to be in the optimal vitamin D status category (plasma 25(OH)D ≥ 75 nmol/L) than other categories [260].

Table 2.4: Sources of vitamin D_2 and D_3‡

Source		Vitamin D content
Cod liver oil		~400–1,000 IU vitamin D_3/teaspoon
Salmon, fresh wild caught		~600–1,000 IU vitamin D_3/3.5 oz
Salmon, fresh farmed		~100–250 IU vitamin D_3/3.5 oz
King salmon, fresh*		233 IU vitamin D_3/100 g
Salmon, canned		~300–600 IU vitamin D_3/3.5 oz
Red salmon, canned*		160 IU vitamin D_3/100 g
Sardines*		440 IU vitamin D_3/100 g
Sardines, canned		~300 IU vitamin D_3/3.5 oz
Mackerel, canned		~250 IU vitamin D_3/3.5 oz
Tuna, canned*		232 IU vitamin D_3/100 g
Kahawai*		200 IU vitamin D_3/100 g
Shiitake mushrooms, fresh		~100 IU vitamin D_2/3.5 oz
Shiitake mushrooms, sun-dried		~1,600 IU vitamin D_2/3.5 oz
Egg yolk*		66 IU vitamin D_3/100 g
Butter*		37 IU vitamin D_3/100 g
Fortified foods		
Fortified milk		100 IU usually vitamin D_3/8 oz,
Fortified orange juice		100 IU vitamin D_3/8 oz
Infant formulas		100 IU vitamin D_3/8 oz $_3$
Fortified yogurts		100 IU usually vitamin D_3/8 oz
Fortified butter		56 IU usually vitamin D_3 /3.5 oz
Fortified margarine		429 IU usually vitamin D_3/3.5 oz
Fortified cheeses		100 IU usually vitamin D_3/3 oz
Fortified breakfast cereals		~100 IU usually vitamin D_3/serving
Supplemental sources		
Over the counter		100-1,000 IU vitamin D_3 or vitamin D_2
Prescription	Multivitamin	300 IU
	Vitamin D_3	50,000 IU†

IU = 25 ng. Oz (ounce); 1 oz = ~28.35 grams. †It is prescribed as a monthly dose; once a month for three months. In cases of moderate/severe vitamin D deficiency, the dosage is one tablet/day for 10 days (loading phase).
‡The sources and contents of the foods included, except for foods with an asterisk, are from US data and adopted and reproduced from [52].
*Adopted and reproduced from New Zealand Food database Version 8, Foodworks 2007, Xyris software.

The clinical implications of these findings are unclear. The increase in vitamin D status does not primarily mean that using contraceptive improves vitamin D nutrition. Genistein and 17β-estradiol have been shown to down-regulate CYP24A1 (24-hydroxylase) and to up-regulate CYP27B1 (1,α-hydroxylase) in human colon and breast cancer cells affecting vitamin D metabolites concentration [262]. These findings were confirmed by a study examining the effect of endogenous fluctuation of oestrogen in pre-menopausal women. The authors concluded that at elevated levels of oestrogen, 25(OH)D is preferentially metabolised to $1,25(OH)_2D$, while at low levels it is predominantly metabolised to $24,25(OH)_2D$ [263]. Therefore, endogenous oestrogen may promote the formation of $1,25(OH)_2D$ and inhibit the catabolism of vitamin D.

Contraceptive use has also been suggested to increase VDBP concentration [79, 261]. Moller et al (2013) in their cross-sectional study, examined the effect of hormonal contraceptives on plasma vitamin D metabolites in 75 healthy Danish women [261]. They found a significant relationship between hormonal contraceptive use and total 25(OH)D and VDBP, but not free 25(OH)D levels indicating that hormonal contraceptives may affect the metabolism of VDBP. However, hormonal contraceptive users had significantly higher daily intake of calcium which may have confounded the results. Higher calcium intake has been associated with higher levels of vitamin D [264]. Furthermore, no significant differences were observed in calcium homeostasis and bone turnover indices between hormonal contraceptive users and non-users, supporting the free hormone hypothesis. Based on the free hormone hypothesis, only hormones liberated from binding proteins are biologically active [71]. It should be noted that the small sample size and the high median plasma 25(OH)D concentration of 79 [64-111] nmol/L may have limited the authors' availability to detect any between group differences.

Vitamin D Binding Protein

VDBP stimulates 25-hydorxylase activity in rabbit liver microsomes and mitochondria [265]. A positive correlation between plasma VDBP and plasma 25(OH)D concentrations has been reported by some studies [261, 266]. The increase in VDBP is associated with an increase in total $1,25(OH)_2D$ and total 25(OH)D concentrations, but not the free form of these metabolites [261, 267, 268]. Based on the free hormone hypothesis, one can postulate that the increase in total 25(OH)D concentrations, but not the free form, should not have any physiological advantage. Powe et al (2011) did not find any significant relationship between BMD and total serum 25(OH)D concentrations in 49 healthy young adults (P=0.236), though

BMD was significantly associated with free and bio-available (free + albumin bound) serum 25(OH)D concentrations (r=0.413, P=0.003 and r=0.441, P=0.002, respectively) [266].

Genetic Background

Environmental factors have explained some of the variation in the vitamin D levels leaving a large proportion to unknown factors [269]. Based on twin and family studies, the heritability of vitamin D status has been confirmed, though there is a large variation in its estimations [270]. With the recent advances in the field of genetics, the contribution of genetics in vitamin D metabolism and regulation has been the focus of many recent clinical trials. These studies have identified associations between serum levels of vitamin D and several genes such as *CYP27B1*, *CYP2R1*, *VDR*, *CYP24A1*, 7-dihydrocholesterol reductase (*DHCR7)* and *GC* [270, 271]. These genes encode 1a-hydroxylase, hydroxylation of vitamin D to 25(OH)D, VDR, inactivation of 1a,25(OH)2D, a reductase catalysing the conversion of 7-dehydrocholesterol to cholesterol in the skin and vitamin D binding protein, accordingly [270, 271]. The relationship between serum 25(OH)D and VDBP and its encoding gene, GC, has been extensively evaluated and the results have been reproduced across different populations.

Apart from carrying vitamin D and its metabolites in the circulation, VDBP scavenges actin [272] and binds to fatty acids [273]. Furthermore, its concentration has been shown to be associated with diseases such as diabetes and cancer (reviewed by Malik et al (2013) [274] and with serum 25(OH)D concentrations [261].

The most commonly studied GC variants are single nucleotide polymorphisms (SNPs) rs7041, rs4588, rs2282679 and rs1155563 [74-78]. It has been indicated that different GC variants have different affinity to 25(OH)D [275, 276] and have different glycosylation pattern affecting their metabolic rate and consequently their half-life [276]. Compared to GC-1f and GC-1s isoforms, GC-2 isoform has been shown to have the lowest affinity to 25(OH)D [275] and to lack trisaccharide glycosylation at position 436 [276].

Several cross-sectional studies have provided further support for the more rapid metabolic rate of the GC-2 variant [74, 276]. Plasma VDBP concentrations were the lowest in carriers of GC-2 allele compared to carriers of GC1-1 and GC1-2 alleles [mean (SE): 4.35 (0.07) μmol/L vs. 4.80 (0.03) μmol/L and 5.22 (0.03) μmol/L, respectively; $P<0.001$) [74]. Low levels of VDBP, in turn, have been associated with lower concentrations of vitamin D in the

circulation [72, 261]. Accordingly, vitamin D status may well be affected by VDBP genotype, and its phenotype [74-76, 277].

Lurisden et al (2005) found that plasma 25(OH)D and 1,25(OH)D concentrations were highest in post-menopausal women with GC1-1, intermediate in GC1-2 and lowest in women with GC2 phenotype [74]. More than half of women with GC2 phenotype had serum 25(OH)D concentrations <50 nmol/L, but no one had plasma PTH levels outside normal range, and all women with GC2 phenotype had BMC, BMD and other bone markers comparable to women with other GC phenotypes. The authors concluded that there may be a different threshold for vitamin D sufficiency for people with different GC phenotypes. It should be emphasised that low levels of VDBP are associated with several diseases such as diabetes and cancer [274] for which vitamin D deficiency is a recognised risk factor [180, 202], and it is known that 25(OH)D concentration in the circulation is affected by GC genotype [76]. Of note is the finding that, in winter, the proportion of the variation in 25(OH)D levels explained by polymorphisms in GC genes was substantially lower in East Asians (-7.0%), and was negligible in Europeans. The largest between-genotype group differences in serum 25(OH)D concentrations have been reported to be in summer [75].

In another study among 3210 elderly Han Chinese, Lu et al (2012) found that carriers of SNPs D432E (rs7041) as well as carriers of T436K (rs4588), rs2282679 or rs1155563 exhibited reduced plasma 25(OH)D concentrations [76]. Similarly, the most recent study by Perna et al (2013) showed that serum 25(OH)D concentrations were lower by 5.1-5.4 nmol/L and 8.8-9.6 nmol/L in heterozygous and homozygous elderly German subjects (n=3741), respectively, carrying the rare allele with SNPs rs4588, rs2282679 or rs1155563 [75]. The T436K polymorphism, in a regression analysis, accounted for approximately 21.0% and 7.0% of the variance in 25(OH)D concentrations in autumn in East Asians and Europeans living in Canada, respectively [78].

These findings have several important implications; first of all, from a public health perspective, it highlights the importance of more individualised approach in the prevention and the treatment of vitamin D deficiency and targeting at risk populations. It is possible that the carriers of rare GC alleles benefit less from UVR and need higher doses of vitamin D supplements if it is proven that they respond differently to vitamin D supplementation (this will be discussed in the next section). Furthermore, from a research view point, this genetic-environment interaction should be taken into account when the relationship between vitamin

D levels and genotype is investigated since this relationship may be more evident when the vitamin D intake or the baseline levels are high.

Diseases & Medications

There are several diseases known to be risk factors for vitamin D deficiency [217]. People with fat mal-absorption syndromes, chronic kidney diseases, hepatic failure, granuloma-forming disorders, some lymphomas and bariatric patients are at higher risk of vitamin D deficiency. These diseases either affect vitamin D absorption or vitamin D metabolism. Also, a wide variety of medications such as glucocorticoids interfere with vitamin D metabolism. Patients on such medications are at high risk of vitamin D deficiency [217].

Response to vitamin D supplements

In response to a given dose of vitamin D supplement, the effect on serum 25(OH)D concentration can differ between individuals. This variation can be explained by several environmental and biological factors. There are several efficacy and safety studies, though few of them have aimed to conduct dose-response trials which identify factors responsible for serum 25(OH)D variation in response to vitamin D supplementation (**Table 2.5**). Findings suggest that basal serum 25(OH)D concentration [31, 32], type of vitamin D supplement [33], dosing regimens [31, 32, 278], body composition [36, 264], age [240], dietary calcium intake [35, 264], hormonal contraceptive use [34], genetic [279, 280], dietary fat content and composition [281] and some diseases and medication [282, 283] may contribute to serum 25(OH)D variation in response to supplementation with vitamin D. Unfortunately, the effect of the majority of these factors on response to vitamin D supplements in a Middle Eastern population is not clear.

Basal 25(OH)D concentration

Baseline 25(OH)D concentration has been consistently shown to make a significant contribution to variance in 25(OH)D response to vitamin D supplementation [7, 31, 32, 269, 278, 284-288]. In response to supplementation with daily 4000 IU vitamin D for 14 days, Trang et al (1998) showed that change in 25(OH)D concentration had a significant inverse correlation with baseline serum 25(OH)D concentrations [289]. The largest increase was seen in subjects in the first tertile (10-34 nmol/L) followed by those in the second tertile (35-49 nmol/L) and then those in the third tertile (50-86 nmol/L); +30.6 ± 16.2, +25.5 ± 11.7 and 13.3 ± 13.9 nmol/L, respectively (P=0.02).

In a study by Bacon et al (2009), deficient subjects (<50 nmol/L) receiving a loading dose of 500,000 IU had larger incremental change in their serum 25(OH)D concentrations at one month than non-deficient subjects (≥ 50 nmol/L), 71.0 [95% CI, 58.0-84.0] vs. 50.0 [95% CI, 38.0-63.0] nmol/L(*P*=0.03), respectively [278]. Similarly, Canto-Costa et al (2006) found that while the mean increase was 25.4 nmol/L in subjects with serum 25(OH)D concentrations <50 nmol/L, it was 13.0 nmol/L in those with serum 25(OH)D concentrations >50 nmol/L (*P*<0.05). The participants were housebound elderly men and women (n=42) and received weekly 7,000 IU vitamin D_3 supplements for 12 weeks [284].

These findings were confirmed by another study which used a lower dose of vitamin D supplementation. DeLappe et al (2006) supplemented women aged >65 years old with daily 800 IU vitamin D_3 and 1000 mg calcium for 3 months. The mean serum 25(OH)D concentration increased significantly at the follow up. The mean increase in serum 25(OH)D concentration was higher in women with vitamin D insufficiency (n=36, <50 nmol/L), compared with vitamin D sufficient subjects (n=15, >50 nmol/L). The mean serum 25(OH)D concentration increased from 28.9±11.9 nmol/L at the baseline to 52.5±26.4 nmol/L at the follow up in vitamin D insufficient subjects, but in sufficient subjects it increased from 73.9±25.2 at baseline to 76.1±22.5 nmol/L at the follow up [290].

Veith et al (2001), however, failed to show any effect of baseline 25(OH)D concentrations on 25(OH)D response to supplementation [291]. The authors assigned participants (n=61; mean baseline serum 25(OH)D concentration, 40.7±15.4 nmol/L) to receive either daily 1000 or daily 4000 IU vitamin D_3 for 2-5 months. The majority of participants (93.5%) had serum 25(OH)D concentrations <40 nmol/L. Hence, due to the very low and narrow range of serum 25(OH)D concentrations and small sample size, the authors may have not enough power to detect any differences across basal 25(OH)D concentration groups.

Type of Vitamin D; D_3 vs. D_2

It has been long believed that the two supplemental forms of vitamin D, D_3 and D_2, are equally effective in elevating or maintaining serum 25(OH)D concentrations. However, emerging evidence suggest that vitamin D_2 is not as potent as vitamin D_3 [33, 150, 289, 292-295], especially in the long-term [295] and when it is administered in bolus doses [296].

Armas et al (2004) assigned healthy middle aged men (33.1±11.5y) with the mean baseline serum 25(OH)D concentration of 79.2±21.1 nmol/L (n=20) to receive a single oral dose of

50,000 IU vitamin D_2 or D_3 [33]. Although serum 25(OH)D concentrations increased significantly in both treatment groups after 28 days, the mean serum 25(OH)D concentration in the vitamin D_3 group was higher by 22.0 nmol/L than vitamin D_2 group. Compared to the area under the curve (AUC) for vitamin D_2, the AUC for vitamin D_3 revealed that the vitamin D_3 was 3-fold more potent. The AUCs to day 28 were 204.7 nmol/L and 150.5 nmol/L for vitamin D_3 and vitamin D_2, respectively. Recruiting the same age group but both genders, Trang et al (1998) gave participants the same accumulative dose but in a shorter period of time. Supplementation with daily 4000 IU vitamin D_2 or D_3 for 14 days resulted in a significant increase in serum 25(OH)D concentrations in both groups. The mean increase in serum 25(OH)D concentration was larger by 10.0 nmol/L in D_3 group compared with D_2 group; 23.3±15.7 nmol/L vs. 13.7±11.4 nmol/L (P=0.03), respectively [289].

The substantial discrimination in favour of vitamin D_3 has also been reported in studies among older persons. Supplementation with a single dose of 300,000 IU vitamin D_3 was more effective in ensuring serum 25(OH)D concentrations >50 nmol/L [294]. This dose of vitamin D_3 ensured serum levels >50 nmol/L in 89% and 100% of subjects 6 and 12 weeks after supplementation, respectively, while nobody in the group treated with vitamin D_2 achieved these levels. Furthermore, serum PTH levels were suppressed within the normal range in 42% of patients in vitamin D_2 group and in all patients in vitamin D_3 group. It should be noted that the route of delivery was different between two vitamin D groups (it was orally in vitamin D_3 group and intramuscularly (IM) in vitamin D_2 group) which may have affected the results. However, based on the AUC, and independent of the route of delivery, this dose of vitamin D_3 was reported to be more potent than D_2 in elevating serum 25(OH)D concentrations and in decreasing serum PTH levels when was administered to vitamin D deficient female patients of nursing home [292].

Brinkley et al (2011) assigned vitamin D deficient patients with the mean age of 53 years old to receive smaller doses of vitamin D_3 or D_2 for one year; daily dose of 1600 IU vitamin D_2 or D_3 or monthly dose of 50,000 IU D_2 or D_3 or matching placebos [293]. The authors showed that the average increase per 100 IU vitamin D_2 was 0.95 nmol/L, while the average increase for vitamin D_3 was 1.45 nmol/L. Supplementation with vitamin D_2 resulted in a significant decrease in mean serum 25(OH)D_3 concentration.

Several studies have shown that supplementation with vitamin D_2 increases serum 25(OH)D_2, but decreases 25(OH)D_3 [295, 297, 298]. Logan et al (2013) showed that supplementation with daily 1000 IU vitamin D_3 for 25 weeks was more effective than vitamin D_2 in elevating and maintaining serum 25(OH)D concentrations during the autumn and winter months in New Zealand [295]. Vitamin D_2 and placebo groups had significantly lower serum 25(OH)D_3 concentration than vitamin D_3 group. Serum 25(OH)D_2 increased though serum 25(OH)D_3 decreased significantly in vitamin D_2 group which was greater by 9.0 nmol/L than the placebo group (P<0.05).

The mechanistic pathways by which vitamin D_2 affects the metabolism of vitamin D_3 is not clear. Armas et al suggested that the up regulation of mechanisms involved in vitamin D_2 metabolism may lead to an increase in the degradation of serum 25(OH)D_3 [33]. There is no evidence for such hypothesis. However, what is evident is that 25-hydroxylase has higher affinity to vitamin D_3 than vitamin D_2 [299]. The rate by which vitamin D_3 is hydroxylated in kidney mitochondria is five times more than that of vitamin D_2 (at a rate of 10 vs. 2 pmol/mg protein X minutes, respectively) [299]. While vitamin D_3 is preferentially 25-hydroxylated vitamin D_2 is 24-hydroxylated and deactivated [300]. This is not the case for vitamin D_3; this molecule must first undergoes 25-hydroxylation, then 24-hydroxylation and then an additional side chain oxidation to be biologically deactivated [301]. Houghton and Veith (2006) in their article suggested that the higher affinity of hepatic hydroxylase, VDBPs and VDR to vitamin D_3 and its metabolites than vitamin D_2 may explain the higher potency of vitamin D_3 [301].

