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OPTIMAL DOSE OF VITAMIN D REPLACEMENT: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS FROM THE MIDDLE EAST AND NORTH AFRICA

by

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Title: Optimal Dose of Vitamin D Replacement: A systematic Review and Meta-analysis of Randomized Controlled Trials from the Middle East and North Africa

Background: Hypovitaminosis D, defined as a 25-hydroxyvitamin D (25(OH)D) level <20 ng/ml, is highly prevalent worldwide, more so in the Middle East and North Africa (MENA) region. Relevant risk factors in adults, specific to the MENA region, include multiparity, clothing style and veiling, season, socio-economic status, urban living and the lack of governmental regulation of food fortification. The latest Institute of Medicine (IOM) recommendations for vitamin D supplementation targeted populations from North America, and may not necessarily apply to the MENA region. The WHO does not have any current guidelines on this topic, with the exception of guidelines in pregnancy.

Objectives: The objectives of this systematic review and meta-analysis are: (1) determine the mean difference in 25(OH)D level reached with low (< 800 IU), moderate (800-2,000 IU) or high (>2,000 IU) daily dose of vitamin D in subjects in MENA countries, by age and reproductive status, and estimate the proportion of subjects who reach a mean 25(OH) D level ≥ 20 ng/ml in above treatment groups; (2) determine the effect of vitamin D supplementation on other outcomes: fracture rates, mortality, hypercalcemia-hypercalciuria, bone mineral density, kidney stones and muscle strength; (3) describe the vitamin D dose response and identify the predictors of 25(OH)D level achieved following supplementation.

Search methodology: A systematic search for English and Non-English articles was conducted using Medline, PubMed, the Cochrane Controlled Trials Register, EMBASE, Popline, Global Health Library, Index Medicus for WHO Eastern Mediterranean without any time restriction; search was updated in July 2015. Additional studies were identified on ClinicalTrial.gov and the WHO registry for clinical trial. Authors were contacted for unpublished data.

Eligibility criteria: We considered randomized clinical trials comparing different doses of oral vitamin D supplementation or placebo in MENA countries, of both genders and all age categories, including pregnant women.

Data collection and analysis: References retrieved were reviewed in duplicate by 2 independent reviewers. We abstracted data, and assessed risk of bias using the Cochrane risk of bias tool in duplicate and independently. We calculated the weighted mean difference (WMD) and 95% Confidence Interval (CI) of 25(OH)D level reached between any two treatment arms (or treatment arm versus placebo), and in each age category, using RevMan version 5.3. We conducted a multivariate meta-regression to identify the significant predictors of 25(OH)D levels following intervention, on STATA version 12.

Results: We identified 25 studies as eligible: 2 in elderly, 13 in adults, 6 in pregnant women, 3 in children, 1 in infants.

In adults, the WMD in 25(OH)D level achieved was 18.3 (14.12;22.49) ng/ml, comparing a high dose (weighted mean dose of 4,856 IU/d) to placebo, and 14.7 (4.57;24.89) ng/ml, comparing an intermediate dose (weighted intermediate dose of 1,750 IU daily) to placebo. Accordingly, 89% and 72%, in the high and intermediate dose groups, respectively, reached the IOM defined desirable level for 25(OH)D level of 20 ng/ml.

In pregnant women, the WMD in 25(OH)D level was 7.89 (4.96-10.81) ng/ml comparing an intermediate (weighted mean dose of 1,800 IU/d) to a low dose (weighted mean dose 300 IU/d). The WMD was 8.5 (5.07-11.93) ng/ml, comparing a high (weighted mean of 3,700 IU/d) to an intermediate dose, and it was 17.27 (15.8-18.73) ng/ml, comparing a high to a low dose. The proportion of pregnant women reaching a 25(OH)D level ≥ 20 ng/ml was 94 %,73 % and 43% in the high, intermediate, and low dose groups, respectively.

In children and adolescents, comparing an intermediate dose (weighted mean dose of 1,870 IU/d) to placebo yielded a significant WMD in 25(OH)D level of 15.77 (8.68;22.87) ng/ml, and 73% reached the desirable 25(OH)D level. A low dose of 200 IU/d did not increase 25(OH)D level significantly, compared to placebo.

Data on the effect of vitamin D supplementation on skeletal and extra-skeletal outcomes and surrogate markers were limited. Interestingly, vitamin D supplementation, even high doses, did not result in a significant change in serum calcium level. An intermediate vitamin D dose in children, compared to placebo, reduced PTH level significantly, WMD -7.00 [-7.38, -6.62] pg/ml.

The meta-regression analysis included 13 placebo arms and 17 intervention arms (65% of which were high dose), in adults and elderly. It showed that vitamin D dose and the baseline 25(OH)D level are the most robust predictors of the 25(OH)D level achieved following intervention. The average increase in 25(OH)D level was 0.44 ng/ml per 100 IU/d vitamin D, and 0.77 ng/ml for each increase in baseline 25(OH)D level by 1 ng/ml.

Conclusion: The IOM vitamin D recommended dietary allowance (600-800 IU/d across all age categories) is not sufficient to allow to the majority of the population in our region to reach the target of 20 ng/ml. Doses that are up to 2-3 folds higher may be required to reach desirable levels. There is a need for additional long term safety data using such doses. Our result will inform region specific vitamin D replacement guidelines in various age groups.

ILLUSTRATIONS

TABLES

ABBREVIATIONS

25(OH)D	25-hydroxyvitamin D
BMD	Bone Mineral Density
BMI	Body Mass Index
CI	Confidence Interval
EIA	Enzyme Immuno- Assay
ELISA	Enzyme Linked Immuno-Sorbent Assay
ES	Endocrine Society
GRADE	Grading of Recommendations, Assessment, Development and Evaluation
HR	Hazard Ratio
HOMA-IR	Homeostatic Model Assessment for Insulin resistance.
HOMA-B	Homeostatic Model Assessment for ß-cell function
HPLC	High Pressure Liquid Chromatography
ICTRP	WHO International Clinical Trials Registry
IMEMR	Index Medicus for WHO Eastern Mediterranean
IOF	International Osteoporosis Foundation
IOM	Institute Of Medicine
Ln	Natural Log
LCMS	Liquid Chromatography Mass Spectrometry
MENA	Middle East and North Africa
MS	Multiple Sclerosis
MAACE	Major Adverse Cardiac and Cerebro-vascular events
NAFLD	Non-Alcoholic Fatty Liver Disease
NICE	National Institute for Health and Clinical Excellence
NYHA	New York Heart Association

PCOS	Polycystic Ovaries Syndrome
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analysis
PTH	Parathyroid hormone
RCT	Randomized Controlled Trials
RDA	Recommended Dietary Allowance
RR	Relative Risk
Sd	Standard Deviation
Sdp	Pooled Standard Deviation
SE	Standard Error
SLE	Systemic Lupus Erythematosis
SOF	Study of Osteoporotic Fracture
UAE	United Arab Emirates
UVB	Ultra Violet B
USPTF	US Preventive Services Task Force
VDR	Vitamin D Receptor
WMD	Weighted Mean Difference

CONTENTS

CHAPTER 1

INTRODUCTION

1. 1. Vitamin D physiology and vitamin D nutritional status

Sun exposure (Ultraviolet B (UVB) radiation) constitutes the major source of vitamin D, implying that season, latitude, time of exposure to sunlight and skin pigmentation significantly affect vitamin D production at the skin, and thus 25(OH)D levels (1). Only few foods contain vitamin D, namely cod liver oil, salmon, sardines and egg yolk (2).

Vitamin D is a pre-hormone. It undergoes hydroxylation at the liver (through 25-hydroxylase) and the kidneys (through 1α-hydroxylase) to produce the active form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), known as calcitriol. 25-hydroxyvitamin D (25(OH)D) and $1,25(OH)_2D$ are both inactivated by 24-hydroxylase. 25(OH)D reflects vitamin D stores and therefore, it is the best indicator of vitamin D nutritional status (3).

Vitamin D can be supplemented, as ergocalciferol (D2) or cholecalciferol (D3). These 2 preparations differ by the composition of their side chain and may differ slightly in their effect on vitamin D status. Although the superiority of vitamin D3 in raising serum 25(OH)D levels, compared to vitamin D2, has been debated in several trials (4-12), it was most recently confirmed in a meta-analysis by Tripkovic et al (13). The latter showed a weighted mean difference of 15% in the increase in 25(OH)D level, favoring D3 form, compared to D2 form (13). Enteral and parenteral preparations are available, with various dosage frequencies. The data on the difference between various dosing and frequency regimens on efficacy and safety is inconclusive (14-16). In one study conducted in elderly following hip fracture, and comparing daily, weekly and monthly dosing frequencies of the same equivalent daily dose of vitamin D,

the three regimens were found to be equally effective in achieving comparable 25(OH)D levels (17).

1.2. Prevalence and causes of hypovitaminosis D

Hypovitaminosis D is a worldwide problem (18, 19). While the highest mean 25-hydroxyvitamin D (25(OH)D) levels in adults and elderly are observed in North America, Asia Pacific and Europe (mean 25(OH)D level range 20.4- 28.9 ng/ml), the Middle East and North Africa (MENA) region, despite being labeled as a "sunny region", registers the lowest values, with mean 25(OH)D levels of 13.6-15.2 ng/ml for the same age category (19, 20). Therefore, more than 50% of the adult population in the MENA region has 25(OH)D levels below 20 ng/ml and indeed, a larger proportion has levels less than 30 ng/ml (21).

In adults, the classical risk factors for hypovitaminosis D are related to the age, female gender, latitude and dark skin pigmentation (21). Other risk factors, specific to the MENA region, have been identified, including multiparity, clothing style and veiling, season, socio-economic status, urban living and the lack of governmental regulation of food fortification (21). In infants, prolonged breast feeding without adequate supplementation is a major determinant of hypovitaminosis D (21). Furthermore, polymorphism of key genes, encoding for enzymes on the metabolic pathway of vitamin D, vitamin D receptor or transport proteins, correlated with vitamin D status in cohorts from Europe, Canada and USA (22, 23). In the MENA region, in addition to genetic variants identified in Saudi rickets cases (21), a recent cohort in Lebanese elderly confirmed that Single Nucleotide Polymorphism (SNP) of vitamin D 25-hydroxlase (cytochrome P450 2R1-CYP2R1) predicted a significant variability in 25(OH)D levels (24).

1.3. Vitamin D deficiency and outcomes

Vitamin D has been traditionally labeled as an essential factor for maintaining calcium and bone metabolism, and ensuring skeletal integrity (3, 25). Recently, the discovery of the widespread distribution of Vitamin D Receptors (VDR) in different tissues explained various effects of vitamin D beyond the skeleton, such as modulating muscle function, a possible anti-carcinogenic effect, and a potential role in cardiovascular, infectious and auto-immune diseases (3, 26).

Therefore, vitamin D deficiency can be implicated in a myriad of skeletal and extra-skeletal consequences. Throughout life cycle, vitamin D deficiency leads to severely depressed calcium and phosphate absorption and secondary hyperparathyroidism, resulting in a compromised bone health with bone loss and increased risk of fractures (2, 27). Rickets in children and osteomalacia in adults are classic consequences of severe vitamin D deficiency (2). While the former leads to leg bowing and short stature, the latter is typically characterized by a throbbing bony pain and increased fracture risk secondary to under-mineralized bone (1, 2). On the other hand, observational studies have associated hypovitaminosis D with extra-skeletal outcomes, including increased risk of infections (tuberculosis and viral infections), cancer (colon cancer), auto-immune diseases (type 1 diabetes mellitus, multiple sclerosis, systemic lupus erythematosis (SLE)) and cardio-vascular diseases (hypertension, coronary artery disease and peripheral vascular disease) (28).

1.4. Impact of hypovitaminosis D in the MENA region

The MENA region registers the highest rates of rickets in children, ranging from 1-27%, rates that are at least 10-folds higher than those reported in Western countries (21). In adults,

several studies from Lebanon, Iran and Israel showed a negative correlation between 25(OH)D levels and parathyroid hormone levels (PTH) (27, 29, 30). In elderly Lebanese men and women, "vitamin D osteopathy" was described, resulting in bone loss, mediated by lean mass and PTH levels (27). The associations of hypovitaminosis D with non-classical outcomes have been also described in MENA countries, including increased risk of type I diabetes (Saudi Arabia, Qatar), metabolic syndrome (Iran), SLE (Iran, Egypt, Israel), and cardiovascular mortality (Iran, Israel) (21).

1.5. Vitamin D supplementation and outcomes

Meta-analyses of randomized controlled trials (RCTs) confirmed that vitamin D supplementation improves skeletal outcomes. In adults, vitamin D supplementation reduces hip fractures by 12-35% and non-vertebral fractures by 7-38%, depending on the vitamin D dose and the presence or absence of concomitant calcium (Table 1) (31-34). In addition, vitamin D supplementation reduces falls by 14-19% (35, 36) and may also improve muscle strength (37-39). In children, vitamin D supplementation improves bone mineral density (BMD) and lean mass (40) and was found to do so at the lumbar spine and total body in a meta-analysis, specifically in a subgroup of children with 25(OH)D level < 14 ng/ml (41). Data on vitamin D supplementation and fracture reduction is derived from Western populations and no data from Non-Western populations.

While the protective effect on musculo-skeletal health has been consistent, with only few exceptions in publications from the same group (42, 43), controversy has been emerging regarding the pleiotropic effects of vitamin D. A PubMed search (2010 -July 2015) revealed over 30 recently published systematic reviews of RCTs trying to illustrate the evidence on vitamin D

supplementation and various outcomes (Table 1). Vitamin D3 supplementation resulted in reduced mortality by 3-12% (44-46). Three meta-analyses assessed the effect of vitamin D supplementation on glucose control and insulin sensitivity (47-49). In one of them, a small but significant improvement in fasting glucose (a decrease by 0.3 mmol/l) and insulin resistance (a decrease by 0.25) was demonstrated in patients with diabetes or insulin resistance (47). Two meta-analysis showed no effect vitamin D supplementation on weight (50, 51). The effect of vitamin D supplementation on asthma and respiratory infections was inconsistent. While some showed a 59% reduction in asthma exacerbation (52) and 36-42% reduction in respiratory infections (53, 54), others did not detect any significant effect (55, 56). Three systematic reviews did not show any protective effect of vitamin D supplementation on cardio-vascular diseases (57-59). Vitamin D supplementation improved depressive symptoms in one meta-analysis (60). However, the effect was neutral in 2 others (61, 62). The effect of vitamin D supplementation on neonatal anthropometric outcomes seems promising, with possible improvement in birth weight and length (63). Conversely, the effect on maternal outcomes is still inconclusive (63).

Before deriving final conclusions regarding the impact of vitamin D supplementation on clinically important health outcomes, specifically non-classical outcomes, it is noteworthy that several of the aforementioned meta-analyses suffer from one or more limitations (Table 1). First, the change in 25(OH)D levels was not consistently documented in many of the trials included in these reviews. Indeed, failure to reach a desirable level may preclude the occurrence of any significant effect of vitamin D administration. Second, the duration of supplementation in some included studies was < 3 months. Since the half-life of vitamin D is of 2 weeks, supplementation for at least 10 weeks is required in order to reach a steady vitamin D status (64). Third, some of

the trials included used high infrequent dosing regimens (every 3, 6 or 12 months); regimens that might not maintain steady 25(OH)D levels for the whole study duration (65). Interestingly, a trial by Sanders et al., revealed an increased risk of hip fracture in patients given a single high vitamin D dose of 500,000 IU once yearly compared to placebo, during a 3.5-year follow-up period; the highest risk was in the first 3 months following dose administration (66). Fifth, the intervention of interest in several included trials was active or synthetic vitamin D. This form of supplementation is not the one recommended for the general population (67, 68). Finally, the quality of evidence derived from systematic reviews depends on the quality of the individual studies that are included. As shown in Table 1, the risk of bias of these systematic reviews, as provided by authors, extended form low to high risk. Indeed, all these aforementioned limitations affect the magnitude and the significance of the reported effects of vitamin D supplementation.

1.6. Vitamin D guidelines: desirable 25(OH)D level and recommended doses

Several scientific societies have issued guidelines on vitamin D supplementation in the general population (Appendix 1). All these guidelines targeted Western populations. The International Osteoporosis Foundation (IOF) was the only society to specifically recognize the Middle East as a region at high risk of hypovitaminosis D, and thus requiring higher supplementation doses (69).

The latest Institute of Medicine (IOM) – 2010, and Endocrine Society (ES) -2011 respective guidelines on vitamin D replacement in the general population, were based on a systematic review of literature (67, 68). The IOM guidelines targeted the general population. Conversely, the ES guidelines targeted subjects at high risk of vitamin D deficiency. Both guidelines defined the desirable 25(OH)D level and the dietary requirements of vitamin D in

each age category, based on data from observational and interventional studies, assessing the effect of vitamin D supplementation on mineral and skeletal outcomes (67, 68). Although both societies considered the same parameters to define desirable levels (3), the target levels differed from 20 ng/ml for the IOM to 30 ng/ml for the ES. While the National Osteoporosis Society adopted the IOM desirable 25(OH)D level of 20 ng/ml (70), Osteoporosis Canada and the IOF guidelines recommended a level of 30 ng/ml (Appendix 1) (69, 71).