Table 2.5: Factors predicting serum 25(OH)D response to vitamin D supplementation

Study	Population Characteristics	Study Design, Duration & Groups	Factors		Description
			Affecting	Not affecting	
Aloia et al (2008) [32]	• Healthy white and African American men and women (n=138)	• RDBPCT • 6 months starting from the mid winter • Dosing at baseline started with 2000 IU D_3/day and 4000 IU D_3/day for those with >50 and ≤ 50 nmol/L, respectively. Then, the intake was modified	• **Ethnicity** • **Baseline level↓↑**	• **Age** • **BMI** • **Body Fat%**	• The goal o • African An Americans however, th between A
Armas et al (2004) [33]	• Healthy male (n=20)	• Randomised control trial • 28 days • Single dose of 50,000 IU vitamin D_2 or D_3 administered orally	• **Type of vitamin D**		• Significant supplemen In D_3 grou day In D_2 grou baseline lev • Based on th more than • The AUC t nmol/L, res
Bacon et al (2009) [278]	• Elderly men and women (n=63)	• Randomised double blind trial • 8 months • Single dose of 500,000 IU (loading dose), loading dose + monthly 50,000 IU or monthly 50,000 IU	• **Baseline level↓↑**	• **Dosing regimens**	• At one mon concentrati non-deficie • All dosing • The pattern at one and monthly do

Table 2.5 continued

Barger-Lux et al (1998) [286]	• Healthy men (n=116)	• Open labelled trial • 8 weeks • 1000, 10,000, or 50,000 IU D_3/day or other vitamin D metabolites	• **Dose↑↑** • **BMI↓↑** • **Baseline level↓↑**		• Significant D dose gro respectivel two higher • In addition contributed response
Biancuzzo et al (2013) [297]	• Healthy male and females (n=34)	• RDBPCT • 11 weeks • 0, 1000 D_2/day or 1000 D_3/day	• **Dose↑↑** • **Type of vitamin D**		• Significant supplemen supplemen • Significant $1,25(OH)_2$ vitamin D_2 Serum 1,25 • Significant s$1,25(OH)_2$ supplemen
Binkley et al (2011) [293]	• Community-dwelling men and women (n=64)	• RDBPCT • One year • 1600 IU D_2 or D_3/day or 50,000 IU D_2 or D_3/month and matching placebos	• **Dose↑↑** • **Type of vitamin D**	• **Frequency of dosing**	• Significant groups • The averag 1.45 nmol/ • D_2 resulted 3-fold grea 25(OH)D l
Blum et al (2008) [69]	• Healthy ambulatory men and women (n=257)	• RPCT • 12 months (one year) • 0 or 700 IU/day vitamin D_3+500 mg/day calcium	• **Dose↑↑** • **Season** • **Baseline levels↓↑** • **Body composition↓↑**		• Significant compared t nmol/L, res • Mean adjus nmol/L in t • The adjuste kg/m² grou

Table 2.5 continued

Canto-Costa et al (2006) [284]	• Homebound elderly male and female (n=42)	• Prospective control intervention trial • 12 weeks • 7,000 IU/week vitamin D_3	• **Baseline levels↓↑**	• **Body fat%**	• The increas across body 19.0±17.0 • Those with 25.4 nmol/ nmol/L
DeLappe et al (2006) [290]	• Females (n=114)	• Prospective cohort intervention trail • 3 months • 800 IU D_3/day+1000 mg /day calcium	• **Baseline level↓↑**		• Significant follow-up (follow-up) • The mean i per day • The mean i was higher s25(OH)D which incr sufficient s 76.1±22.5
Fu et al (2009) [279]	• Healthy adults (n=98)	• Open label un-blinded intervention trial • 600 and 4000 IU vitamin D_3/day for one year	• **Dose↑↑** • **T436K genotype**	• **Gender** • **BMI** • **D432E genotype** • **Age**	• Contributio 22% and 8 • Serum 25(carriers hav TT allele c respectivel
Gallagher et al (2012) [36]	• Postmenopaus al healthy white women with vitamin D insufficiency (n=163)	• RPCT • One year starting late winter and early spring • 0, 400, 800, 1600, 2400, 3200, 4000 and 4800 IU vitamin D_3+1200-1400 mg/day calcium	• **Dose** • **BMI↓↑**		• A curviline nmol/L in • Significant dose of vita • At 12 mont weight than obese subje

Table 2.5 continued

Gallagher et al (2013) [264]	• Healthy postmenopausal African American women with vitamin D insufficiency (n=110)	• RDBPCT • One year • 0, 800, 1600, 2400 and 4800 IU/day vitamin D_3+1200-1400 mg/day calcium	• **Calcium↑↑** • **BMI↓↑**	• **Ethnicity**	• Calcium w 9.5 nmol/L mg increas • BMI was a BMI<30 kg nmol/L inc BMI≥ 30 k nmol/L inc • The slope BMI<30 co • Unrelated t associated • Response v Americans
Giusti et al (2010) [302]	• Community-dwelling elderly women with secondary hyperparathyroidism and vitamin D deficiency (n=59)	• Randomised control trial • 6 months • 300,000 IU vitamin D_3/every 3 months or 1000 IU vitamin D_3/day+1500 mg calcium	• **Frequency of dosing↓↑** • **Duration ↑↑** • **BMI↓↑**	• **Baseline level**	• Mean incre compared t nmol/L) • Larger prop levels >75 • Larger prop nmol/L at 3 of the high • BMI (10% variability) of s25(OH)

Table 2.5 continued

Goussous et al (2005) [288]	• Healthy ambulatory men and postmenopausal women (n=52)	• Randomised placebo trail • 3 months • 800 IU vitamin D_3/day (all subjects)+2 x 500 mg/day or placebo	• **Baseline level↓↑**	• **Calcium**	• Significant (+16.2±14. control gro • No signific calcium an response) • No signific difference
Hackman et al (2010) [303]	• Hospitalised men and women patients with s25(OH)D ≤ 50 nmol/L (n=26)	• Open labelled prospective randomised trial • 3±1 months • 50,000 IU vitamin D_3/day for 10 days or 3,000 IU vitamin D_3/day for one month followed by 1,000 IU D_3/day for two months+500 mg/day calcium citrate (all patients)		• **Dosing regimen** • **Season**	• Increase in groups (me dose group • Low and hi 77.0±15.0 • 90.0% & 6 & 57.0% o >50 and ≥
Harris et al (2002) [285]	• Healthy young & old men (n=50)	• Randomised control trail, • 8 weeks • 800 IU D_3/day	• **Dose↑↑** • **Baseline levels↓↑**	• **Age**	• Significant groups whi and old) • Baseline va plasma leve

Table 2.5 continued

Hashemipour et al (2010) [304]	• Healthy men and women (n=33)	• RDBPCT • 4 months • Single dose of 0, 300,000 or 600,000 IU vitamin D_3 administered IM	• **Duration** • **Dose↑↑**	• S25(OH)D not after 2 • Mean incre greater tha
Heaney et al (2003) [38]	• Healthy men (n=67)	• RPCT • 5 months (20 weeks) during winter • 0, 1000, 5000, and 10,000 IU vitamin D_3/day	• **Dose↑↑**	• Significant s25(OH)D the two hig • Serum PTH change in t significantl
Hollis & Wagner (2004) [298]	• Fully lactating mothers (n=18)	• Randomised controlled trial • 4 months • 1600 or 3400 IU D_2/day + 400 IU D_3/day	• **Dose↑↑**	• Significant treatment g • S25(OH)D serum 25(O
Leventis et al (2009) [294]	• Rheumatology outpatients with vitamin D deficiency (n=69)	• Intervention trial • 24 weeks • Single dose of 300,000 IU D_2 administered IM or single dose of 300,000 IU D_3 administered orally	• **Type of vitamin D**	• Significant weeks in b significantl • In D_2 group different ti achieved le • PTH suppr patients wi D_2 and D_3

Table 2.5 continued

Logan et al (2013) [295]	• Healthy men and women (n=61)	• RDBPCT • 25 weeks beginning in winter and ending in winter • 0, 1,000 IU/day D_2 or 1000/day D_3	• **Dose↑↑** • **Type of vitamin D** • **Season**		• Total serum significantl compared v • Serum leve compared v • There was after the in • In D_2 group significantl
Nelson et al (2009) [287]	• Healthy pre-menopausal women (n=112)	• Randomised double blind placebo trial • 21 weeks (winter) • 0 or 800 IU/day D_3	• **Dose↑↑** • **Baseline level↓↑** • **Body fat%↓↑** • **Season** • **Oestrogen dose↓↑**		• Significant groups (fro group and group) • Achieving was seen in (67.4±22.8 fat (29.9%±
Nimitphong et al (2013) [280]	• Healthy adults (n=39)	• Un-blinded randomised control trial • 400 IU vitamin D_2 or D_3/day plus 675 mg calcium every day for 3 months	• **Type of vitamin** • **VDBP genetic variants (rs4588 SNP)**	• **BMI**	• D_3 tended t (P=0.08) • D_2 decreas • The increm significantl with carrie
Nugent et al (2010) [305]	• Inpatient females (n=90)	• Interventional control trial • 3 months starting in winter • Single dose of 300,000 IU vitamin D_3 IM or no intervention	• **Dose↑↑**		• Significant treatment c nmol/L), h • Significant

Table 2.5 continued

Putman et al (2013) [306]	• Healthy adolescents with vitamin D sufficiency (n=53)	• Double-blind, randomised clinical trial • 11 weeks in fall and winter • 200, 1000 IU D_3/day	• **Prior treatment**	• **Dose** • **Age** • **Compliance** • **Season** • **Baseline level**	• No signific significant • Change in prior treatn season of e disappeare vitamin D
Rajakumar et al (2008) [307]	• Obese & non-obese African American children (n=41)	• Open label non-randomised pre-post comparison • One month during winter • 400 IU/day vitamin D_3		• **BMI**	• S25(OH)D and non-ob • The thresh treatment v children; ef cohort was nmol/L and
Romagnoli et al (2008) [292]	• Female patient residence of nursing homes with vitamin D deficiency (n=32)	• Prospective randomised intervention • 60 days • Single dose of 300,000 IU vitamin D_2 or D_3 administered orally or IM	• **Route of delivery** • **Type of vitamin D**		• Significant groups at 6 • Sharp incre supplemen • Rapid and gradual inc • Based on th • At 60 days supplemen independen

Table 2.5 continued

Saadi et al (2007) [7]	• Healthy nulliparous and lactating women (n=178)	• Open-labelled, randomised, parallel group trial • 3 months • 2,000 IU vitamin D_2/day or 60,000 IU vitamin D_2/month	• **Frequency of dosing↑↑** • **Baseline levels↓↑** • **Weight↓↑**		• All women monthly re • Despite usi women and women had concentrati in s25(OH) IU vitamin respectivel
Talwar et al (2007) [31]	• Healthy African postmenopausal women (n=208)	• RPCT, • 36 months (3 years) • 0 or 800 IU vitamin D_3/day for the first 2 years and then 2000 IU/day for the third year in the vitamin D group+1200-1500 mg calcium in both groups	• **Dose↑↑** • **Duration** • **Season** • **Baseline levels↓↑**	• **Age** • **Weight** • **BMI** • **Body Fat%**	• Significant (+22%) in • Only 60% at 27 month of interven
Trang et al (1998) [289]	• Healthy men and women (n=72)	• Randomised double blind trial • 14 days • 4000 IU/day D_2 or 4000 IU/day D_3	• **Type of vitamin D** • **Baseline level↓↑**		• Both treatm and D_3 incr and 23.3 n • The largest s25(OH)D (50-86 nm s25(OH)D second tert

Table 2.5 continued

Veith et al (2001) [291]	• Healthy men and women (n=61)	• Randomised intervention trial • 2-5 months • 1000 or 4000 IU D_3/day	• **Dose↑↑**	• **Weight** • **Baseline level**	• Significant from 3 mo low and hig • The lower serum leve respectivel effective in
Veith et al (2004) [308]	• Thyroid clinic outpatients (n=64)	• Blinded randomised trial • 6 months • 600 or 4000 IU D_3/day	• **Dose↑↑** • **Duration↑↑**		• Significant increase in nmol/L, res • The media and high do vitamin D/ • The greate • Significant
Zabihiyeganeh et al (2013) [309]	• Patients with s25(OH)D<75 nmol/ (n=79)	• Open labelled randomised clinical trial 6 months • Single 300,000 IU D_3 administered IM or in 6 divided doses administered orally (50,000 IU D_3/week for 4 weeks then monthly for 2 months)	• **Route of delivery**		• Significant treatment g • Change in group than nmol/L), bu months • After 6 mo levels <50 • Oral supple supplemen

Table 2.5 continued

Zhao et al (2012) [310]	• Non-Hispanic white postmenopausal women (n=1063)	• RDBPCT • One year • Placebo, 1100 IU/day vitamin D + 1400 mg/day calcium or 1400 mg calcium/day only	• **Dose↑↑** • **Baseline level↓↑** • **Season** • **Baseline calcium level** • **BMI**	• All five pre • Dose, base remained s group
Zwart et al (2011) [311]	• Healthy Antarctic men and women workers (n=41)	• Un-masked controlled intervention trial • 6 months during winter • 2000 IU/day or10,000 IU/week vitamin D (type of vitamin D was not specified)	• **Baseline levels↓↑** • **BMI↓↑**	• Significant 6 months • All particip concentrati • Those with compared t

RDBPCT, randomised, double-blind, placebo-controlled trial; IU, international unit; BMI, body mass index; %, percent; s25(OH)D, serum
RPCT, randomised, placebo-controlled trial; Ca, calcium; PTH, parathyroid hormone; IM, intramuscularly; mg/day, milligram per day; VD
nucleotide polymorphism; iPTH, intact parathyroid hormone;
↓↑**,** lower predicting factor is associated with increased 25(OH)D in the circulation
↑↑**,** higher predicting factor is associated with increased 25(OH)D in the circulation

Dosing Regimen (Dose, Route & Duration)

Evidence shows that higher doses are associated with less increase in serum 25(OH)D concentrations per unit of vitamin D compared with lower doses of vitamin D [38, 308]. On the other hand, over the treatment period, higher doses administered either orally or intramuscularly result in greater increase in serum 25(OH)D concentrations and the increase is dose-dependent [38, 286, 298, 304]. Furthermore, serum 25(OH)D concentrations reach the plateau faster [278]. In a study by Veith et al (2004), supplementation with either daily 600 or daily 4000 IU vitamin D increased serum 25(OH)D concentrations significantly over the study period of 6 months though the increase was larger in the high dose group (112.0±41.0 vs. 79.0±30.0 nmol/L, respectively). However, the increment in serum 25(OH)D concentrations per 40 IU vitamin D was 4 times more in the low dose group than the high dose group; 2.2 vs. 0.6 nmol/L [308].

Similarly, Heaney et al (2003) assigned healthy men to receive either 1000, 5000 or 10,000 IU vitamin D per day for 5 months during winter, the increase in serum 25(OH)D concentration per 40 IU vitamin D input in the 5000 and 10,000 IU groups were 0.736 and 0.636 nmol/L, respectively [38]. However, serum 25(OH)D concentrations increased dose-dependently. Serum PTH levels increased in placebo, did not change in 1000 and 5000 IU dose groups and decreased significantly in the 10,000 IU group indicating the efficacy of the higher dosage. Moreover, Barger-Lux et al (1998) showed that larger doses of vitamin D (10,000 and 50,000 IU vitamin D daily), but not lower dose (1000 IU), significantly suppressed circulating PTH levels, probably because larger doses showed a greater potency in increasing serum 25(OH)D concentrations [286].

Taking 1000 IU vitamin D daily has been also shown to be less effective in achieving serum levels ≥ 75 nmol/L compared with 4000 IU per day [291] and 300,000 IU vitamin D every 3 months (a daily dose of approximately 3333 IU) [302]. Subjects in these trials were vitamin D deficient and needed a loading dose which is certainly larger than 1000 IU per day. The dosing frequency may influence the response to supplementation through its effect on compliance rate. An intermittent regimen was more effective in ensuring a higher compliance rate; 80% and 100% in the daily and intermittent regimens, respectively [302].

Similarly, several other trials have reported the higher rates of compliance with regimens of less frequent vitamin D supplementation [293, 311]. The compliance rate was less in subjects receiving daily regimen (1600 IU vitamin D_2 or D_3) than monthly regimen (50,000 IU

vitamin D_2 or D_3); daily D_2 & D_3, 95.4% & 91.6% and monthly D_2 & D_3, 99.4% & 98.9%, respectively [293].

Vitamin D supplements can be administered either orally or IM. To ensure adherence to medication, some researchers have used the IM form of vitamin D supplement. Vitamin D supplements administered IM are often found in bolus dosages, and are useful for patients with absorption disorders and with low compliance and in areas where oral supplements are not available. However, there are some concerns about the safety [305] and effectiveness of vitamin D administered IM [292, 309]. Zabihiyeganeh et al (2013) confirmed that serum 25(OH)D response to oral supplementation was marginally better than IM supplements over a 3-month period [309]. The mean change in serum 25(OH)D concentration was significantly higher in the oral group than IM group at 3-months (+90.0±11.2 vs. 58.8±8.9 nmol/L, respectively; P=0.03). It should be noted that despite having the same accumulative dose of 300,000 IU vitamin D, the dosing regimens were completely different across treatment groups; IM group received a single 300,000 IU vitamin D but oral group received weekly 50,000 IU vitamin D for 4 weeks and then monthly for 2 months. When subjects were followed up after 6 months, there was no significant difference in serum 25(OH)D between oral and IM groups. However, the proportion of subjects attaining adequate serum 25(OH)D levels of ≥ 75 nmol/L was almost significantly higher in oral group than IM group (65.0% vs. 43.6%, respectively, P=0.06).

Comparing single dose of 300,000 IU vitamin D_2 and D_3 administered IM or orally, Romagnoli et al (2008) showed that the IM route was not as effective as oral route [292]. After 3 days, serum 25(OH)D concentrations increased significantly in those taking supplements via oral route. At 30-day, the change was generally larger in oral group and was significantly larger in those taking vitamin D_3 supplement orally; D_3 oral 119.50±18.25 vs. D_3 IM 39.8±28.3 nmol/L and D_2 oral 43.35±11.95 vs. D_2 IM 12.7±11.2 nmol/L (P<0.001). Furthermore, at 60-days, those taking vitamin D supplements IM had lower, but not significantly, mean serum 25(OH)D concentrations. The authors suggested that a longer period would be needed to observe significant changes in serum 25(OH)D concentrations when vitamin D supplements are administered IM.

Several trials have shown that serum 25(OH)D response to vitamin D supplementation reach the peak at 3-months [31, 309], while others suggest that 6 months is needed to see a greater biological response. Talwar et al (2007) assigned healthy post-menopausal women to receive

either daily 800 IU for two years followed by daily 2000 IU vitamin D for one year or placebo through the entire study period. Mean serum 25(OH)D concentration in the active arm increased significantly at 3 months and 27 months (3 months after the initiation of the second dose), but decreased at 24 and 36 months indicating that serum 25(OH)D concentrations reach the peak after 3 months of supplementation.

In another study, however, serum 25(OH)D concentrations increased and PTH levels decreased significantly at 6 months compared with 3 months ($P<0.001$) [302]. Furthermore, the proportion of cases reaching serum 25(OH) levels of >75 nmol/L was higher at 6 months, but not 3 months. Unrelated to the change in serum 25(OH)D concentrations, bone turn over markers decreased significantly at two different time points but the improvement was greater at 6 months. Based on the dose response curve examining the relationship between vitamin D supplements and serum 25(OH)D concentrations and PTH levels, the slope of 6 months did not differ significantly from that of 12 months [264]. Accordingly, one can conclude that a period of 6 months would be enough to see the maximum biochemical response.

BMI or Body Fat Percentage

Higher body fat percentage or higher BMI have been associated with smaller increases in serum 25(OH)D concentrations in response to vitamin D supplementation [7, 36, 69, 264, 286, 287, 302, 307, 311]. Blum et al (2008) assigned healthy ambulatory men and women aged ≥ 65 years old to receive daily 700 IU vitamin D or placebo for one year. The change in serum 25(OH)D concentrations was significantly inversely associated with BMI, central body fat, weight and waist circumference [69]. After one year, the mean adjusted serum 25(OH)D concentrations was higher in subjects with BMI <25 kg/m² than those with BMI ≥ 30 kg/m² (57.0±14.0 vs. 40.8±5.3 nmol/L, respectively) despite having comparable baseline levels. The adjusted change was 20% less in those with ≥ 30 compared to those in the 25 kg/m² category.

Recruiting the same age group, Gallagher et al (2012) and Gallagher, Peacock, Yalamanchili, & Smith (2013) found that BMI was a significant predictor of serum 25(OH)D response to vitamin D supplementation in healthy postmenopausal white and African American women, respectively [36, 264]. Serum 25(OH)D concentrations were higher in normal weight than overweight (a difference of 12.2 nmol/L) and obese women (a difference of 17.7 nmol/L) [36]. At the 12-month time point, in African American women with BMI <30 kg/m², every 1000 IU increase in the dose resulted in a 13.0 nmol/L increase in serum 25(OH)D concentration while in women with BMI ≥ 30 kg/m², the same dose resulted in a 10.3 nmol/L

increase. The slope of dose-response at the 12-month time point was 2.9 nmol/L higher in BMI category <30 compared to BMI ≥ 30 [264].

An effect of BMI and percentage body fat on serum 25(OH)D response to supplementation has also been reported in younger subjects [287, 311] and children [307]. Change in mean serum 25(OH)D concentration after 6 months, but not 3 months, was negatively associated with BMI among healthy Antarctic men and women workers with the mean age of 40.1±10.0 years old; those with BMI>28 kg/m² responded poorly to treatment compared to those with BMI <28 kg/m² (P<0.03) [311]. Using body fat percentage as a better measure of body fat stores, Nelson et al (2009) showed that achieving optimal serum 25(OH)D concentrations in winter time was associated with lower percentage body fat (29.9%±7.1% vs. 35.4%±7.4%) [287].

However, there are a few studies which failed to show any relationship between anthropometric measures and response to treatment [31, 269, 284, 291]. These studies have been limited by high mean BMI of study population (29.5±4.0 kg/m²) [31], small participant numbers (n=42) [284] and using body weight instead of more reliable measures of body composition/body fat such as BMI [291].These limitations may have prevented the authors detecting any effect of body composition on response to vitamin D supplementation.

Aging

Although aging has frequently been reported to be associated with lower levels of 25(OH)D, it seems to have no or very minor effect on response to supplementation. Comparing healthy men aged 18-35 years old with men aged 62-79 years old, Harris & Dawson-Hughes (2002) showed that supplementation with 800 IU vitamin D per day for 8 weeks resulted in a significant and comparable increase in mean serum 25(OH)D concentrations in both age groups [285]. Similar findings were reported by others [31, 32].

Veith et al (2003), however, showed that elderly (>70 years old) had consistently higher levels of PTH than younger people (<50 years old), and serum 25(OH)D concentrations of >100 nmol/L in elderly was associated with PTH levels comparable to the PTH levels of younger people with serum 25(OH)D concentrations near 70 nmol/L. The authors concluded that in order to overcome high levels of PTH, older people may need more vitamin D to have higher serum 25(OH)D concentrations [240].