The IOM defined the Recommended Dietary Allowance (RDA) for vitamin D, corresponding to the dose that would allow to ≥ 97.5% of participants to reach a desirable 25(OH)D level ≥ 20 ng/ml, in each age category. The ES guidelines have collected a wealth of RCTs conducted in each age category but suggested to use doses higher than those used in the cited RCTs in order to reach their target of 25(OH)D level (67). Appendix 2 details the studies that were used by the IOM and ES to derive their respective recommendations.

The recommended vitamin D doses varied widely between societies (Appendix 1); for example, in individuals >65 years, the recommended doses ranged between 400 IU/d, for the National Institute for Health and Clinical Excellence (NICE) (72) and Swiss guidelines (73), and 1,500 - 2,000 IU/d, for the ES guidelines (67) (Appendix 1). The U.S. Preventive Services Task Force (USPTF) guidelines, based on a systematic review and meta-analysis of vitamin D supplementation on skeletal outcomes, only recommended vitamin D supplementation for fall prevention, at doses around 800 IU/d, but not for fracture prevention (74).

1.7. Vitamin D U-Shaped response curve

While the interest was toward defining a desirable 25(OH)D cutoff, ensuring adequate bone mineralization and fracture risk reduction (67, 68), recent evidence from observational

studies on vitamin D status and extra-skeletal outcomes suggests a U-shaped response of vitamin D and a desirable safe range, rather than a single cutoff. Ensrud et al. concluded that, in older women of the Study of Osteoporotic Fractures (SOF) cohort, the risk of frailty increased when 25(OH)D level fell outside the range of 20-30 ng/ml (75). A wider range of 25(OH)D level of 18-60 ng/ml was found to decrease mortality, including breast cancer mortality, after adjustment for various predictors such as age, race, smoking status, disease severity (76-78). The incidence of major adverse cardiac and cerebrovascular events (MAACE) decreased following cardiac surgery in adults when 25(OH)D level ranged between 30 and 40 ng/ml (79). In Canadian children, respiratory wheezes were more common and lung function was depressed when 25(OH)D level was <20ng/ml or >30ng/ml (80). Although causal relationship between vitamin D level and various outcomes still needs to be confirmed, these observational data suggest that a 25(OH)D level range of 20-40 ng/ml seems safe, allowing optimal musculo-skeletal health, in addition to possible contribution to improvements in cardiovascular, cancer and mortality outcomes (3).

1.8. Vitamin D dose-response curve

The increase in 25(OH)D level in response to supplementation was assessed in several trials. Following vitamin D supplementation, 25(OH)D level increased by 0.37-1.2 ng/ml/mcg (81-83), and reached a plateau at 45 ng/ml, at doses ≥ 3,200 IU daily (84). In obese individuals, the increments are lower, estimated at 0.2 ng/ml/mcg (85, 86). Indeed, in addition to the vitamin D supplementation dose, other variables affect the increment in 25(OH)D level following intervention. Seven systematic reviews conducted a meta-regression analysis in order to assess the vitamin D dose response while adjusting for the predictors that significantly affect the

25(OH)D level achieved following intervention (68, 87-92) (Table 2). All these reviews included mostly trials conducted in Western countries and only few trials from Asia and Africa were included in 2 of them (87, 92). None of them addressed the MENA countries specifically. Autier et al, Shab-bidar et al and McNally et al reviews focused on specific age categories, adults or children/adolescents (89, 90, 92); the other papers included in the same analysis trials from all age categories (68, 87, 88, 91). The IOM and Cashamn's reports assessed the effect of total vitamin D intake, including dietary and supplements, and limited their analysis to studies conducted in winter season, where UVB radiation from sun exposure is reduced to minimal (68, 88). The other systematic reviews did not take into account the effect of dietary vitamin D on the 25(OH)D level reached. Indeed, this is related to the inconsistent reporting of such information in individual trials. With the exception of Seamens et al, Shab-bidar et al and McNally et al, logarithmic transformation of the vitamin D dose resulted in a better prediction model of the level reached, compared to the non-logarithmic variable. With the exception of Seamans et al, baseline 25(OH)D level and age were consistently assessed in addition to the dose, as covariates affecting vitamin D status; other predictors were also evaluated, in decreasing order of the frequency: duration, latitude/country, type of vitamin D supplementation (D2, D3), concomitant calcium intake, type of population/ethnicity, disease status and study quality. The dose was consistently found to be a positive predictor. Baseline 25(OH)D level was a negative predictor when the outcome assessed was the change in 25(OH)D level (89-91). Conversely, it was a positive predictor when the outcome assessed was the 25(OH)D level achieved (92). Age was found to positively affect 25(OH)D level in 2 reviews (90, 91). However, findings from the review by McNally et al., that focused on neonates, infants, children and adolescents, showed that the age negatively affects the achieved 25(OH)D level, although it did not reach statistical

significance (92). The effect of the duration of supplementation was variable (90, 92). Latitude, concomitant calcium supplementation (versus no calcium) and vitamin D2 (compared to vitamin D3) were negative predictors of the increments in 25(OH)D level across the board (Table 2). The effect of concomitant calcium supplementation has been controversial. Some suggested possible decreased compliance to vitamin D supplementation, secondary to calcium side effects (91). Others showed increased 25(OH)D level, related to an inhibitory effect of calcium on vitamin D metabolism (93).

1.9. Vitamin D assay variability and impact on results

There are several vitamin D assays available nowadays to measure 25(OH)D levels and assess vitamin D status (94). The old protein binding assays have been recently replaced by the rapid automated immunoassays. The chromatographic methods using high performance liquid chromatography (HPLC) are less commonly used. Liquid Chromatography Tandem –Mass Spectrometry (LCMS) is considered the gold standard method for measurement of 25(OH)D levels (3). Within and between assay variability result in large differences in the measured 25(OH)D levels, translated into a positive or negative bias of the actual 25(OH)D level (bias range -15% ; + 30%), according to the DEQAS report, July 2014 (3). This report showed also that the accuracy of HPLC and LCMS assays was the best, of 10%, but still did not reach the desirable 5% accuracy (3). Therefore, laboratories participation in vitamin D standardization programs is recommended, as it has been highlighted in several vitamin D replacement guidelines (Appendix 1).

The current vitamin D replacement guidelines target Western populations, who have higher 25(OH)D levels, compared to the MENA region (18). No systematic reviews have assessed the vitamin D dose response relationship in trials conducted in our region. However, few randomized trials studies from Asia in general, and Lebanon in particular, reveal that the current recommended doses to reach desirable 25(OHD) levels would not be sufficient in our populations (21, 95).

The proposed review addresses the effect and the predictors of vitamin D supplementation on serum 25(OH)D levels and on various skeletal and non-skeletal outcomes, in MENA population, across all life cycle. It tries to evaluate the applicability of the IOM RDA to subjects from MENA countries.

1.10. Thesis objectives

The main objectives are:

(1) Define the mean difference in 25(OH)D level reached with low (<800 IU), intermediate (800-2,000 IU) or high (>2,000 IU) daily dose of vitamin D in subjects in the MENA countries, by age and reproductive status.

(2) Compare the effect of vitamin D supplementation, by dose and age category, on other outcomes: BMD, fall and muscle parameters, kidney stones, hypercalcemiemia/ hypercalciuria, mortality, metabolic parameters.

(3) Define the dose response of vitamin D supplementation in this region and identify the potential predictors affecting 25(OH)D level reached following intervention. This will allow the development of region specific recommendations in term of recommended vitamin D doses to reach desirable levels.

1.11. Thesis Hypothesis

Individuals form the MENA region require different doses of vitamin D supplementation compared to Western populations, in order to achieve desirable 25(OH)D of 20 ng/ml level, and to ensure the skeletal and extra-skeletal beneficial effects of vitamin D. This is anticipated in light if their specific risk factors for vitamin D deficiency, including multiparity, lifestyle and concealed clothing style, in addition to genetic polymorphism in the vitamin D enzymatic pathways.