Dietary Calcium Intake

There are very few trials examining the effect of dietary calcium intake on serum 25(OH)D response to vitamin D supplementation, and the results are mixed. Most dose-response and efficacy trials administer calcium supplements alongside vitamin D supplements to ensure daily calcium intake of 1200-1500 mg and to minimize the confounding effect of dietary calcium intake on response to supplementation. Increased intake of calcium which results in a slight increase in serum calcium levels is frequently associated with lower levels of serum PTH [312]. The decrease in PTH levels results in a decrease in production of $1,25(OH)_2D$ by the kidneys, and an increase in the levels of 25(OH)D in the circulation [66].The increase in 25(OH)D levels could be explained by several mechanistic pathways: (1) inhibition of 25-hydroxylase by $1,25(OH)_2D$ as a result of negative feedback loop (2) decrease in the use of 25(OH)D as a substrate, and (3) delayed metabolic clearance of 25(OH)D in the liver [312]. Thomas, Need & Nordin (2010) showed that supplementation with 1000 mg calcium for one week with additional 1000 IU vitamin D daily for 7 weeks raised the mean serum 25(OH)D concentration more effectively than vitamin D or calcium alone [312].

Similar results were reported in dose-response trials conducted to determine the effect of different dosages of vitamin D supplement on serum 25(OH)D concentrations [264]. Using a multivariate model, Gallagher et al (2013) [264] showed that total calcium intake (diet + supplement) was a significant covariate. Every 1000 mg increase in calcium intake was associated with a 9.5 nmol/L increase in serum 25(OH)D concentrations in vitamin D deficient postmenopausal African American women supplemented with vitamin D.

In another study, however, Bell, Show and Turner (1987) showed that calcium intake had opposite effect [313]. Compared to the vitamin D only group, addition of 2000 mg calcium per day to daily 100,000 IU vitamin D for four days resulted in a significantly less increase in mean serum 25(OH)D concentration. The increment in calcium group was less than half of that observed in the control group (63% vs. 133%, respectively; $P<0.02$).

Goussous et al (2005), in contrast to the latter trials, could not detect any effect of calcium intake on response to supplementation [288]. The researchers assigned elderly men and women to receive both 800 IU vitamin D_3 and 1000 mg calcium or 800 IU vitamin D_3 and placebo per day for 3 months. Serum 25(OH)D concentrations increased significantly in both groups, and the mean increase was comparable in both groups (+16.2±14.8 in the calcium group and +16.6±17.4 nmol/L in control group, $P>0.05$).

Oestrogen

Several cross-sectional studies have shown that oral contraceptive use may influence baseline levels of 25(OH)D. However, apart from one trial [287], there is no other intervention trials investigating the effect of oral contraceptives on serum 25(OH)D response to supplementation with vitamin D. Nelson et al (2009) assigned healthy pre-menopausal women to receive 800 IU vitamin D or placebo for 21 weeks [287]. Factors influencing response to supplementation were treatment dose, baseline 25(OH)D, summer increase and estrogen dose; the odds ratio for using higher dosages of estrogen and having larger change in serum 25(OH)D concentrations was 1.08 (P=0.01).

Genetic

The relationship between VDR and VDBP genotype and levels of 25(OH)D in circulation has been examined by several studies [279, 280, 314-316], though, very few have examined the effect of VDBP genotype on 25(OH)D response to vitamin D supplementation [279, 280]. In an open-label un-blinded randomised intervention trial, Fu et al (2009) examined the contribution of VDBP D432E and T436K SNPs to variation of 25(OH)D response to either 600 IU/day or 4000 IU/day vitamin D for one year [279]. The presence of 436K allele was associated with lower serum 25(OH)D concentrations at baseline. However, the percentage increase in serum 25(OH)D concentration from baseline in both groups was in opposite direction; those with KK genotype had the largest increase followed by TK and then TT genotypes. In a multiple linear regression model, dose and 436K, but not 432E contributed significantly to overall variance, 22% (P<0.0001) and 8.5% (P<0.001), respectively. It should be noted that baseline 25(OH)D levels were not included in this model. The observed pattern could be due to the lower baseline 25(OH)D concentrations in carriers of 436K allele. It is well documented that baseline 25(OH)D concentration is a significant predictor of 25(OH)D response to vitamin D supplementation [7, 31, 32, 269, 284-288].

Furthermore, the increase in 25(OH)D following vitamin D supplementation also appears to be partly vitamin D-type specific. Serum 25(OH)D response to supplementation with vitamin D was examined in 39 healthy adults given 400 IU/day vitamin D_3 or vitamin D_2 [280]. The percentage increase in serum 25(OH)D and 25(OH)D_3 following supplementation with vitamin D_3, but not with vitamin D_2, was significantly affected by rs4588 genotype. Compared to CA and AA alleles, participants homozygous for GC2 allele (CC) had a significantly larger increase in 25(OH)D and 25(OH)D_3 (5.84±3.07 nmol/L for 25(OH)D and

6.09±3.03 nmol/L for 25(OH)D_3 vs. 22.58±6.18 nmol/L for 25(OH)D (P<0.01) and 22.98±6.00 nmol/L for 25(OH)D_3 (P<0.01), respectively). It should be noted that there are some limitations to this study; lack of a control arm may have prevented the authors to control for confounding effects of lifestyle factors. Furthermore, there was not enough power to detect any small changes associated with vitamin D_2 supplements because of the small sample size.

Dietary Fat Content and Fat Composition

Dietary fat content and fat composition has been suggested to affect vitamin D absorption. Vitamin D is a fat soluble vitamin and it is plausible to suggest that amount of fat in diet improves its absorption. Mulligan recruited vitamin D patients who were taking vitamin D supplement on an empty stomach or with a small meal but did not achieve an adequate rise in serum 25(OH)D concentrations (n=17) [317]. The patients were instructed to take their supplements with the largest meal of day which may contain more fat. Mean serum 25(OH)D concentration increased by 56.7±36.7% (from 76.25±11.75 at baseline to 118.00±27.25 nmol/L after diet modification). This trial had some limitations including its small sample size and the lack of a control group. In a systematic review evaluating the vehicle substances on vitamin D bioavailability, Grossmann et al (2010) concluded that compared to vitamin D as powder or dissolved in ethanol, solubilised vitamin D in a small amount of fish oil produced greater change in 25(OH)D concentrations (mean change of 4.05, 2.75 and 0.5 nmol/L per 100 IU/day vitamin D in fish oil, powder and ethanol, respectively) [318].

The fat composition of diet has also been shown to influence the absorption of vitamin D [281]. However, the results from epidemiological trials are mixed. In a study by Niramitmahapanya et al (2011), while the researchers failed to show any relationship between dietary fat content and the response to supplementation, they could show that fat composition was significantly associated with response to supplementation [281]. The increment in plasma 25(OH)D concentration was positively associated with mono unsaturated fatty acids (MUFA, P=0.016) and with the ratio of MUFA/PUFA (P=0.014), but negatively with poly unsaturated fatty acids (PUFA, P=0.038). In contrast, a very recent randomised controlled trial showed that treatment with n-3 PUFA did not affect serum 25(OH)D concentrations [319].

The mechanisms by which type of fatty acids may influence vitamin D absorption are not known. Niramitmahapanya et al (2011) suggested that fatty acids such as PUFA, linoleic and

linolenic acid may increase solubility of vitamin D in the micelles which in turn may increase the micelles size. As a consequence, vitamin D may stay longer in the micelles and may have difficulty in passing the intestinal mucosa [281].

Diseases & Medications

Evidence shows that people who have diseases influencing the absorption and metabolism of vitamin D such as intestinal mal-absorption, kidney and liver diseases as well as those taking medications such as antiepileptic drugs, glucocorticoids, bile acid sequestrants and lipase inhibitors are more likely to have low vitamin D status.

A significant proportion of people with cystic fibrosis (35.5±10.1 nmol/L) [320], celiac diseases (44.5±18.0 nmol/L) [321], and Crohn's disease (56.9±23.7 nmol/L) [282, 322] have serum 25(OH)D concentrations ≤ 75 nmol/L, suggesting that a larger proportion of these patients need vitamin D supplementation. In an open-labelled prospective clinical trial, Yang et al (2012) showed that patients with mild-to-moderate Crohn's disease had a mean serum 25(OH)D concentration of 40.0±25.0 nmol/L at baseline [282]. To achieve serum 25(OH)D levels of >100 nmol/L, the majority of these patients (78%) needed the maximal dose of vitamin D supplement (daily 5000 IU) for 24 weeks rather than daily 1000 IU vitamin D for 2 weeks. These findings are suggestive of the reduced response to supplementation in these patients mainly due to malabsorption.

With respect to medications affecting serum 25(OH)D concentrations and consequently the response to supplementation, Robien et al (2013) in their review concluded that the available evidence is insufficient though evidence for some medications such as antiepileptic drugs, glucocorticoids, orlistat and cholestyramine are stronger [283]. Antiepileptic drugs and glucocorticoids have been shown to reduce serum 25(OH)D concentrations only when the exogenous sources of vitamin D is limited [283]. Orlistat, a lipase inhibitor, and cholestyramine, a bile acid sequestrant, may cause malabsorption of fat and consequently impairment of vitamin D absorption. Treatment of obese adolescents with Orlistat was shown to significantly decrease serum 25(OH)D concentrations (-7.0±8.5 nmol/L, $P<0.01$) one month after the start of treatment [323]. A limitation to this study was that it lacked a control group. In another randomised controlled trial [324], decrease in mean serum 25(OH)D concentration was observed in both treatment and placebo, groups. In agreement with Robien et al's opinion, it is plausible to say that reduced dietary fat intake may have influenced serum 25(OH)D concentrations.

Compared to the control group, administration of cholestyramine to prepubertal children with familial hypercholesterolemia for one year resulted in a significant decrease in mean serum 25(OH)D concentrations [325]. The significant decrease was observed in all seasons (P=0.04). Another RCT, however, reported no significant differences in circulating 25(OH)D concentrations between treatment and control groups after 7-10 years [326].

Vitamin D Deficiency: Prevention, Treatment & Management

Supplementation with vitamin D

Vitamin D supplements are used to prevent and treat vitamin D deficiency in at risk populations and are found in two forms, vitamin D_2 and D_3, and various dosages and administered via different routes. The supplemental form of vitamin D prescribed by general practitioners (GPs) to at risk populations in New Zealand is monthly 50,000 IU vitamin D_3 which is administered orally [29]. It should be noted that the treatment of vitamin D deficiency is totally a different approach; it starts with a loading phase which then moves into the maintenance phase [327]. In the loading phase which is required for a rapid correction of vitamin D deficiency, a total of 280,000-300,000 IU vitamin D should be given during 6-10 weeks depending on the availability of vitamin D preparations. In New Zealand, 50,000 IU vitamin D preparation is available as such this supplement is advised to be given daily for 10 days.

In the maintenance phase which starts usually one month after loading phase, dosages of 800-2000 and sometimes up to 4000 IU vitamin D is administered daily or intermittently. Larger doses are given in less frequent dosing regimens [327]. In New Zealand, 50,000 IU vitamin D is given monthly which is equivalent to 1666 IU per day [29].

Efficacy of dosing regimens using monthly accumulative dosages of ≥ 50,000 IU vitamin D in Middle Eastern populations

Several studies investigating the effects of vitamin D supplementation on different clinical and biochemical outcomes in Middle Eastern populations have questioned the adequacy of supplementation with an accumulative dose of ≤ 50,000 IU/month (administered either as intermittent larger doses or daily smaller doses) vitamin D in optimising serum 25(OH)D concentrations (**Table 2.6**) [22].

From these findings (**Table 2.6**), one can suggest that a dose of more than 50,000 IU/month is required to optimise serum 25(OH)D concentrations. These trials show that a very large proportion of Middle Eastern populations have chronic vitamin D deficiency/insufficiency [7, 328-331]. This is why, in part, these populations need larger doses of vitamin D supplements. Interestingly, although supplementation with large doses resulted in a significant increase in serum 25(OH)D concentrations, which reached to >250 nmol/L in some cases. This did not cause any clinical toxicity such as hypercalcaemia or hypercalciurea [331, 332]. Furthermore, evidence shows that the larger the dose, the greater the observed improvement in clinical and biochemical outcomes is [332].

Soheilykhah et al (2012) [332] assigned pregnant women to receive either 200 IU/day, 50,000 IU/month or 50,000 IU/every two weeks vitamin D starting at the gestational age of less than 12 weeks for 5 months. Serum 25(OH)D increased significantly in all groups. The change in serum 25(OH)D concentration was significantly larger in those taking the largest dose, followed by 50,000 IU/month and then 200 IU/day, and serum 25(OH)D concentrations <75 nmol/L was observed in 37.5%, 60.5% and 88.6% of women in each group, respectively. Furthermore, 50,000 IU vitamin D/every two weeks improved insulin resistance significantly with no adverse effects.

Saadi et al (2007) also showed that supplementation with 2000 IU/day or 60,000 IU/month vitamin D for 3 months did not optimise serum 25(OH)D concentrations (≥ 50 nmol/L) in 70% of women [7]. Type of vitamin D may have affected the response because the supplemental form was D_2. Vitamin D_2 is biologically less potent than vitamin D_3 [33, 289, 292-295]. In another study from Saudi Arabia, Al-Daghri et al assigned type 2 diabetic male and female Saudi subjects to receive 2000 IU vitamin D3 daily for 18 months [22]. Serum 25(OH)D concentrations improved significantly but remained at suboptimal levels in 22% of participants. It should be noted that 2000 IU/day or 60,000 IU/month vitamin D is larger than the dose being used in all at risk populations in New Zealand (50,000 IU/month).

On the other hand, in studies where participants used 100,000 IU vitamin D, a larger proportion of subjects reached the sufficient levels of serum 25(OH)D. Supplementation with 300,000 IU vitamin D administered either as a single dose or in 6 divided doses over 3 months, which equates to 100,000 IU/month, reduced the proportion of subjects with serum 25(OH)D levels <50 and <75 nmol/L to 15.2% and 41.8%, respectively [309]. Yet, some participants remained vitamin D deficient/insufficient suggesting that doses larger than

100,000 IU/month are needed in these populations. Why these populations respond poorly to vitamin D supplements in such large doses and what are those factors affecting the response to supplementation, are among many questions remaining unanswered in these populations.

Monitoring and assessment of serum 25(OH)D concentrations in New Zealand

Testing serum 25(OH)D concentrations is expensive in New Zealand, and District Health Boards (DHBs) are reluctant to fund vitamin D measurements. However, the number of requests for vitamin D measurements in Auckland has increased over the past decade; from 8500 in 2000 to 32,800 in 2010 [333]. Based on the 2012 Consensus Statement on Vitamin D and Sun Exposure in New Zealand, serum 25(OH)D in patients with severe symptomatic vitamin D deficiency such as confirmed and suspected osteomalacia, osteoporosis and unexplained chronic limb pain, in patients with evidence of metabolic bone disease and in patients with unexplained low serum calcium or phosphate or raised alkaline phophatase should be investigated [29]. Furthermore, routine monitoring and baseline assessment of serum 25(OH)D in asymptomatic individuals who are at high risk of vitamin D deficiency like Middle Eastern women is not recommended [29, 327]. These women might be given supplements, if they are recognised by their GPs (personal observation), without consideration of their baseline levels.

What does the current approach mean to New Zealand and to at risk populations?

This blanket approach may increase health disparities in New Zealand. At the national level, unfortunately, New Zealand policy makers and health system are not responding well to include the growing population of refugees/immigrants and their ethnic groups as health population [334]. Reflecting the national policy, there is a large gap in our knowledge about this population. There is no/little data on demographics (ethnicity data, they are all included in “other” category) and health status and needs of this population (lack of population-based funding and health research targeting this population) [334]. Furthermore, there are limited resources such as interpreters and funding to respond to the increase in the immigrants population [334]. Having limited knowledge and skills for providing culturally relevant information and care for some refugees/immigrants (Middle Eastern population) by our health professionals and GPs [334] may put this population at greater risk of diseases compared to other populations. This, in turn, may lead to an increase in health disparities in New Zealand.

Table 2.6: Efficacy of supplementation with 50,000 – 100,000 IU vitamin D in Middle Eastern populations

Study	Population	Treatment	Vitamin D deficiency		
			Threshold (nmol/L)	Baseline (Proportion of Subject%)	Follow-up (Proportion Subject%
Al-Daghri et al (2012) [22]	Saudi men and women patients with type 2 diabetes mellitus and the mean age of 53.9±10.2 (y) (n=92)	2,000 IU vitamin D_3 administered daily to all patients for 18 months	<50	100.0	22.0 (18 mo)
Dawodu et al (2012) [124]	Healthy Arab pregnant women (n=162)	400 (group1), 2000 (group 2), 4000 IU (group 3) vitamin D_3 administered daily starting at 12-18 weeks of gestation and continued to delivery	<50	98.2	Group 1: 52.4 Group 2: 24.4 Group 3: 9.3
Ghazi et al (2010) [328]	School age Iranian boys and girls with the mean age of 16.3±1.3 (y) (n=207)	50,000 IU vitamin D_3 administered orally every month (group 1), every two months (group 2) or placebo (group 3) for 6 months	<50	Girls: 97.0 (all groups) Boys: 77.0 (all groups)	Girls: 63.0 (6 mo, all Boys: 16.0 (6 mo, all
Hosseinzadeh-Shamsi-Anar et al (2012) [329]	Iranian mothers with gestational diabetes and the mean age of 30.1±5.1 (y) (n=45)	Single dose of 300,000 IU vitamin D_3 administered IM and control	<35	79.2	4.2 (3 mo, all

Table 2.6 continued

Saadi et al (2007) [7]	Healthy Emirati nulliparous and lactating women with the mean age of 29.6±6.1(y) and 23.8± 5.2 (y) in lactating and nulliparous women, respectively (n=178)	2,000 IU vitamin D_2 administered daily or 60,000 IU vitamin D_2 administered monthly for 3 months	<50	Lactating 98.9 (all) Nulliparous 99.0 (all)	Lactating 72.9 (3 mo, all Nulliparou 65.2 (3 mo, all
Soheilykhah et al (2012) [332]	Pregnant Iranian women with the gestational age of less than 12 weeks and the mean age of 26±4.5 (y) (n=120)	200/day (group 1), 50,000/month (group 2)or 50,000/every two weeks (group 3) vitamin D_3 for 5 months (to delivery)	<75	99.1	Group 1: 88.6 Group 2: 60.5 Group 3: 37.5
Zabihiyeganeh et al (2013) [309]	Iranian men and women with serum 25(OH)D concentrations<75 nmol/L and the mean age of 48.9±1.4 (y) (n=92)	Single dose of 300,000 IU vitamin D_3 administered IM (group 1), or 300,000 IU vitamin D_3 administered orally in 6 divided doses over 3 months period (group 2)	<50 <75	NS 100	15.2 (3 mo, all 13.9 (6 mo, all 41.8 (3 mo, all 45.6 (6 mo, all

The data for active arm/s have been presented in this table; 25(OH)D, 25-hydroxy vitamin D; SD, standard deviation; n, number; IU, in
month
†range
*Mean±SEM

More than one third of refugees in New Zealand are of Middle Eastern origin [16, 335]. In general, the health of refugees reflects the population health pattern of countries where they have come from [335]. In the long term, the health of refugees replicates what is seen in other low-socioeconomic groups such as the Pacific population [334]. Women of Middle Eastern origin are most likely to come to New Zealand with already established health issues such as vitamin D deficiency. These women in their home country are culturally/legally obliged to have a very conservative clothing style which limits their exposure to sun light. Limited sun exposure is a strong and well known risk factor for vitamin D deficiency [232]. If these women are not identified by their GPs as a risk group for vitamin D deficiency because the GPs have no/little knowledge about these women's background and lifestyle, vitamin D deficiency remains undiagnosed and untreated. Even if they are identified they may be given inadequate dose of vitamin D supplements or may be given culturally irrelevant recommendations such as having sensible sun exposure. Further complicating the issue are those factors affecting response to vitamin D supplements. If these factors, discussed in the previous section, are not accounted for by physicians while treating vitamin D deficiency, these women will remain vitamin D deficient. Chronic vitamin D deficiency in the short- and long-term could exacerbate other clinical outcomes linked to vitamin D deficiency in this population who are already exhibiting a higher prevalence of chronic diseases such as CVD, diabetes and depression than other populations in New Zealand [16].

Although, the case stated here was based on a theoretical reasoning, we could demonstrate that this is what is happening in this population [17]. In a pilot study of 42 women of Middle Eastern origin living in Auckland, 75% of women were vitamin deficient and all had serum 25(OH)D concentrations <50 nmol/L despite using vitamin D supplements; 21% and 37% of subjects were using prescribed vitamin D supplements (50,000 IU/month) and any sort of vitamin D containing multivitamins, respectively. These results are suggestive that (1) approximately 80% of subjects were not identified for vitamin D supplementation by GPs, and (2) those identified were not properly treated for vitamin D deficiency.

Furthermore, our findings agree with those from the Middle Eastern region in that vitamin D deficiency is highly prevalent in this population [8-12, 19-21], and in that the majority of participants did not respond well to supplementation (60,000 IU/month or 2000 IU/day which is more than 50,000 IU/month prescribed in New Zealand) [7, 22]. Accordingly, it is not logical to use a one-size-fits-all approach for vitamin D supplements and recommend one

dose (50,000 IU/month) to all at risk groups without considering those factors affecting the response to vitamin D supplements; as it appears in the 2012 “Consensus Statement on Vitamin D and Sun Exposure in New Zealand” [29].

Chapter 3: Methods

Study Design and Population

This study was a randomised double blind controlled trial among 62 healthy pre-menopausal women of Middle Eastern origin, aged 20+ and living in Auckland, New Zealand (**Figure 3.1**). The study commenced in late June 2013 (approaching winter) and concluded in late January (approaching summer) 2014. Women were invited to participate in the study by leaflets. Leaflets were distributed in a number of venues around Auckland such as North Shore Islamic Centre, Iman Foundation, Ahlul Bayt Foundation and Ahlul Bayt Islamic Centre. A number of talks were given to social and community gathering on different occasions. The researcher then contacted respondents and interviewed them about exclusion and inclusion criteria.

It was calculated that 22 subjects would be required for each arm of the trial to demonstrate a significant difference at 80% power and 5% significance. We assumed that within group SD was 25 nmol/L (as the data from our pilot study was not normally distributed we could not refer to the SD of our study population. Accordingly, we adopted the average SD from studies from the Middle Eastern region [8, 12, 229]], the difference between and within dose groups in a two tailed analysis was 22 nmol/L [7, 22, 31, 36], and uniform withdrawal rate across all groups was 10%. The following formula [336] was used to calculate the sample size:

$N=2\alpha^2K/(\mu_2-\mu_1)^2$

N=Sample size

α=SD

K=7.9; constant, which is a function of the statistical significance of 5% and the statistical power of 80%

$(\mu_2-\mu_1)$=the difference in serum 25(OH)D concentrations between and within groups

Inclusion and Exclusion Criteria

Women were eligible to participate if they: (1) were 20 years old and older, (2) were in pre-menopausal stage (having regular menstrual periods during the last year; derived from https://www.aace.com/files/menopause.pdf on fifth of March 2013), and (3) were of Middle Eastern origin, or both parents were born in Middle Eastern countries, to name, Iran, Iraq, Kuwait, Lebanon, Syria, and Saudi Arabia.

Volunteers were excluded if they had illnesses such as digestive disorders, kidney diseases, and liver diseases or taking medications such as glucocorticoids and anti-epileptic medicines. These diseases or medications are known to affect vitamin D absorption or metabolism [217]. Also, volunteers were excluded if they had hypercalcaemia, hypercalciuria, sarcoidosis, or renal osteodystrophy with hyperphosphatemia [29, 337, 338]. Furthermore, subjects were excluded if they had major systemic illnesses such as atherosclerosis or cardiac function impairment [29]. Women with bleeding disorders or taking blood thinning medication were not eligible to participate (for the participant's safety). Finally, treatment with vitamin D within the last 6 months (other than multivitamins) was another exclusion criterion.

Funding and Ethics

The initial funding for the study was provided by Massey University.

Ethical approval was granted by the Health and Disability Ethics Committee:, Reference No. 13/STH/40. Volunteers were provided with an information sheet explaining the study protocol in detail and signed an informed consent form for participation in the study.

Setting

The study took place in Auckland, New Zealand. Auckland is New Zealand's largest city with a population of just over 1 million. The majority of Middle Eastern immigrants settle in Auckland [16].

Methodological Procedures

All laboratory tests and participants' examinations were done in the Human Nutrition Research Facility at Massey University, Albany, New Zealand.

Participants who met the eligibility criteria signed informed consent forms and were randomly assigned to 1 of 2 monthly vitamin doses [50,000 IU+placebo, and 100,000 IU per month (2x50,000 IU)] or placebo (2xplacebos) for 6 months. We used a period of 6 months for intervention as no difference has been seen in incremental increase in serum 25(OH)D concentrations between 6 and 12 months of supplementation with vitamin D [36]. The randomisation scheme was generated using the Web site Randomization.com (http://www.randomization.com) by using the letters A to C.

The primary investigator dispensed study medication to the participants and managed the allocation record. Each pill dispenser contained 6 compartments, one for each month (**Figure**

3.2). Each dispenser had a label with letters A to C, the study number, the date dispensed and participant's identification number. Each compartment had a label with the date of use (reminder) and contained vitamin D tablets and matching placebo tablets. A third party (not involved with the research group placed the allocated treatments into pill dispensers and labelled them. Monthly text messages were sent to the participants to remind them of taking their supplements. Randomisation and allocation were fully concealed from the researchers and the subjects, unless a serious adverse event. After six month (end of study) and following analysis of data, both the researchers and the subjects became un-blinded.

Study tablets, Cal.D.Forte®, contained 50,000 IU vitamin D_3 and were provided by PSM HealthCare Ltd trading (as API Consumer Brands, Auckland). The placebo was provided by the same company, was identical to vitamin D tablets and contained no vitamin D. We used vitamin D_3 because it is biologically more active and more effective in improving serum 25(OH)D concentrations than vitamin D_2 [33]. Furthermore, it is the only form which is prescribed by GPs and is subsidised in New Zealand. Participants were advised to keep pill dispensers tightly closed and keep them at room temperature. Participants were also asked to take vitamin D supplements with food to increase the absorption [317]. At the 6-month follow up, compliance was calculated by counting the empty compartments.

Participants were screened in winter to minimize the effect of sun exposure. Our placebo arm also helped us to determine if there was any seasonal effect on serum 25(OH)D concentrations.

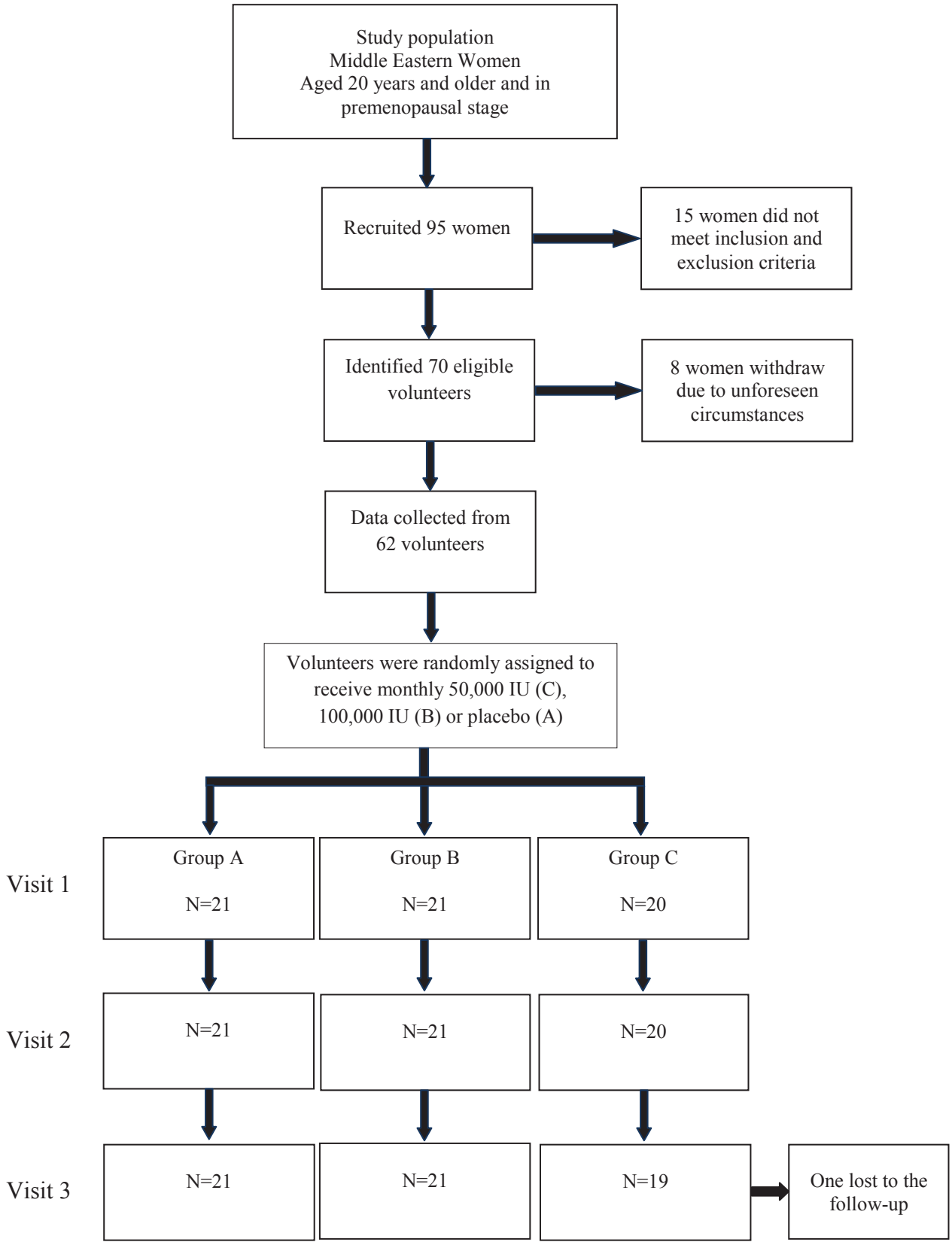

Figure 3.1: Study design and study population selection

Once recruited, participants were invited for a baseline blood test prior to being given their supply of supplements. Also, the information on demographics, medical history, skin colour, physical activity level and anthropometry was simultaneously entered in the corresponding forms, and participants were asked to fill in a 4-day food diary (three working days and one weekend day). At the 3- and 6-month visits another blood sample was taken to check for hypercalcaemia, also, to measure serum 25(OH)D concentrations. At these visit, participants were asked to fill in the change of lifestyle questionnaire to record any changes in lifestyle between the two visits. Once the intervention was completed, at the 6-month visit, participants were weighed again, body composition and blood pressure were measured, and the final blood sample was taken. To obtain a highly accurate measure of blood levels of 25(OH)D and to minimize a high between people variations, it would be recommended to measure serum 25(OH)D concentrations at their peak which is 3 days after final supplements being used [33]. However, this was not feasible due to practical considerations. Alternatively, blood samples were collected 10-14 days after the taking of the tablets, to allow subjects to return to more of a steady state. For a presentation of the study procedure refer to **Figure 3.3**.

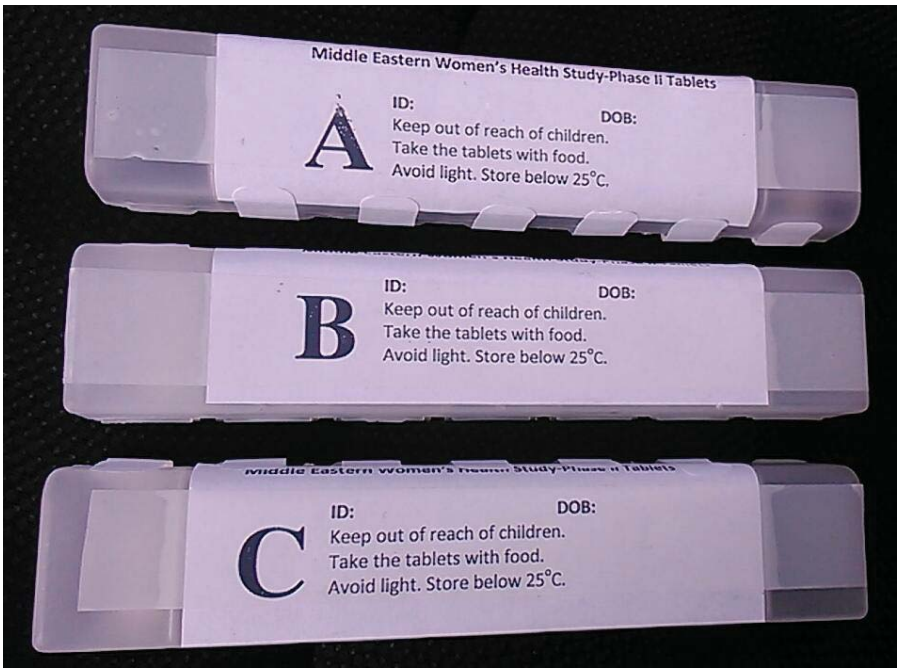

Figure 3.2: Labelled pill dispensers

Blood Sampling and Processing

Registered phlebotomists took venous blood samples using a sterile vacutainer Flashback needle and needle holder. Overnight fasting for blood sample tests was not required, but some restrictions were required for measuring body fat percentage. Serum was used for the analysis of 25(OH)D and calcium. The blood was protected from light allowing clotting for 30 minutes and centrifuged for 10 minutes at 2000 gram at 4°C within 2 hours of sampling. Aliquots of serum were collected in Eppendorf tubes and stored at -80° C until the analysis of serum 25(OH)D at the end of the study in one batch (**Figure 3.4**). Every participant's serum calcium was analysed independently of other participants at 3 and 6 months, after each visit. Serum 25(OH)D was measured using Siemens ADVIA Centaur® Vitamin D Total assay. It has been shown that the ADVIA Centaur Vitamin D Total Assay has good correlation with routine laboratory Liquid Chromatography-Tandem Mass Spectrometry [339] which is currently regarded as the gold standard. Calcium and albumin were measured by a Flex reagent cartridge system by Siemens Health care Diagnostics (Siemens Australia and New Zealand) with a CV of 2.2 – 3.0%.

Vitamin D status was determined by measuring circulating serum 25(OH)D concentrations. Serum 25(OH)D has been shown to be a good indicator of both dietary vitamin D intake and vitamin D synthesised by UVB radiation [233]. The cut-off levels of ≥ 50 nmol/L, 25-50 nmol/L, 12.5-25 nmol/L, and <12.5 nmol/L were used to determine vitamin D sufficiency, insufficiency, deficiency and severe deficiency, respectively [18]. We also used cut-offs of ≥ 75 nmol/L to define vitamin D sufficiency [44, 99]. Hypercalaemia was defined as a serum calcium level greater than 2.63 mmol/L (>10.5 mg/dl) [337].

Questionnaires

Data regarding demographics, medical history, skin colour, calcium intake, change in lifestyle and physical level were collected by self-administered questionnaires and interview based questionnaire, respectively. Translation (in Arabic and Persian) was available if it was required. The questionnaires (attached as appendices 3, 4 and 5) were checked for completeness, while other measurements and blood sampling were performed.

Visit One – Baseline Measures:

- Collect consent forms
- Complete questionnaires: (1) participant details and demographic questionnaire, (2) medical history questionnaire, (3) Fitzpatrick skin colour scale questionnaire and (4) physical activity level questionnaire
- Bioelectrical Impedance Analysis for weight, BMI and body composition,
- Anthropometry (height)
- Blood test
- Instructions on how to fill in questionnaires
- Instructions on completion of food diary

Baseline Blood Measures:

- Serum 25(OH)D
- Serum Calcium

Randomisation to Two Intervention and One Placebo Arm

Randomisation Performed by Third Party Not Associated With the Study

Visit Two – 3-months Measures:

- Blood test
- Change of lifestyle questionnaire

Visit 2 Blood Measures:

- Serum 25(OH)D
- Serum Calcium

Visit Three – 6-month (Endpoint) Measures:

- Bioelectrical Impedance Analysis for weight, BMI and body composition
- Blood pressure
- Blood test
- Change of lifestyle questionnaire

Visit 3 Blood Measures:

- Serum 25(OH)D
- Serum Calcium

Figure 3.3: A presentation of study procedure

In this study, a self-report instrument, New Zealand Physical Activity Questionnaire Short Form (NZPAQ-SF), was chosen to estimate physical activity level. Although self-report instruments overestimate physical activity levels, this instrument has been validated against NZPAQ-Long Form, monitoring heart rate in a multi-ethnic adult New Zealand sample and several other instruments, and the results have been shown to be comparable to international survey instruments such as International Physical Activity Questionnaire (IPAQ) [340].

Skin colour was tested using subjective assessment and Fitzpatrick skin colour scale questionnaire. For subjective assessment, participants were asked to indicate their skin colour

whether it was dark, brown, medium, olive, or fair. Self –report of natural skin colour has been reported to have a moderate to high correlation with skin reflectance spectrophotometer which is a gold standard (r=-0.7, P<0.001) [341]. Fitzpatrick skin type has also been shown to significantly correlate with self-report of skin colour (r=0.63, P<0.001) [341].

Data regarding dietary calcium intake was collected by a 4-day food diary, including one weekend day. Instructions on how to accurately complete the food diary were recorded in the computer and participants were asked to listen to them. Instructions were also explained verbally in details if it was requested. Also, clearly written instructions were provided with the food diary. Participants were given a free-post, pre-addressed envelope for the return of the booklet. Average dietary calcium intake was assessed over four reported days using FoodWorks Professional Edition 7. All questionnaires appended (Appendices 3-6).

In the present study, a 4-day food diary was chosen because of the high respondent burden and time-consuming characteristics of longer food diaries such as 7-day food diary. Because of the high within-person variation in nutrient intakes, it is recommended to record dietary intakes over a longer period of time to have a highly accurate estimate of intake. However, if a 4-day food diary covers different days randomly, it gives us more accurate estimates [342].

Figure 3.4: Labelled Eppendorf tubes, aliquots of serum were collected in these tubes and stored at -80° C

Anthropometric and Body Composition Measurements

Height was measured using a standard stadiometer. Two measurements for height were taken by the researcher, and then the average was recorded.

Weight, BMI and body fat percentage were measured using bioelectrical impedance analyser (BIA, Inbody 230). Body Mass Index was defined as weight (kg) divided by height squared (m^2). We used BIA method because it has less respondent burden compared to BodPod (clothing requirement) and DEXA (x-ray radiation). Comparing Bioelectrical impedance with DEXA, Thomson et al (2007) [343] and Karelis et al (2013) [344] reported a good agreement between these two methods. To standardise and have accurate measurements, participants were required to abstain from food within 2 hours prior to testing, from caffeinated drinks, physical activity, and showering/bathing/sauna within 4 hours, from alcohol within 24 hours [345], and from blood donation within 48 hours prior to testing [345].

Provision of Results to Participants

Following the completion of the trial and the receipt of blood results, each participant received a feedback form. This contained average daily calcium intake, anthropometric and body composition measurement, blood pressure and blood results (baseline and current vitamin D status). Also, participants were informed if they were taking the active (50,000 IU or 100,000 IU) or placebo dose. If serum 25(OH)D concentration was outside normal range, the participant was advised to visit her GP.

E-mails informing the progress of the study were sent to all participants at intervals during the study with the goal of keeping them interested and up-dated.

Data Handling and Statistical Analysis

All data were entered into three Microsoft Excel spreadsheets; one for name and address details, one for name and unique Subject Number, and the last one for all other data. All entries were double checked by the researcher.

Before commencement of statistical analysis the data were cleaned and checked for coding errors. Statistical analysis was performed using SPSS software (version 21, SPSS Inc, Chicago IL.). Analysis included all participants (intent to treat) except for one missing person. The data was checked for outliers. In case of presence of any outlier that case was excluded from the analyses. We had an outlier with baseline serum 25(OH)D concentration

of 189 nmol/L which was excluded from analyses. To note, this case was included in the analyses determining baseline characteristics. Additional analyses were performed with participants who were adherent to the protocol which was defined as having an adherence of 80% or higher during the study period (per protocol). There was no difference in outcomes between intent to treat and per protocol analyses as such the results of intent to treat analyses were reported here.

Descriptive statistics were used for baseline population characteristics: frequencies, mean ± SD, geometric mean [95% CI, lower bound-upper bound] or median [25^{th} – 75^{th} percentiles]. Depending on the normality of distribution, Kruskal-Wallis tests were performed to examine between groups differences, and Spearman's for correlation if data were not normally distributed otherwise, independent T-test and one-way independent ANOVA and Pearson's correlation were used. For multiple comparison, a Boneferroni correction was applied and all effects were reported at 0.05/number of comparisons significance. Effect size was calculated using $Z/\sqrt{n}$ and $\sqrt{t^2/(t^2+df)}$ formulas for non-parametric and parametric tests, respectively [346]. A value of 0.1, 0.3, and 0.5 represents small, medium and large size effect, respectively [346].

Mixed model design was used to explore the dose-response curve for serum 25(OH)D. Dose as continuous and time as categorical (baseline, 3, and 6 months) were included as fixed effects, and participant was included as a random effect. Covariance structures were compared by using the "Akaike information criterion". We also compared different models by looking at the difference in the "-2 Log Likelihood (-2LL)" and compared it with the critical values for the chi-square statistics with the comparable degree of freedom [346]. The autoregressive structure, the compound structure and variance components had similar Akaike information values, so the variance structure was chosen.

Also, a linear regression was used to determine vitamin D response as a function of predictor variables including dose, baseline values, percentage body fat, dietary calcium intake, the use of contraceptives containing oestrogen and dressing code. All assumptions for regression analysis were met (multi-colinearity, independence of variables (Durbin-Watson), normally distributed residuals, and the same variance of the dependent variable for all values).

Serum 25(OH)D concentration, the primary outcome, was assessed as continuous, categorical (tertiles) and dichotomous [first cut off: sufficient level of 25(OH)D $\geq$ 50 nmol/L vs.

insufficient level of 25(OH)D <50 nmol/L, second cut off: sufficient level of ≥ 75 nmol/L vs. insufficient level of < 75 nmol/L]. A two tailed *P* – value of <0.05 was considered significant.

Chapter 4: Results

Baseline Characteristics

Sixty-one participants completed the study (**Figure 3.1**) out of 62 initial recruits. One participant in the 50,000 IU group moved to overseas and was lost to the follow-up at 6-months visit. This participant was included in the baseline analysis, only, thus the analysis of the intervention trial included 61 participants. The median follow up of all 61 randomly assigned participants was 163 days (range, 157 to 197 days).