CHAPTER 2

DATA AND METHODS

The protocol of this systematic review was developed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidance (96) and has been published in the PROSPERO registry; protocol registration number CRD42014010488 (97). The PRISMA statement outlines recommendations for reporting systematic reviews to ensure their transparency. It includes 27 items describing the title, abstract, background, objectives, methods, results (including risk of bias), discussion, limitations, in addition to funding agency (96) (see Appendix 3 for a checklist of items to include while reporting a systematic review and meta-analysis, adapted from the PRISMA statement (96))

2.1. Data sources

We identified eligible studies by searching electronic databases using the relevant Mesh Terms and keywords related to Vitamin D, MENA and RCTs. We applied the search strategy to Medline (1946 till present), Embase, PubMed and Cochrane Library without time or language limitation. The search was initially conducted in May 2014, and updated in July 2015. Relevant Mesh terms included: Vitamin D, Vitamin D Deficiency, randomized controlled trial, and all MENA region countries. These were specifically defined according to the World Bank definition and include Middle East, Northern Africa, Algeria, Bahrain, Djibouti, Egypt, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Libya, Malta, Morocco, Oman, Palestine, Qatar, Saudi Arabia, Syria, Tunisia, United Arab Emirates, West Bank and Gaza, Yemen. Similarly, we applied the search to Popline and to other databases relevant to the MENA region, including Index Medicus for WHO

Eastern Mediterranean (IMEMR), and Global Health (see Appendix 4 for full details on search strategy). In addition, we searched trials registries in 2014, the ClinicalTrial.gov. and the WHO international Clinical Trials Registry (ICTRP), for registered and potentially completed trials, and contacted the primary investigators for preliminary results. The response rate of the primary investigators of these trials was 40%. Finally, we screened the references lists of all systematic reviews of RCTs on the topic that were published in the last 10 years.

In March 2014, we contacted experts in the field, involved in the development of International vitamin D guidelines, Professors Paul Lips, Michael Holick and Roger Bouillon, for queries about any trial that could be relevant to our review and that may not have been caught by our search.

2.2.Eligibility criteria

2.2.1. Type of studies

Inclusion criteria:

-RCTs reporting pre and post intervention 25(OH)D level.

-Published or unpublished data (access to unpublished data by contact of principal investigators or corresponding authors).

-No publication date restriction.

Exclusion criteria:

-Prospective interventional studies that are not randomized.

-Studies that did not report pre or post intervention 25(OH)D level.

2.2.2. Type of participants

Inclusion criteria:

-Studies conducted in the Middle East and North Africa including the countries identified based on the World Bank definition, as detailed in Section 2.1 and search strategy Appendix 4).

-Studies of healthy, community dwelling individuals.

-Studies of healthy individuals given vitamin D as a preventive measure of certain diseases or individuals with diseases that have no reason to have altered vitamin D metabolism.

-Studies of both sexes, at all age groups, including pregnant women.

Exclusion criteria:

-Studies conducted on participants from MENA descent but who were living in Western countries.

-Studies on rickets in children and osteomalacia in adults characterized by low 25(OH)D, below 15 ng/ml with evidence of laboratory and radiologic abnormalities, as these individuals require higher doses of vitamin D supplementation (higher than the doses recommended for the general population).

-Studies of institutionalized and hospitalized individuals; this would only apply to elderly and their needs would be different.

-Studies of individuals with chronic illnesses [chronic kidney disease (GFR $\leq$30 ml/min), chronic advanced liver disease, heart failure (New York Heart association (NYHA) class $\geq$ 3)]

-Studies of individuals with conditions or on drug therapy that might affect vitamin D metabolism and vitamin D binding protein /metabolism (anticonvulsants, steroids, anti-fungal, malabsorption, bypass surgery).

2.2.3. Type of intervention

Inclusion criteria

-Studies administering vitamin D (D2 or D3) supplementation of any dose, given orally, daily, weekly or monthly, with or without calcium supplementation, compared to placebo, or to a different vitamin D dose.

Exclusion criteria

-Studies that used synthetic or active vitamin D supplementation, as this type of supplementation is not recommended for the general population (67, 68).

-Studies that used vitamin D supplementation given intra-muscularly as the intra-muscular preparations have a more delayed peak in 25(OH)D level that can occur at 120 days (9).

-Studies that gave vitamin D supplementation for a duration of less than 3 months. 25(OH)D has a half-life of 2 weeks and at least 10 weeks are needed to reach a steady state (98).

-Studies that gave vitamin D supplementation spaced more than 1 month, given that 25(OH)D levels cannot be maintained with infrequent dosing (at intervals of more than one month duration) (17, 65).

-Studies that used vitamin D supplementation as fortified food as the content of vitamin D in such preparations cannot be assessed accurately (99).

In case of missing data on 25(OH)D level or other essential variables, we tried to contact the corresponding authors, by email, in order to get the needed information; If such information was not available or we did not get any reply (following the first email and 2 reminders), the study was excluded.

2.3. Outcome measures

2.3.1. Primary outcome measures:

Mean difference in serum 25(OH)D level reached between any two treatment arms, be it between vitamin D groups themselves (high versus low dose, high versus intermediate dose, intermediate versus low dose), or a vitamin D group and placebo, in each age category and reproductive status.

2.3.2. Secondary outcome measures:

Comparing between the different treatment groups and in each age category:

-The incidence of hypercalciuria / hypercalcemia.

-The incidence of kidney stones.

-The incidence of hip fracture.

-The incidence of fall and imbalance.

-The mean difference in serum calcium level.

-The mean difference in urinary calcium level

-The mean difference in serum PTH level.

-Metabolic parameters measured as the mean difference in serum fasting blood glucose, glycosylated hemoglobin (HbA1c), LDL, HDL, triglycerides (TG), insulin sensitivity

parameters, such as insulin level and the Homeostasis Model Assessment (HOMA) index, HOMA for insulin resistance (HOMA-IR) and HOMA for ß-cell function (HOMA-B).

-The mean difference in bone mineral density at the hip, lumbar spine and forearm.

-Muscle parameters measured as the mean difference in muscle strength and other muscle parameters.

-Mortality measured as the incidence of all-cause mortality.

-Other adverse events (other than hypercalcemia, hypercalciuria, kidney stones, if present).

2.4. Study selection:

References retrieved in the search strategy were reviewed in duplicate and independently by reviewers. One reviewer (MC) screened all references. Two other reviewers (SG and KS) partook references screening and each one of them screened half of the references. Screening of abstracts was done based on our eligibility criteria. We retrieved the full text of citations included by at least one reviewer. Full texts were screened in duplicate and independent manner, using a standardized screening form. A calibration exercise was done on a sample of abstracts and full texts to make sure reviewers screening is standardized. Disagreements were resolved by discussion with an expert in this topic, GEHF (Thesis advisor).

2.5. Data collection process:

We developed a priori a data collection form. It was pilot tested on 4 randomly included articles and refined accordingly. Data abstraction was done in duplicate and independently. One

review author (MC) extracted relevant data from all included studies. Two other review authors (SG and KS), independently, partook data extraction of included studies divided in half. Disagreement between reviewers was resolved by discussion; if agreement was not reached, an expert author (GEHF) intervened to make a decision. This rarely if ever occurred. In case of missing data, the corresponding author of the paper was contacted by email. Non published data form 6 studies were sought. We would like to acknowledge the corresponding authors of several RCTs, Drs A. Dawodu, M. Al-Sofiani, A. Sadiya, M. Taheri, T. Niyestani, M. Shakiba, for sharing with us unpublished data.

In case of Non-English articles, translation into English was implemented by H. Hoballah. For trials published only as abstracts, we contacted the corresponding author by email to get the full text (one email and two reminders); in the case where we did not get any reply, the trial was excluded.

2.6. Analysis Plan

2.6.1. Standard meta-analysis

A meta-analysis aims at giving an overall effect size estimate or combined effect of 2 or more studies (100). Pooling of data takes into consideration study weights. In a fixed-effect model, the weight depends on the inverse variance within studies. In a random-effect model, the weight depends on within study variability, which is equal to the inverse variance, and between study variability, which corresponds to tau^2 (100). While the fixed-effect model assumes that the effect size is the same in all studies and any difference observed is due to chance, the random-effect model assumes that each study has a different effect size and, accordingly, it gives more

balanced weights to the studies and results in wider confidence intervals of the effect size (100). Therefore, it provides more conservative estimates and is the method chosen here-in.

A simple random effects meta-analysis was done using RevMan (version 5.3) when at least 2 studies were available for an outcome, in each predefined comparison (placebo versus high dose (> 2,000 IU/d), placebo versus low dose (< 800 IU/d), placebo versus intermediate dose (800-2,000 IU daily), low dose versus high dose, low dose versus intermediate dose and intermediate dose versus high dose), and in each predefined age category (infants 0-1 year, children and adolescents 1-18 years, adults 18-65 years, elderly > 65 years) and in pregnant women.

Continuous outcomes were expressed as mean differences (MD) with 95% confidence interval (CI). Dichotomous outcomes were expressed as relative risk (RR) or hazard ratio (HR) with 95% CI.

For the primary outcome, we calculated the weighted mean difference (WMD) and 95% CI of 25(OH)D level reached between any two treatment arms (or treatment arm versus placebo), and in each age category, using RevMan.

In addition, in each comparison, we calculated the weighted mean baseline 25(OH)D level, the weighted mean 25(OH)D level reached after the intervention and the weighted mean dose administered. The weights of these variables were based on the sample size.

Calculation of means and pooled standard deviations (Sdp), based on sample size, used the following formulas:

Weighted mean = (n1 m1+ n2 m2 + …. + ni mi)/ (n1+n2+..+ni) (101)

$Sdp = [(n1-1)(Sd1)^2 + (n2-1)(Sd2)^2 + \ldots + (ni-1)(Sdi)^2] / [(n1-1)+(n2-2)+\ldots+(ni-1)]$ (by extrapolation from the formula used to calculate pooled standard deviation in independent t-test, assuming equal variances (102)

where "n" is the number of participants in each arm, "m" is the mean level, "Sd" is the standard deviation of the level in each arm.

In each of the aforementioned groups and assuming normality of the distribution of 25(OH)D level, we calculated the proportion of subjects reaching 25(OH)D ≥ 20 ng/ml, based on the calculated weighted mean 25(OH)D level reached at the end of the intervention. To check on the validity of this method, we calculated the proportion of subjects reaching a certain 25(OH)D cutoff in published papers, Dawodu et al 2013 (103), Hollis et al 2011 (104) and El Hajj Fuleihan et al 2015 (105), and compared the obtained results, using our above described method, to the ones reported in these publications (Appendix 5). The calculated proportions differed from the reported ones only by 1- 4%. Finally, we calculated the RR of the event, namely reaching 25(OH)D level ≥ 20 ng/ml at the end of the intervention, in each comparison and age category. The proportion of subjects reaching a 25(OH)D level ≥ 20 ng/ml was calculated by dividing the number of events (an event being the fact of reaching a 25(OH)D level ≥ 20 ng/ml) by the total number of subjects in every vitamin D or placebo group. We have also used data from El Hajj Fuleihan et al.(105) and explored the normality of the distribution of 25(OH)D levels on 222 participants. The stem and leaf plots showed a normal distribution of these levels.

We calculated the mean difference in serum calcium and PTH, fasting blood glucose, HbA1c, HDL, LDL, and TG level, HOMA-IR and HOMA-B in vitamin D groups compared to placebo groups or in different vitamin D groups, when data on these variables were present in at least two studies in the same comparison.

Quantitative analysis was done based on a complete case analysis. Statistical heterogeneity between studies was assessed using Chi square with significance at p-value ≤ 0.05. The quantitative assessment of heterogeneity was done using I^2.

2.6.2. Additional analyses

Subgroup analyses were pre-specified, based on covariates that we expected them to affect the response to vitamin D supplementation, as follows:

-Baseline 25(OH)D level < 20 ng/ml versus ≥ 20 ng/ml, since lower baseline vitamin D levels respond better to vitamin D supplementation (64, 90)

-BMI < 30 kg/m^2 or ≥ 30 kg/m^2 , since obesity negatively affects the response to vitamin D supplementation (85).

In addition, we did sub-group analysis based on the supplementation duration category (3 months versus > 3 months of supplementation). The latter sub-group analysis was not predefined in our protocol.

Analysis based on gender and the presence or absence of concomitant calcium supplementation was not conducted for the lack of the needed relevant data in the included studies.

2.6.3. Meta-regression

A meta-regression is "an extension of the standard meta-analysis" (106). While the meta-analysis quantifies variability between studies, which corresponds to the heterogeneity, the meta-regression explores and identifies the covariates behind this heterogeneity (107). Similar to a simple regression, a meta-regression assesses the relationship between a dependent variable and

one or more covariates (predictors)(108). However, it uses weighted data from studies, rather than individuals' data from a single study (108). Accordingly, each study represents one data point (108). Most commonly the unit of analysis is the study; however, sometimes, one arm, a treatment or control arm, is the unit of analysis (109, 110).

Similar to the simple meta-analysis, there are 2 models of meta-regression: a fixed-effect model and a random-effect model (107). In the former, data are weighted by the inverse variances; in the latter, data weights are based on within and between study variances, and they are equivalent to $1/(\sigma_i^2 + \tau^2)$, where σ_i^2 is the standard error of the effect estimate and τ^2 represents between study variance (107). A meta-regression can be a linear or a logistic regression model. It can be performed on STATA, SPSS and SAS (110). The output of the meta-regression on STATA include the following parameters (107, 108):

- I_{res}^2: represents the percentage of the residual variation attributable to between-study heterogeneity.

-Adjusted R^2: represents the proportion of between-study variance attributable to the covariate(s); it can be negative when the variability explained by the covariate is less than that due to chance.

-τ^2: represents an estimate of the remaining between-study variance; τ is defined by the Cochrane Group as "the standard deviation of the underlying effects between studies"(111).

-Wald test for the overall model. It is a joint test for all the covariates included in the model. This test statistic is compared to the appropriate *F* distribution to derive p-value. It shows only in case of multivariate analysis since in a single variate analysis the p-value of the model would be the same as the one showing in the regression table.

-Regression coefficient: reflects how the dependent variable changes with every one unit change of the covariate. According to the sign of the regression coefficient, the covariate can be a positive or a negative predictor of the outcome.

2.6.3.1. Meta-regression in our case

Our published protocol specified "the meta-regression" as part of our pre-planned analysis. However, we decided on the outcome, predictors and regression model after data abstraction. The meta-regression was only performed in adults and elderly due to the lack of sufficient publications in the other age categories.

In adults and elderly groups, we identified 15 eligible studies (17 intervention arms and 13 placebo arms). Based on literature review, the dependent outcome could be the achieved 25(OH)D level per arm, the change in 25(OH)D level per arm or the mean difference in 25(OH)D level achieved between arms (Table 2). The first two options require the use of arm data. The latter option requires the use of study data. We chose the achieved 25(OH)D level as the dependent variable for our regression model, since this outcome is clinically relevant and easy to apply in practice, and required the least assumptions, given the data we had from individual studies.

The data for the meta-regression analysis was derived from intervention and placebo/control arms; the latter arms reflect the change in 25(OH)D level in response to environmental factors. We calculated within study variances, inverse of standard error $(SE)^2$, based on study arm standard deviation (SD). Between studies variances were calculated automatically on the STATA software version 12.

First, we performed graphical exploration of the data and curve fitting of the vitamin D dose versus the achieved 25(OH)D level, with and without natural logarithmic transformation of the vitamin D dose, to identify the best model.

Second, we performed single variable random-effects meta-regression of the 25(OH)D level reached versus all the covariates identified in previous meta-regressions, as predictors of the vitamin D response to supplementation, and for which we had enough data from the included studies (Table 2). These predictors were, in addition to the dose: baseline 25(OH)D level ng/ml, supplementation duration (continuous or categorical variable, 3 months versus > 3 months of supplementation), age (years), BMI (kg/m^2), presence or absence of concomitant calcium supplementation, latitude and risk of bias. The effect of the type of vitamin D (D2 versus D3) was not assessed, since all the studies, except one, administered vitamin D3. We could not assess the effect of the publication year, as all the studies were published recently, in the period 2012-2015. There was a large variability in the vitamin D assays used and therefore, we could not assess their effects either.

Third, we performed a multivariate random-effects meta-regression. Since the evaluation of covariates requires the presence of 10 studies for each covariate (108), we were only powered to assess the effect of three of them. We included in the multivariate model the 3 most robust predictors (highest adjusted R^2), significantly affecting 25(OH)D level in the univariate analysis at a p-value of 0.1.

Finally, as a sensitivity analysis, we forced in the model other predictors that are clinically relevant and that were consistently evaluated in previous similar reviews (Table 2).

2.7. Risk of bias across studies

We assessed the risk of bias of the included studies using the Cochrane Collaboration's tool for bias assessment (112). This tool includes 7 domains: sequence generation and allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), in addition to other sources of bias that may threaten the study validity (112). A summary assessment for each important outcome within and across studies is provided based on the risk of bias results in all the domains. In our review, we assessed the risk of bias for the primary outcome, the mean difference in 25(OH)D level achieved (112). Selective outcome reporting was assessed by searching for the availability of a published protocol for each included study published after 2009. Otherwise, the methods section was scrutinized and the reported pre-planned outcomes identified in the methods section were compared with the outcomes cited under "Results". Publication bias was assessed by performing a funnel plot of the included studies. For each trial, we plotted the effect by the inverse of its standard error. The symmetry of the funnel plot was checked visually.