Table 4.1 shows the baseline demographic profile and laboratory values of all study population and the treatment groups. There were no significant differences between the 3 groups. Half of the participants had tertiary education. The majority of women were students or employed [30.6% (19/62) and 43.5% (27/62), respectively]. Approximately, 85% had Fitzpatrick skin type V and VI and no one had skin type I and II. Four women were taking multivitamins containing both supplemental calcium and vitamin D, and 11 were taking multivitamins containing vitamin D only at baseline (vitamin D content ranging from 100 to 800 IU per tablet). Nobody was using blood pressure or lipid-lowering medications.

Adherence

Adherence was measured at the final visit as a percentage; (number of pills supplied minus number of pills returned)/number of pills supplied X 100. The mean adherence was 97.8%. Approximately, 10% of women did not adhere to the trial protocol, missing one or two monthly doses; 6.6% (4/61, 3 from group B and 1 from group C) of women missed one monthly dose and 3.3% (2/61, one from group B and one from group C) missed two monthly doses.

Use of other medications

Over the study period, 4.8% (1/21), 31.8% (6/19) and 38.1% (8/21) of subjects assigned to the placebo, 50,000 IU and 100,000 IU groups used some kind of antibiotics, respectively (P=0.03). Other medications used during the study period were Ibuprofen, Paracetamol, Panadol, Tramadol, Adult cold, Diclofenac, Loratadine, Venlafaxine, Citalopram, Fluoxetine, Venlafaxine, Loxalate, Alprazolam, Isotane, Ventolin, and Levothyroxine.

Primary outcome findings

The geometric mean [95% CI, lower bound-upper bound] baseline serum 25(OH)D concentration was 43.8 [39.6-48.9] nmol/L. Of 62 subjects, 6 (9.7%) had serum 25(OH)D concentration less than 25 nmol/L, 31 (50.0%) had concentrations of 25 to less than 50

nmol/L and 23 (37.1%) had concentrations of 50 to less than 75 nmol/L. Only 2 subjects had serum 25(OH)D of 75 nmol/L or more. One subject had serum 25(OH)D concentration of 189 nmol/L. There were no significant differences among the groups neither in geometric mean nor in the prevalence of serum 25(OH)D status based on the two cut-offs of ≥ 50 nmol/L and ≥ 75 nmol/L (**Table 4.2**).

In the mixed model analyses, we found a significant effect of time of follow up, F (2, 74.0) = 11.3, P<0.001, dose of treatment, F (1, 169.5) = 46.9, P<0.001, and the interaction between time of follow up and dose of treatment, F (2, 73.6) = 11.7, P<0.001 (**Figure 4.1**). This interaction indicated that the dose-response curve differed between at least the two time points. The dose-response curves for 3 and 6 months serum 25(OH)D concentrations were not identical. The coefficients for time and the interaction between time and dose at 3 months were different from that at 6 months on the basis of the pair wise *P* values between 3 months and 6 months (*P*<0.001 and *P*=0.02, respectively).

Supplementation with 50,000 IU/month and 100,000 IU raised the mean serum 25(OH)D concentrations from a baseline of 44.0±16.0 and 48.0±11.0 nmol/L to 70.0±15.0 and 82.0±17.0 nmol/L at 6 months, respectively (*P*<0.001 for both treatment groups; mean change of 25.2±16.7 and 34.4±20.2 nmol/L, respectively). The mean serum 25(OH)D concentration of women assigned to placebo group increased from 45.0±18.0 nmol/L at baseline to 54.0±18.0 nmol/L at 6 months (*P*<0.01; a mean change of 7.9±14.2 nmol/L).

The 6 months slopes for both doses (change in serum 25(OH)D concentration over the 6 months divided by the dose assigned) were 0.4±0.2 nmol/L/40 IU for the higher dose and 0.6±0.4 nmol/L/40 IU for the lower dose.

Table 4.1: Baseline Characteristics

Characteristic	Study Group				
	All participants (n=62)	**Placebo (n=21)**	**50,000 IU (n=20)**	**100,000 IU (n=21)**	***P*-value†**
Serum 25(OH)D, nmol/L[1]	43.8 [39.6-48.9]	45.2 [35.5-56.8]	40.9 [33.8-49.9]	46.1 [40.4-52.5]	0.65
Serum 25(OH)D, nmol/L[2]	46.0±15.0	45.0±18.0	44.0±16.0	48.0±11.0	
Age, y*	36.7±9.0	37.1±8.5	35.1±10.1	36.1±8.6	0.78
Weight, kg**	63.4[56.1-72.0]	63.8[55.0-69.5]	64.4[57.5-68.7]	59.1[57.7-73.0]	0.91
BMI, kg/m²**	24.7[22.0-28.1]	24.3[22.0-27.6]	24.5[22.1-27.4]	24.9[21.7-28.5]	0.92
BFP*	36.9±7.6	37.0±6.5	36.8±8.1	37.0±8.3	1.00
BP (Systolic), mm/Hg*	107.8±11.3	108.5±10.6	109.3±11.7	105.8±11.6	0.59
BP (Diastolic), mm/Hg*	70.4±9.5	71.5±7.9	71.6±10.9	68.2±9.6	0.44
Ethnicity					
Persian, n (%)	48 (77.4)	16 (76.2)	15 (75.0)	17 (81.0)	0.89
Arab, n (%)	14 (22.6)	5 (23.8)	5 (25.0)	4 (19.0)	
Clothing style Islamic, n (%)	19 (30.6)	6 (28.6)	7 (35.0)	6 (28.6)	0.88
Physical activity[δ]					—
Low, n (%)	38 (61.3)	16 (76.2)	12 (60.0)	10 (47.6)	
Medium, n (%)	9 (14.5)	1 (4.8)	3 (15.0)	5 (23.8)	
High, n (%)	15 (24.2)	4 (19.0)	5 (25.0)	6 (28.6)	
Contraceptive users, n (%)	7 (11.3)	1 (4.8)	3 (15.0)	3 (14.3)	—
Smoking, Smokers, n (%)	11 (17.7)	3 (14.3)	4 (20.0)	4 (19.0)	0.88
Calcium intake (diet + supplements), mg/d‡	528.5 [468.7-595.9]	487.8 [387.6-614.0]	539.2 [432.7-678.6]	561.2 [445.9-706.3]	0.65
Serum calcium, mmol/L**	2.25 [2.19-2.28]	2.23 [2.19-2.27]	2.27 [2.22-2.31]	2.23 [2.19-2.29]	0.35

25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; BFP, body fat percentage; BP, blood pressure;
[1]All subjects were included and values are reported as anti log means [95% CI, lower bound-upper bound]
[2]An outlier was excluded and values are reported as means ± SD.
*****Values are reported as means ± SD.
******Values are reported as medians [25th – 75th percentile].
†P values <0.05 are considered significant; for normally distributed data and non-normally distributed data, one-way ANOVA and Kruskal-Wallis tests, respectively, were performed; for categorical variables, chi-square was performed. — *P* value for some variables has not been reported because the chi-square test was not performed due to the violation of chi-square assumptions.
‡Derived from 4-day food diary; 54 volunteers (18 per treatment group) returned their food diaries. Values are reported as anti log means [95% CI, lower bound-upper bound].
[δ]Low, less than 2.5 hours of moderate physical activity per week; medium, 2.5-5 hours of moderate physical activity per week; high, equal or more than 5 hours of physical activity per week

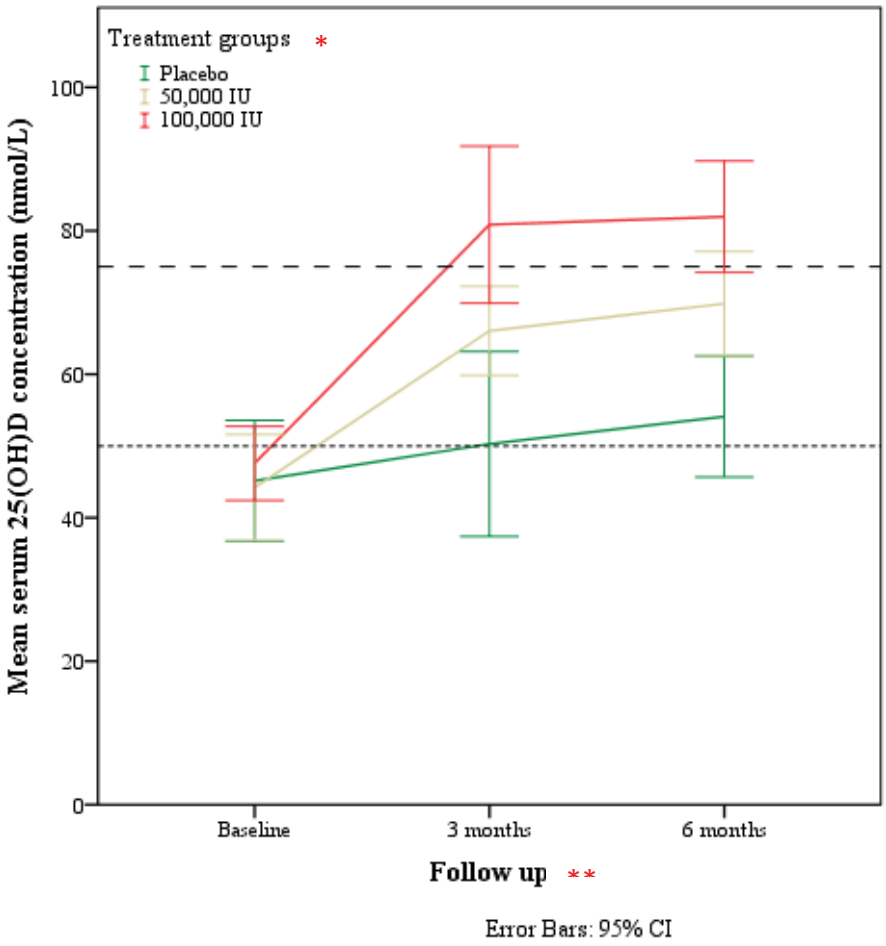

Figure 1: The dose response curve to vitamin D supplementation. The pattern of change in serum 25(OH)D concentrations over the study period (baseline, 3 months and 6 months) within each treatment groups have been presented. Reference lines at 50 (dotted line) and 75 nmol/L (hyphened line) were added for clarification. * Significant effect of dose of treatment F (1, 169.5) = 46.9, P<0.001. ** Significant effect of time of follow up F (2, 74.0) = 11.3, P<0.001. We also found a significant interaction between time of follow up and dose of treatment, F (2, 73.6) = 11.7, P<0.001. 25(OH)D, 25 hydroxyvitamin D; IU, international unit.

Because we found a significant interaction between time and dose in the mixed effects model, we compared group differences at each time point to examine this relationship. We explored interactions between treatment group and covariates in further analyses. We found a significant interaction between group and dressing code at 3 months, F (1, 61.0) = 17.1, P<0.001, and 6 months, F (1, 60.0) = 11.7, P<0.01, but not at the baseline (P>0.05). Because of the significant interaction between dressing code and dose of vitamin D, the two serum 25(OH)D curves for two dressing codes were not parallel at all time-points. Accordingly, we compared group differences at each time point in stratified groups based on dressing code.

Women with Islamic dressing code

Of 62 subjects, the 19 subjects who had Islamic dressing code were evenly distributed across treatment groups (approximately 29-35% of each treatment group). These women had lower mean serum 25(OH)D than women with non-Islamic dressing code at baseline in the placebo group at all time points (baseline, P=0.03; 3 months, P<0.01; 6 months, P<0.01). Serum 25(OH)D concentration increased from baseline with supplementation and reached a plateau at 3 months in women in both treatment groups (**Figure 4.2**). At 3 months, the mean serum 25(OH)D in women with Islamic dressing code in the 50,000 IU (P<0.01) and 100,000 IU (P<0.001) groups were higher than in the placebo group, but there was no statistically significant difference between the 50,000 IU and 100,000 IU groups. At 6 months, the mean serum 25(OH)D in the 100,000 IU was significantly higher than the placebo and 50,000 IU (P<0.001 and P=0.02, respectively). With regard to the within group differences, the mean serum 25(OH)D in women receiving monthly vitamin D supplementation was significantly higher at 3- and 6-months follow ups compared with the baseline level.

Women with non-Islamic dressing code

At the two follow ups, women with non-Islamic dressing code in the 100,000 IU and 50,000 IU group had significantly higher mean serum 25(OH)D than the placebo group (P<0.05). With regard to the within group differences, the mean serum 25(OH)D in women receiving monthly vitamin D supplementation was significantly higher at 3- and 6-months follow ups compared with the baseline level.

In women with non-Islamic dressing code, the mean ± SD serum 25(OH)D in placebo group increased from 50.6±17.7 nmol/L at baseline to 60.0±16.0 nmol/L at the 6-months follow up (a difference of 10.1±15.1 nmol/L, P > 0.05).

The difference between women with Islamic vs. non-Islamic dressing code in the serum 25(OH)D responses to the different dose treatments

We performed further analyses to explore the effect of dressing code on serum 25(OH)D response to the different treatments. We did not find any significant interaction between treatment group and dressing code and any significant effect of dressing code on response to vitamin D supplementation. The mean change in serum 25(OH)D after 6 months was comparable in both dressing code groups though the mean incremental change was larger in women with non-Islamic dressing code assigned to the placebo and 50,000 IU groups, but not 100,000 IU group, than those with Islamic dressing code [8.5±15.8 vs. 6.3±11.0 nmol/L in the placebo group (P=0.07); and 28.6±18.7 vs. 19.4±11.6 nmol/L in the 50,000 IU group (P=0.07), respectively]. Women with Islamic dressing code assigned to the 100,000 IU had larger mean incremental change in serum 25(OH)D than those with non-Islamic dressing code [40.2±25.0 vs. 32.1±18.4 nmol/L (P=0.6), respectively].

Proportion of subjects with serum 25(OH)D concentrations ≥ 50 and ≥ 75 nmol/L

The proportion of subjects reaching serum 25(OH)D above cut-off points of ≥ 50 and ≥ 75 nmol/L is shown in **figure 4.1** and **table 4.2**. At 6-months visit, the percentage of subjects reaching ≥ 50 nmol/L increased in all groups. However, the pattern of change was different across groups. In the placebo group, the proportion remained unchanged at 3-months visit but increased afterward. All subjects in the 50,000 IU and 100,000 IU groups, except for one in the 50,000 IU group, had serum 25(OH)D concentrations ≥ 50 at 3-months visit.

At 3-months visit, serum 25(OH)D concentration increased to ≥ 75 in 30.0 and 47.6% of study groups in the 50,000 IU and 100,000 IU groups, respectively (P<0.001). This proportion remained unchanged in the 50,000 IU group, but increased to 66.7% in the 100,000 IU group at 6 months.

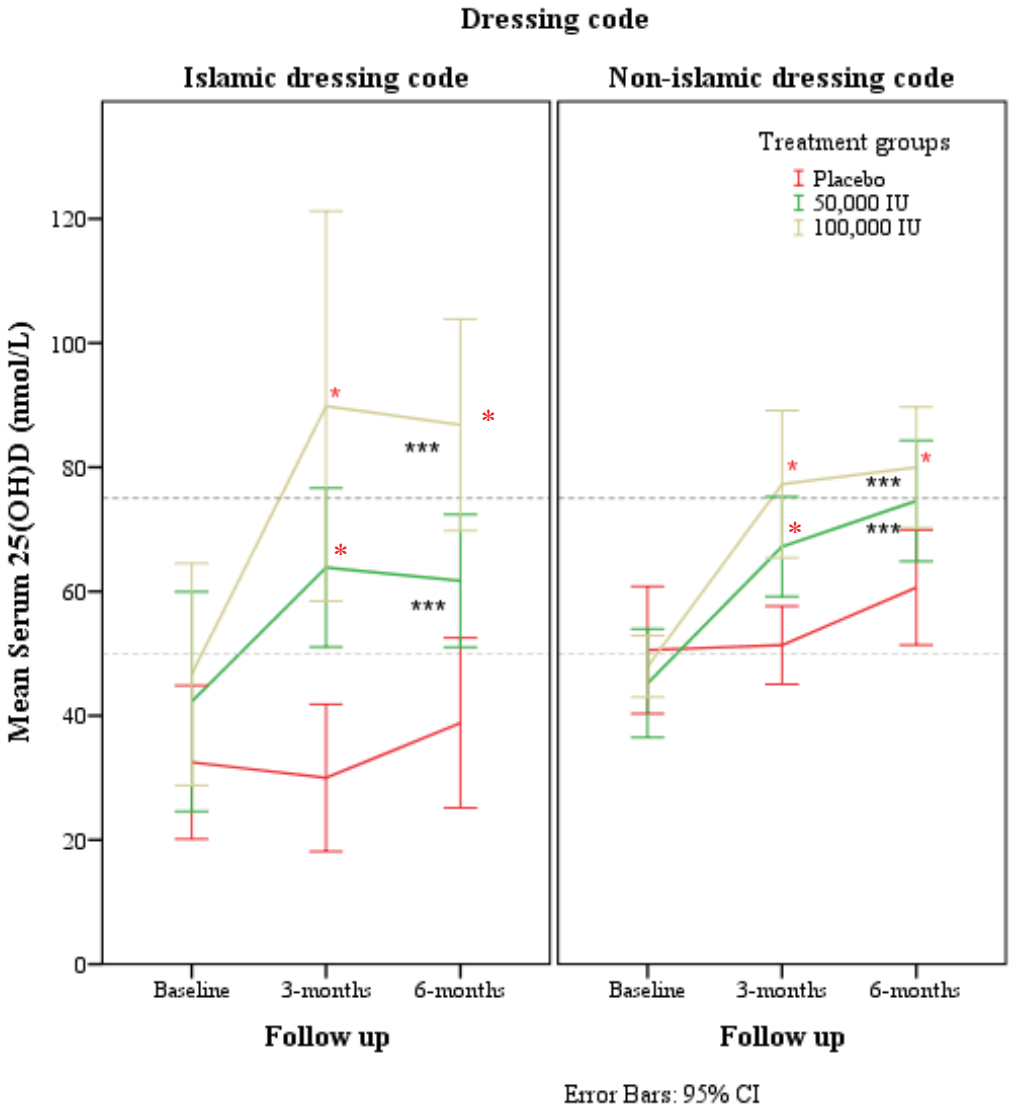

Figure 4.2: The pattern of change in serum 25(OH)D concentration over the study period stratified by dressing code. The graph was stratified by dressing code because of the interaction between dressing code and treatment, F (1, 61.0) = 17.1, P<0.001, and 6 months, F (1, 60.0) = 11.7, P<0.01, but not at the baseline (P>0.05). Reference lines at 50 and 75 nmol/L were added for clarification. * Significant differences between placebo other treatment groups (P<0.01). *** Significant differences within each group over the study period (P<0.05) otherwise, not stated, the difference over the study period is not significant. BFP and baselines serum 25(OH)D was considered as covariates and were controlled for in the analyses. 25(OH)D, 25 hydroxyvitamin D; IU, international unit.

Table 4.2: Percentage of participants with serum 25(OH)D concentrations ≥ 75 nmol/L and ≥ 50 nmol/L at baseline, after 3 and 6 months in three groups

Variable*	Study Group			
	Placebo (n=21)	50,000 IU (n=20)	100,000 IU (n=21)	*P*-value†
Serum 25(OH)D ≥ 75 nmol/L				
Baseline	9.5 (2)	0.0 (0)	0.0 (0)	─
3 months	4.8 (1)	30.0 (6)	47.6 (10)	0.007
6 months	14.3 (3)	31.6 (6)	66.7 (14)	0.002
Serum 25(OH)D ≥ 50 nmol/L				
Baseline	33.3 (7)	40.0 (8)	47.6 (10)	0.64
3 months	33.3 (7)	95.0 (19)	100.0 (21)	<0.001
6 months	61.9 (13)	94.7 (18)	100.0 (21)	─

25(OH)D, 25-hydroxyvitamin D
*Values are reported as percentage (number).
†Chi-square was performed because the variables were categorical. ─ *P* value for some variables has not been reported because of the violation of chi-square assumptions.

Predictors of change in serum 25(OH)D concentration after 6-months

Figure 4.3 shows the distribution of participants according to absolute change in serum 25(OH)D concentrations from baseline to 6 months in all groups. There was a wide variability in absolute change of serum 25(OH)D concentrations in both treatment groups. The minimum and maximum change in serum 25(OH)D concentrations in the placebo, 50,000 and 100,000 IU groups were -14.0 and 37.0, 3.0 and 57.0, and 1.0 and 80.0 nmol/L, respectively.

The model was explored for outliers. There was an outlier which biased our model as such that case was excluded from the analysis. For every 10 cases, one variable can be included in a regression model [346]; because we had 62 cases, we could include six predictor variables in our regression model. The model was designed to be adjusted for known covariates (baseline serum 25(OH)D, dressing code, age, the use of contraceptives containing oestrogen, BFP, ethnicity, BMI and weight).