2.8. Assessment of the quality of evidence:

The quality of evidence for the primary outcome, the mean difference in 25(OH)D level achieved, was assessed using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) methodology (113). This methodology takes into consideration study design, risk of bias, in addition to inconsistency, indirectness and imprecision of results to rate the quality of the evidence (114). Evidence form randomized controlled trials start as a high quality. However, it can be downgraded to moderate or low quality of evidence in the presence

of one or more of the following: inconsistency of the results, imprecise results (wide confidence interval), indirectness of the results or high risk of bias (113, 114). Conversely, observational studies start as low quality and can be upgraded to moderate or high quality, in the presence of a large magnitude of the intervention effect and evidence of a dose response (113, 114).

CHAPTER 3

RESULTS

The search strategy identified a total of 4,961 citations (Figure 1). After duplicate removal, we were left with 4,280 citations for title and abstract screening. We identified 227 citations as eligible, for which we retrieved and screened the full text. 202 articles were excluded. The most common reasons for exclusion were: trials conducted outside the MENA region (34 studies), duration of the intervention of less than 3 months or supplementation administered less frequently than once monthly (31 studies), studies not RCTs (23 studies), the use of active vitamin D preparations (23 studies), the presence of chronic illness, most commonly renal disease (22 studies), vitamin D dose or level not reported (14 studies), the use of parenteral vitamin D supplementation (12 studies), vitamin D supplementation as fortified food (12 studies) (Figure 1). Appendix 6 lists the excluded trials and the specific reason for exclusion of each one.

We identified 25 papers fulfilling our inclusion criteria and these are summarized in Table 3. Therefore, our systematic review included the following:

-2 studies in elderly.

-13 studies in adults.

-6 studies in pregnant women.

-3 studies in children and adolescents.

-1 study in infants.

Seventeen studies were from Iran, two studies from Lebanon, two studies from Saudi Arabia, two studies from Israel, two studies from the United Arab Emirates (UAE). We did not identify

any study conducted in the North Africa region. Across different age groups, vitamin D supplementation was administered for at least 3 months (7 studies in adults, 1 study in pregnant women, 1 study in children and adolescents) and for a maximum of 12 months (2 studies in elderly, 1 study in adults and 1 study in children).

3.1. Objective 1: Effect of vitamin D supplementation on 25(OH)D level

3.1.1. Elderly:

In elderly, we identified 2 eligible studies, a small one from Israel (N randomized = 47) (115) and a larger one from Lebanon (N randomized = 257) (105). The mean age of participants was 66 years in the former and 71 years in the latter.

Breslavsky et al administered in diabetic elderly Israeli men and women an intermediate vitamin D dose of 1,000 IU daily (N = 19 completed the study) versus placebo (N = 13 completed the study) over 12 months. While the baseline 25(OH)D level was 12.9 (10.7) ng/ml and 10.8(6.6) ng/ml, in the intermediate and placebo groups, respectively, the achieved 25(OH)D level was 17.6(11.5) and 14(5.9) ng/ml, in these groups respectively; a statistically non-significant difference between the achieved levels, p-value 0.299 (115). The loss to follow up rate in this trial was high, 20% in the intervention group and 43% in the control group.

El Hajj Fuleihan et al. compared, in non-diabetic Lebanese elderly, a low vitamin D dose of 600 IU daily (N = 112 completed the study) to a high dose of 3,750 IU daily (N = 110 completed the study), administered concomitantly with daily calcium supplementation (calcium citrate 1,000 mg daily) over a period of 12 months. At study entry, the baseline 25(OH)D level was 20.3 (7.5) ng/ml, implying that 54% of participants in each treatment arm fell above the 25(OH)D cutoff of 20 ng/ml. After the intervention, the 25(OH)D levels achieved were 36(9.7)

ng/ml and 26(6.9) ng/ml in the high and low dose, respectively. As reported by the authors, 98% and 83% of Lebanese elderly reached a 25(OH)D level of 20 ng/ml following supplementation (105). This study used the HPLC method to measure 25(OH)D level.

The 2 studies administered different doses of vitamin D and we, therefore, could not pool their results. Compliance to the intervention was not discussed in the first study, while it reached 91.7 and 93.5%, in the low and high dose groups respectively, in the second one.

3.1.2. Adults:

In adults, we identified 13 eligible studies, 9 of them compared a high dose versus placebo and 2 of them compared an intermediate dose versus placebo; these studies were included in the meta-analysis. The remaining 2 studies were not included in the meta-analysis given that the administered vitamin D doses fell into different comparisons (Table 3). The range of mean age for these studies was 27-58 years. The vitamin D equivalent daily dose varied from 400 to 7,140 IU daily. Nine studies were from Iran, 2 studies from Saudi Arabia, 1 study from Israel and 1 study from UAE. Only one study was conducted in healthy non obese adults. Six studies were conducted in diabetic patients. The remaining studies were conducted in patients with Polycystic Ovary Syndrome (PCOS) (2 studies), Multiple Sclerosis (MS) (1 study), Non-Alcoholic Fatty Liver Disease (NAFLD) (1 study) and obesity (1 study) (Table3). All these trials were published in 2012-2015. Most studies did not administer calcium concomitantly with vitamin D, with the exception of Firouzabadi and Hosseini. Most studies included both genders. Study subjects were overweight or obese, with mean BMI >25 kg/m^2. Vitamin D assays used were variable and none of the studies used HPLC. Compliance to vitamin D supplementation was described only in 3 studies, and varied between 87 and 97%. The rate of participants lost to

follow up was high in 2 studies, ranging between 31-56%, and it was not reported in 2 other studies (See table 3 for further details).

3.1.2.1. High dose (> 2,000 IU/d) versus placebo comparison

Nine studies were included in this comparison (116-124). The total number of participants was 342 in the high dose group and 328 in the placebo group. The intervention lasted for 3-4 months, with the exception of the trial by Firouzabadi et al. that extended over a 6-months period (119). The weighted mean vitamin D supplementation dose was 4,856 IU daily. The Weighted Mean Difference (WMD) in 25(OH)D achieved between high dose and placebo groups was 18.30 (14.12;22.49) ng/ml, favoring the high dose; Tau^2 =30.59; Chi^2=97.85, df=8 ($p<0.001$); I^2= 92% (Figure 2 A). The calculated weighted mean baseline 25(OH)D level was 15 ng/ml. The calculated weighted mean 25(OH)D level achieved was 38 ng/ml (Sdp=14.4ng/ml). Accordingly, the proportion of participants who reached a 25(OH)D level of 20 ng/ml in the high dose group was estimated at 89.4%.

3.1.2.2. Intermediate dose (800-2,000 IU/d) versus placebo comparison:

Two studies compared an intermediate dose of vitamin D versus placebo. The total number of participants was 153 in the intermediate arm, and 150 in the placebo arm. The duration of supplementation was 6 months in both studies. The intermediate dose was 1,000 IU/d in one of them (125) and 2,000 IU/d in the other one (126), with a weighted mean vitamin D dose of 1,750 IU daily. The weighted mean difference (WMD) in 25(OH)D level reached was 14.7 (4.57;24.89) ng/ml, favoring the intermediate dose; Tau^2 =49.05; Chi^2 =11.38, df=1 ($p<0.001$); I^2=91% (Figure 2 B). Starting from a weighted mean baseline 25(OH)D level of 11.5

ng/ml, the calculated weighted mean 25(OH)D level reached in the intermediate dose group was 29.8 ng/ml (Sdp=16.25 ng/ml). Accordingly, we estimated the proportion of individuals reaching the target level to be 72%.

Noteworthy that in the placebo arms, the calculated weighted increment in 25(OH)D level was around at 3-5 ng/ml.

Two remaining 2 studies were not included in the meta-analysis, as the administered vitamin D doses fell into different comparisons (127, 128). Golan et al compared a high dose of 4,370 IU/d to a low dose of 800 IU/d, in patients with MS (127). Staring at 20 ng/ml, 25(OH)D level reached 40 ng/ml in the high dose group and 22.6 ng/ml in the high and low dose groups, respectively (127). Ghavamzadeh et al compared a low dose of 400 IU/d to placebo. The 25(OH)D level was 8.6-8.9 ng/ml at baseline. It increased by 10 ng/ml in the high dose arm and decreased by 0.5 ng/ml in the placebo arm (128).

3.1.2.3. Subgroup analysis

Subgroup analysis was performed in the high dose versus placebo comparison, given the availability of studies in this category.

Subgroup analysis by duration of supplementation, 3 months versus > 3 months, showed a significantly higher WMD in 25(OH)D level achieved of 25.68(16.78;34.47) ng/ml, when vitamin D supplementation was given for 3 months, compared to a WMD of 10.47(9.49; 11.45) ng/ml, when supplementation was administered for > 3 months (p-value <0.001).

Subgroup analysis by baseline BMI (<30 kg/m^2 versus ≥ 30 kg/m^2) showed a tendency for a lower WMD in 25(OH)D level achieved in the obese participants 18.30 (14.12; 22.49)

ng/ml, compared to a WMD of 19.65 (12.04;27.25) ng/ml in overweight and normal weight participants. However, these results did not reach statistical significance (p-value 0.79).