Correlations between the covariates were examined before model entry to determine whether multicollinearity existed. Dressing code was included as this variable showed significance at the bivariate level. Furthermore, dressing code was associated with serum 25(OH)D in our previous study [17]. Ethnicity was not included as it was closely associated with dressing

code which is an indicator of sun exposure. Neither body fat percentage nor BMI or weight correlated with baseline serum 25(OH)D concentration. However, BFP was included in the model as the effect of BFP on response to vitamin D supplementation has been determined by several studies [34, 69]. Furthermore, BFP is a more reliable index of body fat stores than weight or BMI. Dietary calcium intake and the use of contraceptive containing oestrogen have been shown to affect the response to supplementation though the evidence is conflicting [34, 264, 313]. Although, we aimed to include the use of contraceptives containing oestrogen in the model, we did not because there were only 7 women using contraceptives containing oestrogen. BFP was correlated with age (r=0.3, n= 62, P=0.03 (two-tailed)); therefore, age was excluded from the model to avoid multicollinearity. Final covariates included in the model were BFP, calcium intake, baseline serum 25(OH)D and dressing code.

Table 4.3 presents the results of the multivariate regression analysis. Dose (P<0.001), baseline serum 25(OH)D concentration (P<0.001) and baseline BFP (P=0.01) were the only variables to reach statistical significance as predictors of the change in serum 25(OH)D over 6 months.

The effect of dressing code on the change in serum 25(OH)D over 6 months was not significant (P=0.10). Larger dose, lower baseline serum 25(OH)D concentration, lower baseline BFP was associated with larger incremental change in serum 25(OH)D concentration after 6 months of supplementation. The other variable included in the model, dietary calcium intake (diet + supplements) was not an independent determinant of the change in serum 25(OH)D concentration.

As a group, the 4 variables, accounted for 57.6% of the variance in the change in serum 25(OH)D concentrations for this sample. According to the standardized beta coefficients, the dose of treatment was the strongest predictor for serum 25(OH)D followed by baseline serum 25(OH)D concentration and baseline BFP and then dressing code (0.6, -0.4, -0.3, and 0.2, respectively).

We performed post hoc analyses to examine the relationship between baseline serum 25(OH)D concentration and response to supplementation, and to examine the relationship between BFP and serum 25(OH)D concentrations.

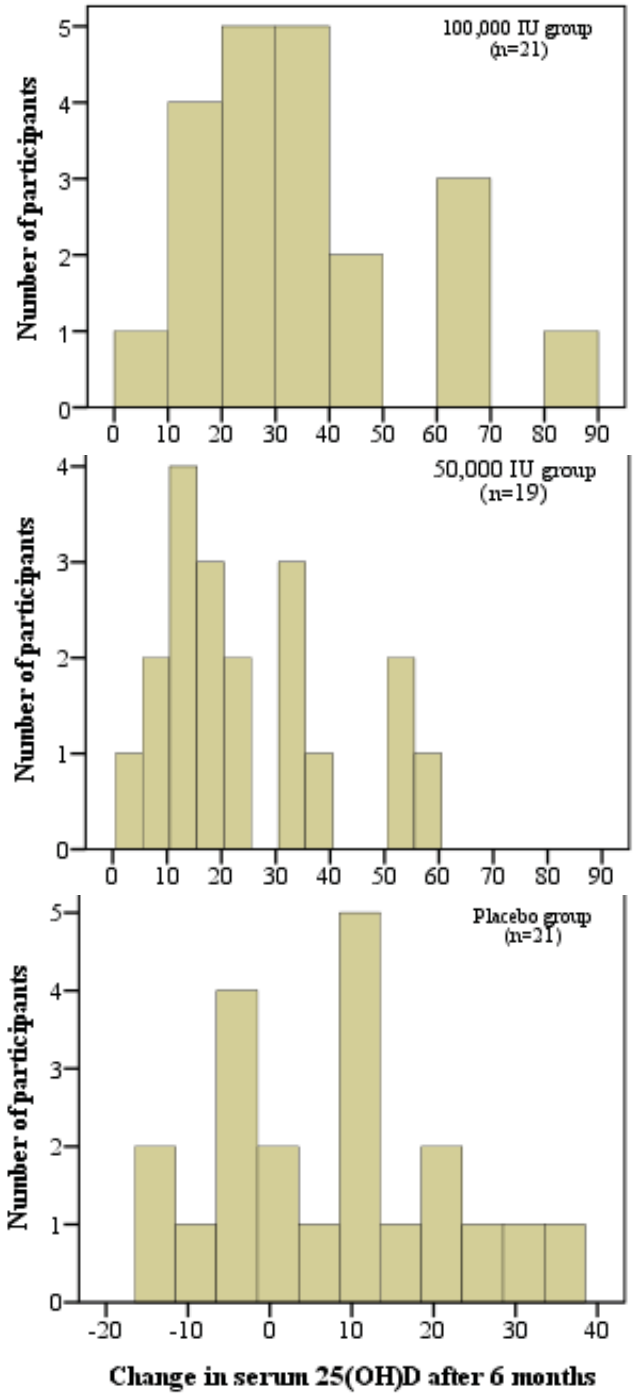

Figure 4.3: Distribution of participants according to absolute change in serum 25(OH)D concentrations (nmol/L) over the study period in all study groups. 25(OH)D, 25 hydroxyvitamin D; IU, international unit.

Table 4.3: Predictors of change in serum 25(OH)D concentrations over study period (6 months)†

Change in serum 25(OH)D Model	Coefficient (*B*)	Standard Error (*B*)	95% CI *B*	Standardized *B*	R^2	*P*-value
Model 1					0.576*	<0.001
Intercept	35.8	13.2	9.3, 62.3			
Dose[1]	14.1	2.4	9.3, 19.0	0.6		<0.001
Baseline serum 25(OH)D concentration[2]	-0.6	0.1	-0.8, -0.3	-0.4		<0.001
BFP[3]	-0.7	0.3	-1.2-, -0.2	-0.3		<0.01
Dressing code[4]	7.3	4.3	-1.4, 15.9	0.2		0.10
Model 2					0.576**	<0.001
Intercept	36.0	14.5	6.7, 65.2			
Dose	14.1	2.4	9.3, 19.0	0.6		<0.001
Baseline serum 25(OH)D concentration	-0.6	0.1	-0.8, -0.3	-0.4		<0.001
BFP	-0.7	0.3	-1.3, -0.2	-0.3		<0.01
Dressing code	7.2	4.4	-1.6, 16.1	0.2		0.10
Calcium intake (diet + supplement)	0.0	0.01	-0.1, 0.1	0.003		0.98

†Backward stepwise technique. In the model dose, baseline serum 25(OH)D concentration (nmol/L), BFP (body fat percentage), dressing code and dietary calcium intake (diet + supplements) (mg) were included.
Valid number of participants: 53.
*F (4, 52) = 16.3, P<0.001; ** F (5, 52) = 12.8, P<0.001
[1]For each increase of one unit in vitamin D of 50,000 IU, serum 25(OH)D is expected to increase by 14.1 nmol/L
[2]For each decrease of one unit in baseline serum 25(OH)D concentration, the change in serum 25(OH)D is expected to increase by 0.6 nmol/L.
[3]For each decrease of one unit in BFP, the change in serum 25(OH)D is expected to increase by 0.7 nmol/L.
[4]Dressing code was coded as 1 = Islamic dressing code and 2 = non-Islamic dressing code; having non-Islamic dressing code was associated with an increase of 7.3 nmol/L in change in serum 25(OH)D concentration after 6 months.
Regression equation: change in serum 25(OH)D after 6 months (nmol/L) = 35.8 + 14.1 x dose – 0.6 x baseline serum 25(OH)D (nmol/L) – 0.7 x BFP + 7.3 x dressing code

The relationship between baseline serum 25(OH)D concentration and response to supplementation

In partial correlation (controlling for the dose of treatment, baseline BFP and dressing code) analyses, baseline serum 25(OH)D concentration correlated significantly with the incremental change in serum 25(OH)D after 3- and 6 months, r = -0.5, df = 56, P<0.001 and r = -0.5, df = 55, P<0.001, respectively.

Further analysis revealed that incremental change in serum 25(OH)D concentration after 6 months were larger in those who had baseline serum 25(OH)D concentrations <50 nmol/L compared with those with concentrations ≥ 50 nmol/L (26.9±21.4 vs. 17.1±16.3 nmol/L, respectively, F (1, 60) = 5.3, P = 0.02). Further analyses revealed that the difference between two categories was significant in the 50,000 IU group only though women with lower BFP had larger incremental change in all groups [32.4±18.3 vs. 15.4±7.1 nmol/L (P=0.01) in the 50,000 IU group and 40.5±21.7 vs. 27.7±17.0 nmol/L (P>0.05) in the 100,000 IU group] (**Figure 4.4**).

Predictive equations to determine the optimal dose

We performed further analyses to construct predictive equations for determining the dose of vitamin D supplements needed to achieve a specific cut off level of serum 25(OH)D in subjects with different baseline 25(OH)D concentrations (<50 vs. ≥ 50 nmol/L). The predictive equations are:

Basal serum 25(OH)D <50 nmol/L: Serum 25(OH)D (nmol/L) at 6 months = 55.3 + 15.9 X dose – 0.6 X BFP

Basal serum 25(OH)D ≥ 50 nmol/L: Serum 25(OH)D (nmol/L) at 6 months = 88.5 + 8.0 X dose – 0.8 X BFP

(1 = 0 IU/month, 2 = 50,000 IU/month, 3 = 100,000 IU/month; to calculate the dose: digits X 50,000 + the whole number which should be replaced by 0, 50,000 or 100,000 if it is 1, 2, or 3, respectively)

Presumably, two subjects with comparable BFP of 33% but with different basal 25(OH)D concentrations of 40 nmol/L and 60 nmol/L would need different doses to achieve a serum 25(OH)D concentration of 75 nmol/L after 6 months; the person with serum 25(OH)D concentration of 40 nmol/L would need larger doses of vitamin D supplementation than the

person with basal serum 25(OH)D concentration of 60 nmol/L (75,000 IU/month vs. 30,000 IU/month, respectively).

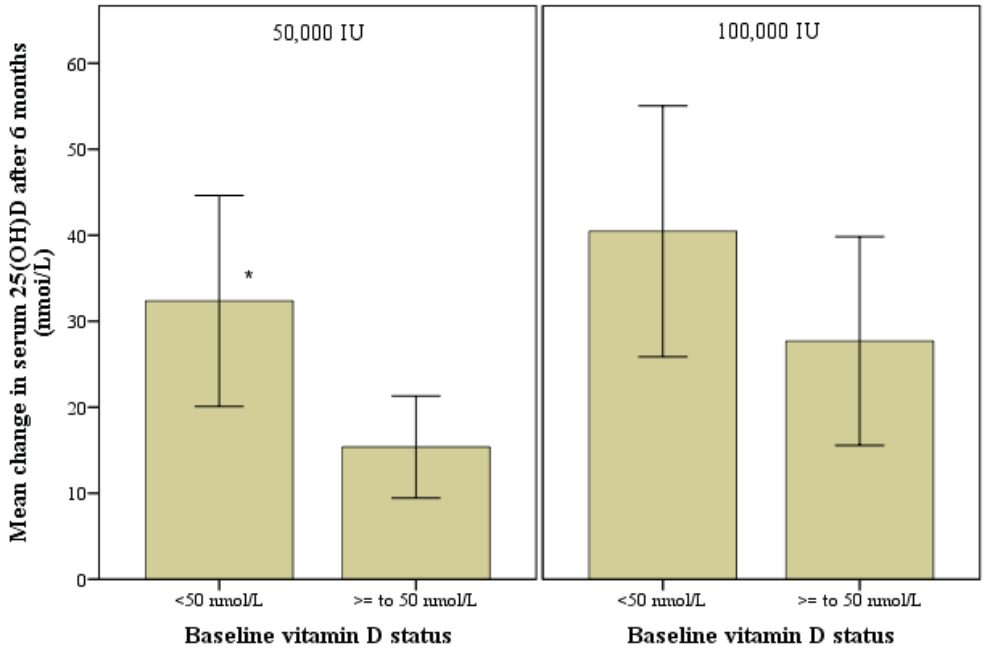

Figure 4.4: The mean change in serum 25(OH)D concentrations over the study period in women with baseline serum 25(OH)D < 50 nmol/L and ≥ 50 nmol/L in both vitamin D supplemented groups. * The difference between two categories in the 50,000 IU group is significant; 32.4±18.3 vs. 15.4±7.1 (*P*=0.01). 25(OH)D, 25 hydroxyvitamin D; IU, international unit.

The relationship between BFP and response to supplementation after 3 and 6 months

We performed post hoc analyses to investigate correlations between serum 25(OH)D concentration at baseline, 3 months and 6 months and anthropometric indices (weight, BMI and BFP). At baseline, neither of these indices correlated with serum 25(OH)D concentration. Except for BFP, neither weight nor BMI correlated with serum 25(OH)D concentration at 3 months and 6 months ($P>0.05$). In partial correlation (controlling for the dose and baseline serum 25(OH)D) analyses, BFP correlated significantly with the change in serum 25(OH)D at 3 months, $r = -0.3$, $df = 57$, $P=0.01$, and at 6 months, $r = -0.3$, $df = 56$, $P<0.01$.

We also compared the change in serum 25(OH)D concentrations at 3 and 6 months in those with BFP ≤ 35% with those with BFP >35% while controlling for the baseline serum 25(OH)D, dressing code and dose of treatment. Using ANCOVA, we found no interaction between dose and BFP categories at 6 months. The change in serum 25(OH)D concentration

was almost significantly larger in women with ≤ BFP (26.5±21.8 nmol/L) than those with > 35% (20.0±18.0 nmol/L), F (1, 60) = 3.2, *P* = 0.08. For the 3 months change, we found a significant interaction between BFP category and dose of treatment. Accordingly, we performed further analyses on groups stratified by dose of treatment. We found an almost significant effect of BFP categories on serum 25(OH)D response to supplementation with 100,000 IU after 3 months only (49.6±35.6 vs. 21.1±15.7 nmol/L, respectively, F (1, 27) = 4.3, *P* = 0.05), though the incremental change was larger among those with ≤ 35% of BFP compared with those having more BFP at all time point and in all treatment groups. **Figure 4.5** presents the mean incremental change in serum 25(OH)D concentration after 3- and 6 months for different treatment groups within BFP categories.

Adverse events

Two subjects in the 50,000 IU group (C), one at 3 months follow up and one at both follow ups reported that they had dizziness within a short period of taking the study tablets. The dizziness remained for 1-2 hours. Other than this no other adverse events were reported.

Among the groups, there were no significant changes in levels of serum calcium, and the mean change in serum calcium did not differ significantly across the study groups. There were no reports of hypercalcemia (serum calcium (corrected for albumin ≥ 2.7 mmol/L) and hypervitaminosis D (serum 25(OH)D > 225 nmol/L).

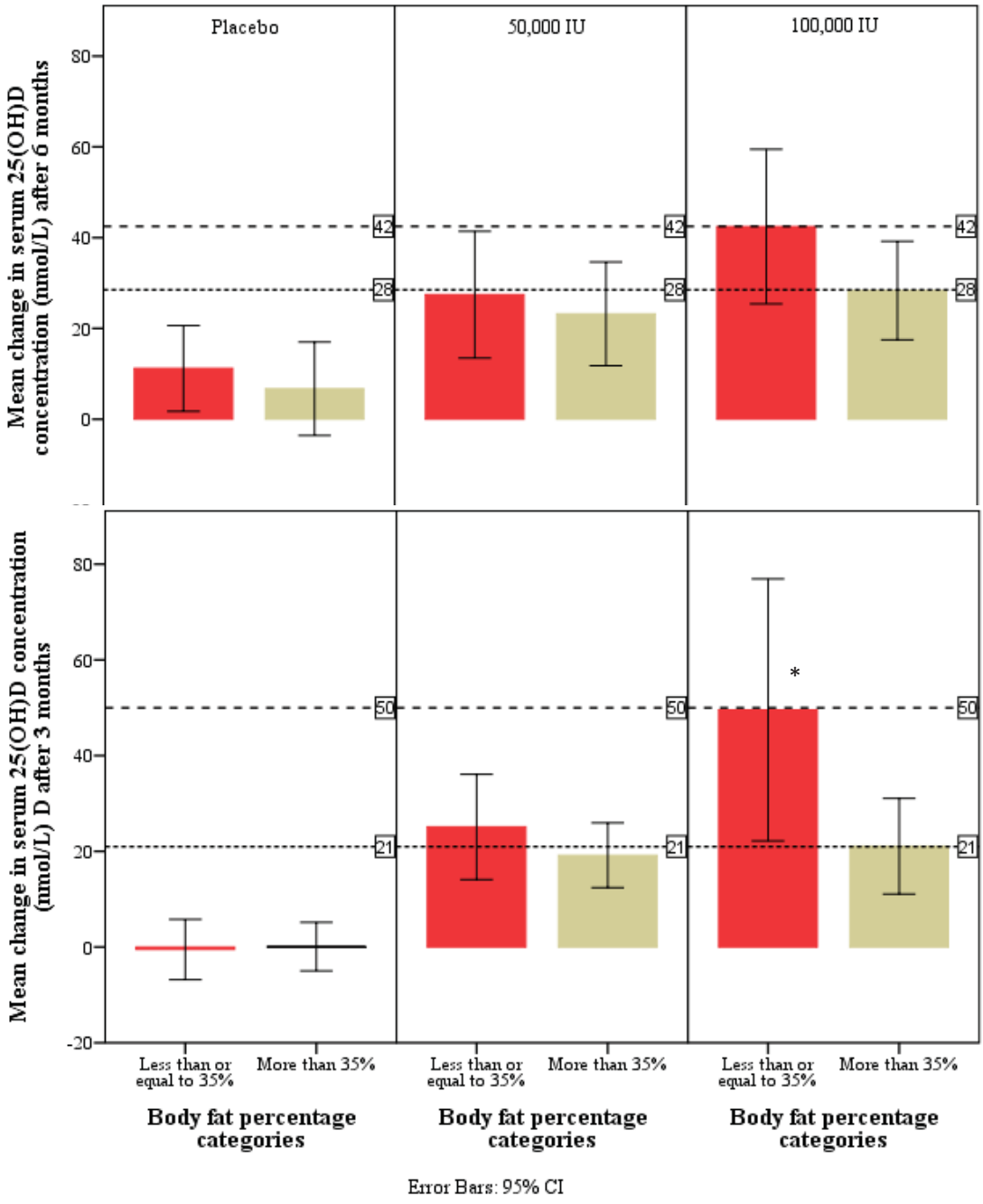

Figure 4.5: Mean change in serum 25(OH)D after 3- and 6 months for different treatment groups within different BFP categories. Reference lines are presented to illustrate the difference between BFP categories in women receiving 100,000 IU vitamin; the small dotted lines indicate that the mean change in women with BFP > 35 increased from 21 at 3 months to 28 nmol/L at 6 months. The hyphened lines indicate that the mean change in women with BFP ≤ 35 decreased from 50 at 3 months to 42 nmol/L at 6 months. *Women with BFP ≤ 35 had an incremental change of 49.6±35.6 nmol/l while women with BFP > 35 had an incremental change of 21.1±15.7 nmol/L, F (1, 27) = 4.3, P = 0.05). 25(OH)D, 25 hydroxyvitamin D; IU, international unit; BFP, body fat percentage.

Chapter 5: Discussion and Conclusions

Discussion

The goal of this double-blind randomised clinical trial was to evaluate the effects of supplementation with monthly 50,000 IU versus 100,000 IU in apparently healthy premenopausal women over the winter though summer months in New Zealand.

In the present study, more than half of the study population had serum 25(OH)D concentrations < 50 nmol/L and only 3.3% had serum 25(OH)D ≥ 75 nmol/L. The results show that a monthly intake of 100,000 IU vitamin D is more effective than 50,000 IU in achieving serum 25(OH)D concentrations of either 50 nmol/L or 75 nmol/L. The mean serum 25(OH)D concentrations reached a plateau after 3 months of supplementation. The slopes for the high and low doses were 0.4±0.2 and 0.6±0.4 nmol/L per 40 IU oral vitamin D input, respectively. There were no reports of hypervitaminosis D (serum 25(OH)D > 225 nmol/L) or hypercalcaemia (serum calcium ≥ 2.7 mmol/L). Of the un-supplemented (placebo) participants, 66.7% had low vitamin D status (serum 25(OH)D < 50 nmol/L) in the beginning (the baseline visit, July-August) and end of winter months (the second visit, September-October). This proportion decreased to 38.1% in summer months (the final follow up, December through February).

To our knowledge, this is the first study comparing monthly 50,000 IU and 100,000 IU by mapping the time course of serum 25(OH)D from winter to summer among Middle Eastern women living in Auckland, New Zealand. In the present study, serum 25(OH)D concentration increased from baseline with supplementation and reached a plateau at 3 months. In agreement with our study, Talwar et al (2007) [31] and von Hurst et al (2010) [206] reported that serum 25(OH)D concentrations reached the peak 3 months after the initiation of each dosing regimens. The study populations received daily 800 IU for two years followed by daily 2000 IU vitamin D for one year or placebo throughout the study period, and daily 4000 IU for 6 months, respectively.

Participants assigned to the 50,000 IU and 100,000 IU groups exhibited significantly higher serum 25(OH)D concentrations than the placebo group. While the difference between the two treatment groups was not statistically significant, concentrations in the 50,000 IU group (70.0±15.0 nmol/L) were lower relative to the 100,000 IU group (82.0±17.0 nmol/L). Comparable to our results, Bacon et al (2009) utilising the same vitamin D tablets but in different dosing regimens over 8 months found that in those receiving monthly 50,000 IU and

500,000 IU (loading dose)+monthly 50,000 IU (equating to approximately 110,000 IU per month), serum 25(OH)D concentrations reached the plateau of 70.0 and 86.0 nmol/L, respectively [278].

Moreover, the proportion of subjects with serum 25(OH)D < 50 nmol/L and < 75 nmol/L decreased more in women receiving monthly 100,000 IU. Although more effective than monthly 50,000 IU, even with this dose, approximately 1/3 of participants still failed to reach optimum serum 25(OH)D concentrations (≥ 75 nmol/L). Other studies also found that accumulative doses of 100,000 to 120,000 IU per month were insufficient to raise serum 25(OH)D concentrations to the desirable concentration of 75 nmol/L or more in all subjects [291, 309]. In Zabihiyeganeh et al's (2013) study, 65.6% of patients who received an accumulative dose of 300,000 IU over 3 months reached serum 25(OH)D concentration of 75 nmol/L or more after 6 months, a finding comparable to ours (66.7%). All subjects in this study had serum 25(OH)D concentrations < 75 nmol/L and were Iranians. These findings indicate that larger doses than 100,000 IU per month are required to shift vitamin D status to ≥ 75 nmol/L in populations with high prevalence of vitamin D insufficiency.

A comparison of our results on vitamin D responses with those in the literature among Caucasians suggests that the response to oral vitamin D supplementation is not blunted in Middle Eastern women. If one presumes that the slope of the response to oral vitamin D is 0.7 nmol/L/1µ oral vitamin D supplemented, as reported by Heaney et al (2003) [38], then by inference, doses of 50,000 IU (1666.7 IU/day = 41.7 microgram) and 100,000 IU (3333.3 IU/day = 83.3 microgram) are expected to raise serum 25(OH)D concentrations by+29.2 and +58.3 nmol/L, respectively. In fact, the mean changes in serum 25(OH)D concentrations over 6 months in our participants were 25.2±16.7 and 34.4±20.2 nmol/L, respectively. Although the observed change in serum 25(OH)D in the 50,000 IU group (a slope of 0.6 nmol/L/1µ oral vitamin D) was comparable to what was calculated in the Heaney et al's paper [38], the incremental change was lower by approximately 24.0 nmol/L in the 100,000 IU group. The slope for this dose in our study was 0.4 nmol/L/1µg oral vitamin D (refer to the **Figure 4.1**). Our finding is in accordance with the findings of Veith et al (2001) [291] in that they also found different slopes for the high and low doses, though the calculated slopes were different. An explanation is that hepatic hydroxylation is a saturable process and with an input above physiological norm of vitamin D, serum vitamin D concentration increases, and the reaction switches from the first order to zero order [347]. It is presumed that the excess vitamin D is

stored in body fat as a native compound and is slowly released [347, 348]. So, larger doses result in flatter slopes compared with the slopes for lower doses. This, therefore, may result in a longer apparent half-life of serum 25(OH)D.

In the current study, lower baseline BFP appeared to be one of the factors causing a better response to oral vitamin D supplementation in premenopausal Middle Eastern women, independent of BFP's effect on baseline serum 25(OH)D concentrations. We did not find any relationship between baseline BFP and baseline serum 25(OH)D concentrations. The difference in serum 25(OH)D response to supplementation based on BFP is consistent with the findings of Nelson et al (2009) [287] and Blum et al (2008) [69]. In the former study, the authors found that achieving optimal serum 25(OH)D concentrations in premenopausal white women in winter time was associated with lower percentage body fat (29.9%±7.1% vs. 35.4%±7.4%) [287]. The authors in the latter study found a significant inverse relationship between the change in serum 25(OH)D concentrations after one year of supplementation with vitamin D and central body fat, waist circumference, weight and BMI [69].

Other studies have also found a significant relationship between other anthropometric indices such as weight and BMI and serum 25(OH)D response to supplementation [36, 69, 264, 311]. In these studies, a lower response was observed in women with baseline BMI ≥ 30 kg/m² than in women with BMI < 30 kg/m² [36] or BMI < 25 kg/m² [69]. In our study, we did not find any differences in serum 25(OH)D response to supplementation among BMI categories. The median BMI of our study population was 24.7 [22.0-28.1] nmol/L and we had a small number of women with BMI ≥ 30 kg/m², but the majority had BFP of more than 30%. The high mean BFP could be attributed to the sedentary lifestyle of these women. The majority of women had less than 2.5 hours of moderate physical activity per week. Due to the limited variability in BMI we did not have enough power to detect any differences across baseline BMI groups. It is worth noting that BMI has a poor sensitivity to detect BFP in some ethnic groups such as Asian-American women [349] and Saudi adults [350]. So, BFP is a better indicator of adipose stores and obesity than BMI or weight in some ethnic groups.

The postulated mechanism for the influence of adipose stores on serum 25(OH)D response to supplementation is that because vitamin D is a fat soluble vitamin, it is stored in body fat for later use [52, 348]; and the larger the adiposity tissue is, the more likely vitamin D is trapped [251]. Experimental support for sequestration comes from human and animal studies [348]; Wortsman et al (2000) [351] exposed both lean and obese individuals with comparable

baseline serum 25(OH)D concentration to whole body UVB or 50,000 IU oral vitamin D_2. After 24 hours, serum 25(OH)D concentrations in obese subjects reached 57% of that in lean subjects exposed to UVB, and was inversely associated with BMI in those receiving oral vitamin D. In support of this study, a study in Wistar rats showed that under supplementation with high dose vitamin D, 25(OH)D concentration in plasma increased rapidly until it reached a plateau [348]. The plasma 25(OH)D and adipose tissue cholecalciferol accumulation occurred linearly and rapidly, and the accumulated cholecalciferol was released slowly in the circulation in the condition of energy balance. Recent evidence of sequestration of vitamin D in human adipose lends credence to these observations [352].

In the present study, while the mean change in serum 25(OH)D concentration in women with BFP $\leq$ 35% receiving 100,000 IU reached a plateau at 3 months and decreased at 6 months, the dose-matched women with BFP > 35% had a lower and steady increase in their serum 25(OH)D concentrations over the study period (at 3- and 6 months). There are two explanations for such observation; one is that instead of entering the circulation for further metabolism, vitamin D is stored in body fat stores and is released slowly later on, or because of this sequestration, the hepatic hydroxylation does not reach saturation and the incremental change does not reach a steady-state. The assessment of an effect of BFP on serum 25(OH)D response to supplementation was not the primary objective of this study. Accordingly, trials with a more robust experimental design which includes more subjects, have a longer study period and more frequent vitamin D testing while controlling for other confounders such as baseline level are warranted.

In addition to the dose of treatment and BFP, serum 25(OH)D concentration at the baseline proved to be related to the serum 25(OH)D response to the treatment. Baseline serum 25(OH)D concentration was the second most significant predictor of the change in serum 25(OH)D concentrations after 6 months and the weight of this correlation was medium because baseline serum 25(OH)D concentration explained 20.2% of the variance of serum 25(OH)D response. Consistent with our study, other studies reported that baseline 25(OH)D concentration makes a significant contribution to variance in 25(OH)D response to vitamin D supplementation [7, 31, 32, 269, 278, 284-288]: higher increases are seen in those with lower baseline levels.

Garland et al (2011) [353] explored the response to vitamin D supplementation as a function of basal value. Based on their findings, subjects with serum 25(OH)D concentration of 37.5

and 75.0 nmol/L for example, a daily dose of 1666 and 3333 IU vitamin D should result in an increase of 40.0 and 32.0 nmol/L, and 82.5 and 66.7 nmol/L, accordingly. In our study, we also found that the larger increase was seen in subjects with serum 25(OH)D concentration < 50 nmol/L compared with those with serum 25(OH)D concentrations ≥ 50 nmol/L; 32.4±18.3 vs. 15.4±7.1 nmol/L in the 50,000 IU group and 40.5±21.7 vs. 27.7±17.0 nmol/L in the 100,000 IU group, respectively. However, the increment in serum 25(OH)D is not comparable to the values reported by Bacon et al [278] and those calculated by Garland et al [353]. Their observations and calculations, respectively, considered baseline levels only, but the results from the present study indicates that other factors such as BFP and amount of cutaneous synthesised vitamin D should be taken into account when assessing the serum 25(OH)D response to a fixed dose.

In the present study, the increase in serum 25(OH)D concentration was not exclusive to the participants assigned to the treatment groups. The increase in serum 25(OH)D concentration in the placebo group was anticipated because we were approaching summer at the final visit. The increase in serum 25(OH)D concentration was seen in both women with Islamic and non-Islamic dressing code (6.3 vs. 10.7 nmol/L), and as was expected, it was larger in women with non-Islamic dressing code. However, the direction of the dressing code in the pattern of change in serum 25(OH)D concentration in treatment groups was surprising.

As serum 25(OH)D concentrations were slightly lower in women with Islamic dressing code owing to the reduced area exposed to sunlight, one might expect that these women would have a greater response to supplementation after 6 months. However, women with non-Islamic dressing code in the 50,000 IU group had almost significantly greater change in their serum 25(OH)D concentrations. One explanation for such observation could be that compared with their counterparts with Islamic dressing code, women with non-Islamic dressing code may have had greater dermal vitamin D synthesis during the last 3 months (approaching summer). This is supported by our finding that no significant differences in the response were detected between two dressing code categories at 3 months (winter months when the effect of sun exposure is diminished for all). These findings highlight the importance of taking sun exposure indices such as dressing style, lifestyle, skin colour and season into account when predicting an adequate dose of vitamin D for supplementation.

Our findings are in agreement with several other studies from New Zealand (latitude ranging from 35 degrees S to 47 degrees S) in that season has a significant impact on vitamin D

status, though the between-seasons differences are much larger in those studies. The prevalence of vitamin D insufficiency and deficiency increased from 30.0-35.0% and 7.0-8.0% in summer to 61.0-63.0% and 21.0-23.0% in late winter/early spring, respectively [354]. The geometric mean of placebo dropped by 44 nmol/L from winter to summer in a study by Logan et al (2013) [295], and dropped by 30 nmol/L from summer to spring in another study by Rockell et al (2008) [220]. The lower seasonal effect in our study population could be attributed to the more conservative clothing style of Middle Eastern women, having more sun avoidance behaviour or darker skin colour than study populations in the mentioned studies. In our previous study, we found that, apart from those having Islamic dressing code, 11.6% had fully covered clothing style due to cultural norms or religion, and 95.4% protected their skin from sun exposure due to the fear of skin cancer or other skin problems [17]. While the majority of our study population had Fitzpatrick skin type V and VI indicating a dark skin type which rarely or never burns, in the afore-mentioned studies, the majority of participants were of European ethnicity that are known to have light skin colour. Darker skin pigmentation has been associated with lower 25(OH)D concentration in several studies [64, 95, 219, 220].

In the current study, we observed large variability in serum 25(OH)D response to the treatment in both groups. Other studies have also reported similar variability [31, 264]. Overall adherence to treatment was more than 80.0%, as such other factors other than adherence attributed to the variance in response to treatments. Our regression analysis revealed that inclusion of dose of treatment, baseline serum 25(OH)D concentration, BFP and dressing code explained 57.6% of the variability in serum 25(OH)D response to supplementation, leaving 42.4% of the variability to unknown factors.

Response to supplementation is also genetically determined. Several genes has been recently identified which are involved in the metabolism of vitamin D such as genes encoding 1α-hydroxylase, VDR and vitamin D binding protein [270, 271]. The relationship between serum 25(OH)D and VDBP and its encoding gene, GC, has been extensively evaluated and the results have been reproduced across different populations [74-76, 277].

Apart from carriage of vitamin D and its metabolites in the circulation, VDBP has anti-inflammatory and immunomodulatory functions [272], and has the ability to bind to fatty acids [273]. The most commonly studied GC variants are single nucleotide polymorphisms (SNPs) rs7041, rs4588, rs2282679 and rs1155563 [74-78]. It has been indicated that different

GC variants have different affinity to 25(OH)D [275, 276] and have different glycosylation pattern affecting their metabolic rate and consequently their half-life [276]. Compared to GC-1f and GC-1s isoforms, GC-2 isoform has been shown to have the lowest affinity to 25(OH)D [275] and to the lack of trisacharide glycosylation at position 436 [276]. Furthermore, GC-2 has less ability to be converted to macrophage activating factor [355]. Accordingly, one can postulate that GC-2 carriers show lower vitamin D status [279] and be more susceptible to infection [356].

The largest between-genotype group differences in serum 25(OH)D concentrations have been reported to be more apparent in summer when the vitamin D load is high [75]. As such, one can hypothesize that the larger variation in response to supplementation in the current study's larger dose arm (6.0 – 124.0 nmol/L) might be due to genetic variation. Based on self-reported antibiotic use (ranging from 4 to 10 days), we also found a high rate of infection in this treatment group (33.3%). Those women who used antibiotics had smaller change in their serum 25(OH)D concentrations after 3 months compared with non-users (P>0.05). It is well documented that vitamin D has anti-inflammatory and immunomodulatory effect [357], and vitamin D supplementation has been shown to be associated with reduced infection [358]. So, an explanation for such observation could be that the higher rate of infection despite of using a large dose of vitamin D supplement (100,000 IU/month) might be the result of genetic susceptibility to infections or immune diseases; presumably subjects were carriers of GC-2 genotype. Although this hypothesis is compelling, it seems to be a very big assumption and lacks experimental support and warrants further studies.

Vitamin D supplementation was not associated with any adverse events. Other studies have also confirmed our findings in that supplementation with even larger doses of vitamin D is safe [38, 278].

Conclusions

In conclusion, the prevalence of vitamin D deficiency/insufficiency is high in this study population who were of Middle Eastern origin highlighting the significance of the situation in this population in New Zealand. Monthly intake of 100,000 IU vitamin D for 6 months is more effective than 50,000 IU in achieving serum 25(OH)D concentrations of 75 nmol/L, and is associated with no adverse events. However, supplementation with this dose does not ensure a serum 25(OH)D concentration of 75 nmol/L or more in all people. Serum 25(OH)D

response to supplementation was predicted by the dose of treatment, baseline levels of serum 25(OH)D and BFP. A better response to supplementation was observed in those taking the larger dose, having lower baseline levels or having lower BFP. Furthermore, the effect of season and dressing style on vitamin D status was obvious in the placebo group and in different dressing code categories, respectively, which was perhaps expected. By approaching summer (the end of study), serum 25(OH)D concentrations improved in the placebo group, indicating the seasonal effect, and slightly decreased in veiled women receiving vitamin D supplements, indicating the effect of dressing style. But the unexpected finding was a very large variability in serum 25(OH)D response to a fixed dose of vitamin D; this highlights the importance of follow up and measurements of serum 25(OH)D when supplementation is used in clinical practice.

Chapter 6: Executive Summary, Methodological Considerations and Recommendations

The objective of the Middle Eastern Women's Health study-Phase II was to determine the effect of monthly 50,000 IU or 100,000 IU vitamin D supplements on vitamin D status in pre-menopausal Middle Eastern women living in Auckland, New Zealand using a randomised placebo controlled design. Middle Eastern women were selected because of the low vitamin D status of this population, previously determined [17], and the lack of randomised trial determining the adequate dosing regimens in this immigrant group in New Zealand.

The evidence of vitamin D deficiency (<25 nmol/L) and insufficiency (25-50 nmol/L) in this population has been recently determined [17]. The results of current study also revealed that 60.0% and 37.0% of women had serum 25(OH)D concentrations <50 nmol/L and <75 nmol/L, respectively. Our results showed that a monthly intake of 100,000 IU vitamin D is more effective than 50,000 IU in achieving serum 25(OH)D concentrations of 75 nmol/L or more in winter and summer months while no sign of hypercalaemia or hypervitaminosis D is detected. Approximately 66.0% of women receiving monthly 100,000 IU achieved the cut off levels of ≥ 75 nmol/L after 6 months, but the proportion in the 50,000 IU group was half that of the 100,000 IU group. These findings demonstrate that doses larger than monthly 100,000 IU is required to shift Middle Eastern women's vitamin D status to ≥ 75 nmol/L. Consequently, the concern about the 2012 "Consensus Statement on Vitamin D and Sun Exposure in New Zealand" [29] regarding the adequacy of monthly dose of 50,000 IU for all at risk populations is highlighted here.

This study also demonstrated that response to supplementation varies widely, ranging from 1.0 to 80.0 nmol/L. A number of factors contributed to the variance in serum 25(OH)D response to vitamin D supplementation. Apart from the dose of treatment, baseline serum 25(OH)D concentration has consistently been significant determinant of serum 25(OH)D response to supplementation [7, 31, 32, 69, 269, 284-288], a finding confirmed by our study. Because hepatic hydroxylation is a saturable process, larger increases in serum 25(OH)D have been seen in subjects with lower baseline levels of serum 25(OH)D. To note, based on baseline serum 25(OH) levels of < 50 vs. ≥ 50 nmol/L, the mean changes in our study were not comparable to the values obtained from prediction equations from other studies [353] suggesting that response to a fixed dose of supplementation is not a direct function of baseline levels only, and other factors such as BFP may also contribute.

Vitamin D is a fat soluble vitamin, and high doses of supplementation are associated with a greater accumulation of cholecalciferol in adipose tissue and its slower release into the

circulation. Accordingly, we demonstrated that compared with BFP of > 35%, having BFP of ≤ 35% is associated with a greater incremental change in serum 25(OH)D concentrations.

We also demonstrated that dressing style had an effect on the pattern of change in serum 25(OH)D concentrations over time which could be partly attributed to the effect of sun exposure. While mean serum 25(OH)D concentration of women with Islamic dressing code decreased slightly from 3-months to 6-months visit (approaching summer), it increased slightly in women with non-Islamic dressing code. Women with Islamic dressing code had lower mean serum 25(OH)D concentration in the placebo group at all time points compared with those with non-Islamic dressing code. These findings highlight the importance of taking sun exposure indices such as dressing style and season into account when evaluating serum 25(OH)D response to a fixed dose.

These variables accounted for some of the variance in the change in serum 25(OH)D concentrations for this sample, leaving more than 40.0% of the variability to unknown factors. Response to supplementation is also genetically determined. The largest between-genotype group differences in serum 25(OH)D concentrations have been reported to be more apparent in summer or when the vitamin D load is high [75]. As such, one can hypothesize that the larger variation in response to supplementation in the current study's larger dose arm where the vitamin D load is high is due to genetic variation.

Due to the low serum 25(OH)D concentrations, the large BFP, the low physical activity level and the conservative clothing style of this study population, supplementation with monthly 100,000 IU for 6 months may be the most effective means of combating vitamin D deficiency/insufficiency in this population. However, it is important to investigate the reasons for such large variability in serum 25(OH)D response to supplementation with a fixed dose of vitamin D while controlling for known factors, and to examine the efficacy of such dose over a longer follow up period or the efficacy of larger doses over a shorter period of time.

In conclusion, the prevalence of vitamin D insufficiency was high in this study population who were of Middle Eastern origin. The larger dose, 100,000 IU per month, was more effective in achieving serum 25(OH)D levels of ≥ 75 nmol/L. Doses larger than 100,000 IU per month is perhaps required to shift vitamin D status of majority of women to ≥ 75 nmol/L. Serum 25(OH)D response to supplementation was predicted by the dos, baseline serum 25(OH)D concentrations and BFP. The unexpected findings were the very large variability in

serum 25(OH)D response to supplementation and the number of women who did not respond or responded poorly to supplementation.

Hypothesis Outcomes

Our study revealed that:

Hypothesis 1: We rejected that supplementation with monthly 50,000 IU vitamin D_3 for 6 months is inadequate for increasing serum 25(OH)D concentrations ≥ 50 nmol/L in the majority of women. But we accepted that this dose is inadequate for increasing serum 25(OH)D concentrations ≥ 75 nmol/L in the majority of women. We also accepted that supplementation with monthly 100,000 IU vitamin D_3 for 6 months is adequate for increasing serum 25(OH)D concentrations ≥ 50 and ≥ 75 nmol/L in the majority of women.

Hypothesis 2: We accepted that both doses are safe and do not increase serum calcium levels to ≥ 2.7 mmol/L nor serum 25(OH)D levels to >225 nmol/L.

Hypothesis 3: We accepted that both lower baseline serum 25(OH)D concentration and lower BFP are associated with greater serum 25(OH)D response to supplementation with the given doses. However, we rejected that dietary calcium intake has an effect on serum 25(OH)D response to supplementation with given doses. With regard to the use of contraceptives containing oestrogen, we can neither reject nor accept that it has an effect on response to supplementation (due to small number of women using contraceptives containing oestrogen).

Methodological consideration

Strengths

The main strength of this study lies in its design and study population. This was a double-blinded, randomised, placebo controlled trial, which is the gold standard for clinical evidence. The study was sufficiently powered (sample size) to detect a difference in the primary outcome, and was of sufficient duration to investigate the effect of supplementation on serum 25(OH)D concentration. All blood samples (from 3 time points) were sent in one batch to one lab (North Shore Hospital, Auckland, New Zealand), and the analysis of serum 25(OH)D was completed to a high standard, providing accurate information regarding the adequacy of the two supplement doses. Furthermore, the inclusion of placebo and the measurement of serum 25(OH)D concentrations at 3 time points helped us to determine the effect of season and to

control for it while interpreting the results. In addition, the anthropometric and body composition measurements were all carried out according to standardized procedures. A further strength was the relatively high rate of compliance and the very few drop outs (only one subject).