Subgroup analysis by baseline 25(OH)D level, ≤ 20 ng/ml versus > 20 ng/ml also did not yield significant result; WMD of 16.51 (12.39-20.63) ng/ml for those who were at ≤ 20 ng/ml at baseline, compared to a WMD of 28.01 (14.38-41.64) ng/ml for those who were at > 20 ng/ml (p-value 0.11).

3.1.3. Pregnant women

We identified 6 eligible studies conducted in pregnant women (103, 129-133). Five studies were from Iran and one study was from UAE. Four studies were conducted in healthy pregnant women while 2 studies were conducted in women with MS (131) or at risk of pre-eclampsia (132). The BMI of participants varied between 25 and 30 kg/m^2. None of the studies used concomitant calcium supplementation. Vitamin D supplementation was started in the 2nd trimester at 12-20 weeks of gestation, with the exception of Sabet et al. where vitamin D supplementation was started at 28 weeks of gestation (Table 3). Maternal 25(OH)D level at delivery was reported in four studies, 2 of them reported also results on venous cord 25(OH)D level. Etemadifar et al reported maternal 25(OH)D levels at 6 months post-partum and not at delivery (131). Shakiba et al. reported only 25(OH)D levels in venous cord (129). The vitamin D assays used were variable and none of the studies used the highly accurate method HPLC. Three studies (103, 130, 132) allowed to compare a low dose versus a high dose of vitamin D, and 2 studies (103, 130) allowed 2 comparisons: low versus intermediate dose, and intermediate versus high dose of vitamin D. Dawodu et al. trial consisted of three arms: low dose of 400 IU daily, intermediate dose of 2,000 IU daily and high dose of 4,000 IU daily. Soheilykhah et al.

compared a low dose of 200 IU daily, versus an intermediate dose equivalent to 1,660 IU daily and a high dose equivalent to 3,300 IU daily. Karamali et al. compared a low dose of 400 IU daily versus a high dose equivalent to 3,970 IU daily.

Compliance rate was described only in 2 studies. Dawodu et al reported a compliance rate of 86% in the high dose arm, 87% in the intermediate arm and 82% in the low dose arm. Karamali et al reported a 100% compliance rate in both groups. A very high rate of participants lost to follow was noted in the trial by Etemadifar et al. ranging between 59 and 71% (131). In the remaining studies, the rate of loss to follow up was 0-20%.

3.1.3.1. Intermediate dose (800-2,000 IU/d) versus low dose (800 IU/d) comparison

Two studies were included in this comparison (103, 130). The total number of participants was 79 in the intermediate dose group and 77 in the low dose group. The weighted mean intermediate dose administered was 1,832 IU daily and the weighted mean low dose 301 IU daily, started at 12- 16 weeks gestational age and continued until delivery. The WMD in 25(OH)D achieved was 7.82 (4.84-10.80) ng/ml; Tau^2 =0.00; Chi ^{2} =0.89, df=1 (p=0.35); I^2 =0% (Figure 3A). The calculated weighted mean 25(OH)D level at baseline was 7.86 ng/ml. The calculated weighted mean level reached in the intermediate dose group was 26.52(Sdp =10.4) ng/ml and in the low dose group 18.57(Sdp =10.4) ng/ml. Accordingly, we estimated the proportion of pregnant women who reached the target of 20 ng/ml to be 73% and 43% in the intermediate and low dose groups, respectively.

3.1.3.2. High dose (> 2,000 IU/d) versus intermediate dose (800-2,000 IU/d) comparison:

The studies included and detailed in the above comparison allowed also to compare intermediate versus high dose (103, 130). The total number of subjects was 83 in the high dose group and 79 in the intermediate dose group. The weighted mean high dose administered was 3,662 IU daily and the weighted mean intermediate dose 1,836 IU daily, started at 12- 16 weeks gestational age and continued until delivery. The WMD in 25(OH)D level reached between arms was 8.5(5.07-11.93) ng/ml; Tau^2 =0.00; Chi ^{2} =0.84, df=1 (p=0.36); I^2 =0% (Figure 3B). The calculated weight 25(OH)D level reached in the high dose group was 35(Sdp=11.8) ng/ml and in the intermediate dose group was 26.5 (Sdp=10.4) ng/ml. We estimated the proportion of pregnant women who reached the target of 20 ng/ml, at delivery, to be 90 % and 73%, in the high and intermediate dose groups, respectively.

3.1.3.3. High dose (>2,000 IU/d) versus low dose (<800 IU/d) comparison:

Three studies were included in the high versus low dose comparison (103, 130, 132). The total number of pregnant women was 113 in the high dose group and 107 in the low dose group. The weighted mean high dose was 3,638 IU daily and the weighted mean low dose administered was 335 IU daily, started in the second trimester and continued until delivery. The WMD in 25(OH)D level reached was 17.27(15.8-18.73) ng/ml, favoring the high dose; Tau^2 =0.00; Chi ^{2} =0.32, df=1 (p=0.85); I^2 =0% (Figure 3C). The calculated weighted mean baseline level in these studies was 11 ng/ml. The calculated weighted mean 25(OH)D level reached in the high dose group was 35(Sdp=9.5)ng/ml and in the low dose group 18.2 (Sdp=7.5) ng/ml. Therefore, we calculated the proportions of subjects who reach the target 25(OH)D of 20 ng/ml to be 94% in the high dose group and 41% in the low dose group.

3.1.3.4. Venous cord blood 25(OH)D level

Two studies allowed the comparison of the effect of a high dose versus an intermediate dose of vitamin D administered during pregnancy, on the venous cord blood 25(OH)D level (103, 129). The total number of participants was 56 and 59 in the high and intermediate dose group, respectively. The weighted mean intermediate dose was 1,800 IU daily and the weighted mean high dose was 3,876 IU daily, started in the second trimester until delivery. The WMD was 7.08(3.84;10.31) ng/ml favoring the high dose; Tau^2 =0.00; Chi ^{2} =0.00, df=1 (p=0.98); I^2 0% (Figure 4). The estimated proportion of neonates reaching a 25(OH)D level of 20 ng/ml at birth was 80% in the high dose and 55% in the intermediate dose.

3.1.4. Children and adolescents

We identified 3 studies conducted in children and adolescents (40, 134-136). One study (40, 134) was conducted in Lebanon, while the 2 other studies were from Iran (135, 136). The three studies were conducted in healthy school children, girls and boys, and the range of mean age of the children was 9.8-16.5 years. The baseline 25(OH)D level ranged between 10 and 15 ng/ml. The vitamin D assay used was HPLC in one study (135), and immune-assays in the two other studies (40, 136).

Compliance to vitamin D supplementation was only described in the study by El Hajj Fuleihan et al, ranging between 97-98% in the low, intermediate and placebo arms (40). The reported rates of loss to follow up were low, of less than 15%.

3.1.4.1. Intermediate (800-2,000 IU/d) dose versus placebo comparison

Two studies were included in this comparison, El Hajj Fuleihan extended over 12 months (40, 134) and Ghazi et al. extended over 6 months (136). The total number of participants was 183 in the intermediate dose group and 179 in the placebo group (Figure 5A). The weighted mean intermediate dose was 1,870 IU daily. The WMD in 25(OH)D level was 15.77(8.68;22.87), favoring the intermediate dose (Figure 5A);Tau^2 =22.87; Chi ^{2} =7.71, df=1 (p=0.005); I^2 =87%. The calculated weighted mean baseline 25(OH)D level was 14 ng/ml. The calculated weighted mean 25(OH)D level achieved was 31.6(Sdp=18.6) ng/ml in the intermediate dose group, corresponding to 73% of children reaching a 25(OH)D level of 20 ng/ml in this group. In the placebo arm, 25(OH)D level increased by 1.9 ng/ml and the proportion of subjects reaching the target 25(OH)D level of 20 ng/ml was estimated at 28%.

3.1.4.2. Low dose (<800 IU/d) versus placebo comparison

We included 2 studies comparing a low dose (same dose in both studies of 200 IU daily) versus placebo. The total number of participants in the low dose group was 174, and in the placebo group 164. The weighted mean baseline 25(OH)D was 13.4 ng/ml. We did not find any statistically significant difference in the 25(OH)D level achieved between the low dose and the placebo (Figure 5B).

3.1.4.3. Subgroup analysis in children

A subgroup analysis by gender, comparing an intermediate dose of vitamin D versus placebo, did not show any significant difference in the WMD of 25(OH)D achieved.