To our knowledge, this is the first study of this nature to be conducted in the Middle Eastern women living in Auckland, New Zealand. These women are known to have a very conservative clothing style and as such the impact of sun exposure on vitamin D status is limited. Accordingly, the change in serum 25(OH)D concentrations was mainly attributed to the supplement doses. Furthermore, this study gave insight into this relatively new migrant population and showed that majority, if not all, Middle Eastern women may need monthly doses of 100,000 IU or more to meet the recommended level of serum 25(OH)D (≥ 75 nmol/L) and highlighted the significance of factors affecting the response to supplementation.

Limitations

Given that our study population were of Middle Eastern origin and women were recruited regardless of their dressing style, we had two dressing code categories (Islamic and non-Islamic). We had to stratify dosing groups by dressing style because we found a significant interaction between treatment group and dressing style. This resulted in smaller sample size within each treatment groups. This small sample size may have reduced the power of the statistical results especially in our situation where serum 25(OH)D response to a fixed dose varied widely. However, this stratification helped us to determine the effect of dermal synthesis of vitamin D and season on vitamin D status of this population and to consider this effect in interpretation of results regarding the primary outcome.

Further limitation is the assessment of dietary calcium intake in this study. Although a 4-day diet record is a recommended method of assessing dietary calcium intake, approximately 13% of participants did not return their baseline diet records, and most of the returned diaries were either incomplete, returned very late or in other language other than English such as Persian. Thus, the calcium intake of this population might not be highly accurate. Some of our study population were not fluent in English and needed some kind of translation. Of the MELAA groups, Middle Eastern population had the greatest proportion that was not conversant in English, with 50% speaking Arabic [16]. This is a limitation because the researcher burden may increase.

Furthermore, because this study was conducted in healthy premenopausal Middle Eastern women living in Auckland, New Zealand, the results may not apply to other ethnic groups, those living in other regions or countries or those with disease.

Recommendations for Future Research

1) Investigating the long-term safety and adequacy of supplementation with monthly 100,000 IU or more,
2) Determining that supplementation with monthly 100,000 IU for 6 months for how long can ascertain the adequate levels of serum 25(OH)D,
3) Conducting RCT to investigate the effect of low baseline serum 25(OH)D concentration and BFP on response to supplementation,
4) Conducting RCT to address vitamin D deficiency and some already known health conditions in this population,
5) Exploring the genetic determinants of vitamin D status in this population
6) Conducting RCT to investigate the effect of genetic determinants on serum 25(OH)D response to supplementation

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Appendices

Appendix 1-Information for Participants

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MEWH Study-Phase II

The Effect of Monthly 50,000 IU or 100,000 IU Vitamin D Supplements on Vitamin D Status in Pre-menopausal Middle Eastern Women Living in Auckland

Information for Participants

You are invited to take part in an intervention trial will determine the effect of supplementation with monthly 50,000 IU or 100,000 IU vitamin D for 6 months on vitamin D status in pre-menopausal Middle Eastern women living in Auckland.

This information sheet provides you with the background to the research and other important details about what is involved, so please read carefully before deciding whether or not to participate.

The researchers are as follow:

Study manager: Hajar Mazahery, MSc student Institute of Food, Nutrition and Human Health, Massey University Phone: 447 3538 Mobile: 0226872997 Email: h.mazahery@hotmail.com	**Principal investigator:** Dr Pamela von Hurst Institute of Food, Nutrition and Human Health, Massey University Phone: 414 0800 ext 41205 Email: P.R.vonHurst@massey.ac.nz

Why is the research important?

Vitamin D deficiency is linked to poor bone health and muscle strength and other diseases such as cardiovascular diseases and diabetes. These conditions can affect people's short- and long-term health and well being. You may be aware that Middle Eastern women are at

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increased risk of vitamin D deficiency/insufficiency. Because of the many lifestyle risk factors such as conservative clothing style, and deliberate sun exposure avoidance, you may not be getting enough sun exposure for adequate vitamin D production. Vitamin D supplements (50,000 IU) are prescribed by General Practitioners to correct vitamin D deficiency in at risk groups. However, no research has investigated whether this dose of vitamin D supplement is useful for vitamin D deficiency treatment in Middle Eastern women or larger doses is needed, and we would like to invite you to help us determine this.

Additionally, we are interested in looking at factors that might affect a person's response to vitamin D, such as body composition, contraceptive use and dietary calcium intake.

Who are we looking for?

We are looking for 66 Middle Eastern women to participate in this study. To fit in to our study you should:

- Have been born in Middle East (or have parents or grandparents who were born there)
- Be female, 20 years of age or older, and in pre-menopausal state
- Not be pregnant, breastfeeding or planning to be in the near future
- Have no bleeding disorders, kidney, liver or gastro-intestinal problems or diseases
- Not having hypercalcaemia and hypercalciuria
- Not taking blood thinning medication and/or other medications such as glucocorticoids and antileptic medicines

What is going to happen?

After you have read and considered the information provided in this information sheet and have decided to take part in this study, you will be asked to complete a questionnaire to ensure that you fit the inclusion criteria. If you do meet the criteria, you will be invited to attend an appointment at the Human Nutrition Research Unit (HNRU) on the Albany

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campus at Massey University. During the study period, you will be required to visit this unit on 3 occasions; at the beginning, 3-months, and 6-months (end of study). The first and the final visits should take approximately one hour, and the 3-months visit 30 minutes.

Before each visit, you will receive an email or a text message informing your appointment time. We will provide you with a $ 20 petrol voucher to cover your travel costs. Your involvement in this study will include the following:

- You will have a blood sample taken to measure your vitamin D levels and other biomarkers related to the actions of vitamin D.
- You will complete questionnaires about your details, demographics, lifestyle change and medical history.
- Your weight and height will also be measured. Your body composition will also be measured using Bioelectrical Impedence Analysis (BIA, a picture of this instrument is attached to this document). This is a very simple procedure where you stand on a set of foot plates similar to weighing scales, and hold a pair of handles. To allow us to accurately measure your body composition, you should avoid the followings:
  - having food within 2 hours of testing.
  - having caffeinated drinks within 4 hours of testing.
  - doing exercise within 4 hours of testing.
  - taking shower/bath/sauna within 4 hours of testing.
  - drinking alcohol within 24 hours of testing.
  - donating blood within 2 days of testing.
- Your skin colour will be determined
- Your blood pressure will be measured using an automatic digital blood pressure monitor.
- You will complete a four day food dairy including one weekend day within the first week of the first visit. You will be provided with a stamped, addressed envelope to send the four day food diary to the Nutrition Research Unit at Massey University.

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At the first visit, you will be randomly assigned to either the treatments (vitamin D) or placebo group. There will be twice as many people in the vitamin D groups than the placebo group. All participants will take two tablets once a month. Neither you nor the researchers will know which kind of supplement you are taking.

You will be given the opportunity to ask any questions you may have throughout the study.

Throughout the study period, you will be sent a reminder to take your supplement once month.

Blood Samples

All blood samples will be taken by a trained phlebotomist, processed in the onsite laboratory facilities at the HNRU and frozen at – 80°C until they are sent to an accredited laboratory for analysis. Some of the samples may not be analysed immediately after the study and will remain stored in the meantime. Additional analyses of the samples may be undertaken as more information becomes known, but any further analyses will only be conducted as part of this research project.

Risks and Benefits

There are no personal risks to your health, but as vitamin D assists calcium absorption, there is a small risk that increasing vitamin D intake could raise calcium in the blood to higher than normal levels. However, the dose you will take has been safely used in several trials with no negative effects. As a precaution we will be closely monitoring your calcium levels during the study. If we identify any possible abnormalities we will withdraw you from the study and advise you to consult your General Practitioner for further investigation.

The principal benefit of taking part in this study is that you contribute to our better understanding of vitamin D dosages required for optimising serum 25(OH)D concentrations.

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We know that vitamin D deficiency is a real problem in Middle Eastern women, and we need to know more about factors affecting the response to vitamin D supplements in this population. In addition, there will be no charges made for any of the tests that you undertake.

Participant's Right

You are under no obligation to accept this invitation to take part in this study. If you decide to participate, you have the right to:

- Decline to answer any particular question;
- Withdraw from this study (at any time without having to give a reason);
- Ask any questions about this study at any time during participation;
- Provide information on the understanding that your name will not be used unless you give permission to the researcher;
- Be given access to a summary of the project findings when it is concluded.

General

If you want to discuss any aspect of this study you should contact the Principal investigator, Hajar Mazahery (Tel: 447 3538; Mob: 0226872997; Email: h.mazahery@hotmail.com)

If you have any queries or concerns regarding your rights as a participant in this study you may wish to contact Health and Disability Advocate, telephone 0800 555 050

At the conclusion of this study we will provide a report of the outcome to those involved in this study and we will send the results to you by mail.

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Privacy of Data

No material which could personally identify you would be used in any reports on this study. Information collected from you in this study will be stored securely in the Department of Nutrition and will only be available to study personnel. When this study is completed, all material will be destroyed.

Committee Approval Statement

This project has been reviewed and approved by the health and Disability Ethics Committee: Reference 13/STH/40.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Please feel free to contact the researcher if you have any questions about this study

Thank you for considering participating in this study!

Appendix 2-Consent Form

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Institute of Food, Nutrition and Human Health
Massey University
Private Bag 102-904
North Shore Mail Centre
Albany, Auckland
New Zealand

T 09 414 0800
F 09 443 9640

MEWH Study – Phase II

The Effect of Monthly 50,000 IU or 100,000 IU Vitamin D Supplements on Serum 25(OH)D Concentrations in Pre-menopausal Middle Eastern Women Living in Auckland

PARTICIPANT CONSENT FORM

This consent form will be held for a period of five (5) years

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to participate in this study under the conditions set out in the Information Sheet.

Signature: ..... **Date:** .....

Full Name - printed .....

Appendix 3-Details, Demographics, Medical History and Fitzpatrick Skin Colour Questionnaire

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Identification Number .....

Date .....

MEWH Study – Phase II

The effect of monthly 50,000 IU or 100,000 IU vitamin D supplements on serum 25(OH)D levels in premenopausal Middle Eastern women living in Auckland

This questionnaire asks you about your details, demographics, medical history and skin colour

Thank you for participating in this study, if you have any questions please feel free to discuss them with the researcher.

Investigator: Hajar Mazahery

Primary supervisor: Dr Pamela von Hurst

All information you provide will remain strictly confidential

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Identification Number. .....

Participant Details

First Name

.....

Family Name

.....

Name you would like to be called by

.....

Address

.....

.....

Phone (home)

.....

Phone (mobile)

.....

Email

.....

Family Doctor/General Practitioner (GP)

.....

Address **Phone**

.....

This section of the form will be detached once it is completed to ensure confidentiality.

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Identification Number . .....

Participant Demographics

Date of birth

Marital status

Years of education

Highest qualification

Occupation (your job)

Country of birth

Years in New Zealand

Parent's ethnic origin

Mother

Father

How would you describe your skin colour? (Please √ one)

☐ Fair ☐ Medium ☐ Olive ☐ Dark/Brown

☐ Other Please specify: Other ..... Please specify:

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Identification Number. .....

Medical History

Have you ever been diagnosed with any of the following conditions?

Medical condition	√ if "Yes"	Comments
High blood pressure	☐	
Heart disease	☐	
Angina	☐	
Stroke	☐	
Cancer	☐	
Osteoporosis	☐	
Rheumatoid arthritis	☐	
Psoriasis	☐	
Tuberculosis	☐	
Diabetes	☐	
Hypothyroidism	☐	
Hyperthyroidism	☐	
Depression	☐	
Other	☐	**Please specify:**

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Identification Number. .....

Medication & Supplementation

Are you taking any form of hormonal contraceptives (oral, patch, shot, or ring)? If so, which brand?

.....

.....

If yes, have you been on this contraceptive (birth control) for at least four months?

☐ Yes ☐ No

Are you taking any form of medication or supplements? Please list

.....

.....

.....

Do you smoke cigarettes? ☐ Yes ☐ No

If yes, how many per day.....

When was the first day of your last period? .....

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Identification Number .....

Skin Colour (Fitzpatrick Skin Type)

Please answer the following questions about your skin colour (Circle one number).

What is your natural eye colour?

1. Light colours
2. Blue, grey or green
3. Dark
4. Brown
5. Black

What is your natural hair colour?

1. Sandy red
2. Blond
3. Chestnut or dark blond
4. Brown
5. Black

What is the colour of your unexposed skin?

1. Reddish
2. Pale
3. Beige or olive
4. Brown
5. Dark brown

Do you have freckles on unexposed areas?

1. Many
2. Several
3. Few
4. Rare
5. None

What happens when you stay too long in the sun?

1. Painful blisters, peeling
2. Mild blisters, peeling
3. Burn, mild peeling
4. Rare
5. Non burning

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Identification Number .....

Do you turn brown within several hours of sun exposure?

1. Never
2. Seldom
3. Sometimes
4. Often
5. Always

To what degree do you turn brown?

1. Never
2. Light tan
3. Medium tan
4. Dark tan
5. Deep dark

Is your face sensitive to the sun?

1. Very sensitive
2. Sensitive
3. Sometimes
4. Resistant
5. Never have a problem

How often do you tan?

1. Never
2. Seldom
3. Sometimes
4. Often
5. Always

When was your last tan?

1. + 3 months ago
2. 2-3 months ago
3. 1-2 months ago
4. Weeks ago
5. Days

Appendix 4- Change of Lifestyle Questionnaire

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Identification Number. .....

Change of Lifestyle

OVER THE PAST 2-3 MONTHS (SINCE THE LAST VISIT):

Side effect:

Did you experience any side effects from tablets given to you in this study?

☐ Yes ☐ No

If yes, please describe:

.....

.....

Change in contraceptive use:

Do you use a contraceptive pill, patch, shot, or ring?

☐ Yes ☐ No

If yes, has your prescription changed since your last visit?

☐ Yes ☐ No

When did this change happen?

.....

Which brand are you using now?

.....

Have you stopped using a contraceptive pill, patch, shot, or ring since your last visit?

☐ Yes ☐ No

If yes, when did you stop using it?

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Identification Number. .....

Change in medication and supplement use:

Please list **all medications** (including antibiotics) that you took since your last visit?

.....

.....

.....

Apart from study supplements, did you take any **vitamins**, **minerals**, **herbal supplements** or **probiotics** since your last visit?

☐ Yes ☐ No

If yes, which ones, how much, and how often did you take them?

.....

.....

.....

Appendix 5-New Zealand Physical Activity Questionnaire-Short Form

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Identification Number .....

Date .....

New Zealand Physical Activity Questionnaire – Short Form

I am going to ask you about the time you spent being physically active in the last 7 days, from last.....to yesterday. Do not include activity undertaken today.

By 'active' I mean doing anything using your muscles.

Think about activities at work, school or home, getting from place to place, and any activities you did for excercise, sport, recreation or leisure.

'I will ask you separately about brisk walking, moderate activities and vigorous activities.'

Ask questions 1-7 (8 is optional)

Walking

1. During the last 7 days, on how many days did you **walk at a brisk pace** – a brisk pace is a pace at which you are breathing harder than normal? This includes walking at work or school, while getting from place to place, at home and at any activities that you did solely for recreation, sport excersice or leisure.

   Think about brisk walking done at least 10 minutes at a time.

   ☐ .....days per week (GO TO 2)

   ☐ None (GO TO 3)

2. How much did you typically spend walking at a brisk pace on each of those days?

   ☐ .....hours.....minutes

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Moderate physical activity

3. During the last 7 days, on how many days did you **moderate** physical activities? Moderate activities make you breathe harder than normal**, but only a little** – like carrying light loads, bicycling at a regular pace, or other activities like those shown on this care (**Showcard 1- Moderate Physical Activity**). Do not include walking of any kind.

   Think about those activities done at least 10 minutes at a time.

   ☐.....days per week (GO TO 4)

   ☐ None (GO TO 5)

4. How much did you typically spend walking at a brisk pace on each of those days?

   ☐.....hours..... minutes

Vigorous physical activity

5. During the last 7 days, on how many days did you **vigorous physical activities**? 'Vigorous' activities make you breathe **a lot** harder than normal ('huff and puff') – like heavy lifting, digging, aerobic, fast bicycling or other activities like those shown this care (**Showcard 2- Vigorous Physical Activity**)?

   Think only about those activities done at least 10 minutes at a time.

   ☐.....days per week (GO TO 6)

   ☐ None (GO TO 7)

6. How much did you typically spend walking at a brisk pace on each of those days?

   ☐.....hours .....minutes

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Frequency of Activity

7. Thinking about all your activities over the last 7 days (including brisk walking), on how many days did you engage in:

   - At least 30 minutes of moderate activity (including brisk walking) that made you breathe a little harder than normal, OR
   - At least 15 minutes of vigorous activity that made you breathe a lot harder than normal (huff and puff)?

   ☐ days per week

   ☐ None

Stage of Change

Note, this question is optional

8. Describe your regular physical activity over the past six months. Regular physical activity means at least 15 minutes of vigorous activity (makes you huff and puff) or 30 minutes of moderate activity (makes you breathe slightly harder than normal) each day for 5 or more days each week. Include brisk walking.

   ☐ I am not regularly physically active and do not intend to be so in the next 6 months

   ☐ I am not regularly physically active but am thinking about starting in the next 6 months

   ☐ I do some physical activity but not enough to meet the description of regular physical activity

   ☐ I am regularly physically active but only began in the last 6 months

   ☐ I am regularly physically active and have been so for longer than 6 months

Notes:

Appendix 6-Four Day Food Diary

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Identification Number .....

Date .....

MEWH Study-Phase II

The effect of monthly 50,000 IU or 100,000 IU vitamin D supplements on serum 25(OH)D levels in premenopausal Middle Eastern women living in Auckland

4-day Food Diary

When you have completed this diary please return it to Hajar Mazahery.

A stamped, addressed envelope is provided for this purpose.

Principal investigator: Hajar Mazahery
Supervisor: Dr Pamela von Hurst

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What to do?

- Complete this diary for four days, one of which should be a weekend day
- If possible record food at the time of eating or just after – try to avoid doing it from memory at the end of the day
- Include all meals, snacks, and drinks, even tap water
- Include anything you have added to foods such as sauces, gravies, spreads, dressings, etc
- Use a new line for each food and drink. You can use more than one line for food or drink. See the example in the first two pages
- Use as many pages of the booklet as you need.

<u>Describing Food and Drinks</u>

- Provide as much detail as possible about the type of food eaten. For example brand names and varieties/types of food.

Example:

Cheese – Mainland, Edam

Milk – Meadowfresh, Calci-trim

Breakfast cereal – Sanitarium, Natural Muesli

Pasta – Wholegrain

- Give details of all the cooking methods used. For example, fried, grilled, baked, poached, boiled
- When using rice and lentils etc, please note if your measurement is taken before or after cooking
- Record recipes of home-prepared dishes where possible and the proportion of the dish you ate. There are blank pages for you to add recipes or additional information

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Recording the amounts of food you eat

It is important to also record the quantity of each food and drink consumed. This can be done in several ways.

- By using household measures – for example, cups, teaspoons, and table spoons. E.g. 1 cup of frozen peas, 1 heaped teaspoon of sugar.
- By weight marked on the packages – for example, a 425 g tin of baked beans, a 32 g cereal bar, 600 ml Coke.
- Weighing the food – this is an ideal way to get an accurate idea of the quantity of food eaten, in particular for foods such as meat, fruits, vegetables and cheese.
- For bread – describe the size of the slices of bread. E.g. sandwich, medium, toast
- Using comparisons – E.g. meat equal to the size of a pack of cards, a scoop of ice cream, the size of hen's egg.
-

Eat as normally as possible – do not adjust what you would normally eat just because you are keeping a diet record and be honest!

All information provided in this diary will be treated with the strictest confidence. No one outside this study will have access to this

Thank you for taking part in this important study.
We are really grateful for the time you are giving.

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Date..... DAY 1

Time food was eaten	**Complete description of food (food and beverage name, brand, variety, preparation method)**	**Amount consumed (units, measures, weight)**
Example 7.55 am	Bread, toasted (Budget)	2 sandwich slices
" "	Cheese, Feta (O'dior)	35 gr
" "	Milk (Meadow fresh, Calci-trim)	One glass (275 ml)
10 am	Raw apple (gala)	Ate all of apple except the core, Apple was 125 gr

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DAY 1 continued

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed (units, measures, weight)

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Recipes DAY 1

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Date..... ***DAY 1***

Time food was eaten	**Complete description of food (food and beverage name, brand, variety, preparation method)**	**Amount consumed (units, measures, weight)**

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DAY 1 continued

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed (units, measures, weight)

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Recipes DAY 1

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Date..... ***DAY 2***

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed (units, measures, weight)

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DAY 2 continued

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed (units, measures, weight)

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Recipes DAY 2

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Date..... ***DAY 3***

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed (units, measures, weight)

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DAY 3 continued

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed (units, measures, weight)

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Recipes DAY 3

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Date..... ***DAY 4***

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed (units, measures, weight)

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DAY 4 continued

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed (units, measures, weight)

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Recipes DAY 4

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Thank you for your help with the MEWH Study – Phase II