3.1.5. Infants

We identified only one trial in infants form Iran comparing 2 low doses of vitamin D, 400 IU and 200 IU daily, in drops, and foodlet or sprinkles, respectively (137). The baseline 25(OH)D level was 82-88.9 ng/ml. At the end of the study, 25(OH)D level was significantly higher in the drops group, 96.4(32.1) ng/ml, versus 88.5(28.4) ng/ml and 87.4(32) ng/ml, in the foodlet and sprinkles groups, respectively.

There was some variability in the increase in serum 25(OH)D level per 100 IU/d of vitamin D administered, between dose categories within each age group and between age groups. The increments per 100 IU/d vitamin D were least in the high dose arms, being 0.40 ng/ml in elderly, 0.46 ng/ml in adults and 0.66 ng/ml in pregnant women. The increments were highest in the low dose arms, reaching 2.50 ng/ml/100 IU and 3.57 ng/ml/100 IU/d, in adults and pregnant women, respectively.

Table 4 provides a summary of results across all age groups.

3.2. Objective 2: Effect of vitamin D supplementation on other outcomes

We did not identify any study comparing the effect of different vitamin D supplementation doses on fracture risk reduction. Only one study in children and adolescents and another one in elderly, both from Lebanon, assessed the effect of various vitamin D supplementations on bone mineral density (BMD). Therefore, data on skeletal outcomes and surrogate markers were scarce. Data on extra-skeletal outcomes, namely metabolic and cardio-

vascular outcomes, were also limited and only available in trials conducted in adults, comparing a high vitamin D dose versus placebo (see Appendix 7 for full details).

The adverse events of vitamin D including kidney stones, hypercalcemia, hypercalciuria and hypervitaminosis D were poorly reported in individual studies (Table 3). The 2 studies conducted in elderly, reported various adverse events. El Hajj Fuleihan et al reported serious adverse events including the following (one participant for each): in the low dose group: death, stroke, thrombophlebitis, hemorrhoids, glaucoma, disc disease; in the high dose group: death, kidney stone, hypertensive crisis, retinal detachment, knee arthroplasty. Breslavsky et al. reported in the intermediate dose group diarrhea (1 subject) and weakness (1 subject). In adults, 2 studies administering a high dose versus placebo, reported no adverse events (122, 123). All the other studies did not provide any details regarding the adverse events (see table 3). In pregnant women, 3 studies reported no adverse events (103, 130, 131), while in 3 others details on adverse events were missing (129, 132, 133). In children, El Hajj Fuleihan reported, in the intermediate dose group, 3 cases of high 25(OH)D level (103, 161 and 195 ng/ml), without concomitant hypercalcemia (40), and one case of glomerulonephritis in the low dose group (134).

3.2.1. Adults

3.2.1.1. High dose versus placebo:

Serum calcium level was the only mineral parameter, other than 25(OH)D level, with available data following intervention in 5 studies in adults administering a high vitamin D dose (equivalent daily dose range 3,300 IU-7,140) versus placebo (116, 117, 122-124). After 3-4

months of intervention, the WMD in serum calcium level achieved in the high dose group, compared to placebo, did not reach statistical significance.

Data from 3 studies showed that vitamin D supplementation improves insulin sensitivity, WMD in HOMA-IR 0.96(0.32; 1.61), favoring the high dose (117, 120, 123). However, no significant effect was detected on BMI and HbA1c. Pooling data from 2 studies did not show a significant effect of vitamin D supplementation on lipid profile parameters (118, 121). Three studies showed a significant reduction in systolic blood pressure, WMD -3.53 (-6.3; -0.76) mmHg, favoring a high vitamin D dose, but no effect on diastolic blood pressure (117, 118, 121).

3.2.2. Pregnant women

The effect of vitamin D supplementation on serum calcium level was assessed in trials conducted in pregnant women (high versus intermediate versus low doses). The WMD in serum calcium level achieved across various comparisons did not reach statistical significance.

3.2.3. Children and Adolescents

The effect of vitamin D supplementation on PTH level was only assessed in children/adolescents. While an intermediate dose (weighted mean dose of 1,870 IU/d) significantly reduced PTH level with a WMD in PTH level achieved of -7.00 (-7.38;-6.62), favoring the intermediate dose, a low dose did not yield significant results. Similar to other age groups, the effect of an intermediate dose of vitamin D on serum calcium level was not significant.

3.3. Objective 3: Vitamin D dose response in meta-regression analysis

We included 30 independent arms (17 intervention arms and 13 placebo arms) from studies conducted in adults and elderly. Since the assessment of the effect of each covariate requires the presence of 10 units of analysis (studies or arms) (138), we were powered to detect the statistical significant effect of only 2-3 predictors.

We first conducted a univariate random-model meta-regression assessing the effect of various candidate predictors on 25(OH)D level. The linear model was better than the logarithmic model in predicting the effect of the vitamin D dose on the achieved 25(OH)D level (Figure 6), the R^2 being 68% and 49%, respectively. In addition to the dose, baseline 25(OH)D level and duration category (3 months versus > 3months) were significantly associated with the 25(OH)D level achieved post-intervention at a p-value of 0.1 (Table 5). While the dose and the baseline 25(OH)D level were positive predictors, the duration category was a negative predictor of 25(OH)D level achieved post-intervention (Table 5).

We then conducted a multivariate random-effect meta-regression including the three most robust predictors of the 25(OH)D level. This model explained 87% of the variability in 25(OH)D level achieved. The dose and the baseline 25(OH)D were persistently significantly associated with the 25(OH)D level achieved post-intervention, whereas the duration category lost significance (Table 6A). Excluding the duration category from the multivariate model did not alter the model characteristics (Table 6B), and therefore, this predictor was safely removed from the final model. The increase in 25(OH)D level was around 0.44 ng/ml per 100 IU/d vitamin D, and 0.77 per 1 ng/ml increase in baseline 25(OH)D level.

3.3.1. Sensitivity analysis

In a sensitivity analysis, we forced in the multivariate random-effect meta-regression model three predictors that are clinically relevant: age, presence or absence of concomitant calcium supplementation and BMI (Table 7). This model explained 89% of the variability in 25(OH)D level achieved post-intervention. Indeed, the dose, baseline 25(OH)D level and the age were persistently positive predictors. The presence of concomitant calcium supplementation and BMI were negatively associated with 25(OH)D level achieved. Age, calcium supplementation and BMI did not reach statistical significance (Table 7).

Since several previously published meta-regressions on this topic have used the natural logarithmic (Ln) transformation of the vitamin D dose (Table 2), we conducted mutivariates random effects meta-regression using Ln of the vitamin D dose, instead of the dose, as a sensitivity analysis. Results did not differ (see Appendix 8). The model that used the dose, as opposed to the Ln dose was the best predictor of 25(OH)D level achieved (Appendix 8).

3.4. Risk of bias assessment

The risk of bias is illustrated by individual study and by domain in Figures 7 and detailed in Appendix 9.

In adults and elderly, the risk of bias was unclear to high. Only three studies were at low risk of bias across all domains (105, 122, 123), and the study by Al-Sofiani followed closely (117). The random sequence generation and the allocation concealment were poorly described in 6 and 8 studies, respectively (Figure 7A). Several trials published after 2010 did not have a published protocol on trial registries (Appendix 9A).

In pregnant women, two studies were at low risk of bias across all domains (103, 132). Two studies were open label, and accordingly were at high risk of selection and performance bias (130, 131). The remaining two studies did not describe any details related to sequence generation, allocation concealment and blinding. They were rated as having unclear risk of bias (Figure 7B, Appendix 9B).

In children and adolescents, the trial by El Hajj Fuleihan et al was at a low risk of bias across all domains. The trial by Ghazi et al. performed well except for the domains on attrition and reporting bias, and the one by Neyestani et al was at high risk of bias for selection and performance bias (Figure 7C, Appendix 9C).

Publication bias was assessed only in adults in the high vitamin D dose versus placebo comparison (total of 9 trials). The inverted funnel plot of the primary outcome of the mean difference in 25(OH)D level achieved did not suggest a clear publication bias (Figure 8).

3.5. Quality of evidence using GRADE

The quality of evidence using GRADE was assessed for the primary outcome, the mean difference in 25(OH)D level achieved following intervention (see Appendix 10).

In adults, the quality of evidence of the mean difference in 25(OH)D level achieved in high and intermediate vitamin D dose, compared to placebo, was very low to low. Although the evidence was derived from randomized controlled trials, considered as high quality of evidence, it was downgraded because of the high risk of bias and the high heterogeneity in the results, related to the variability in the dose, duration of supplementation and baseline 25(OH)D level. In addition, in the intermediate dose versus placebo comparison, the results were imprecise, WMD

14.73 (4.57-24.89) ng/ml; wide confidence interval where the WMD in 25(OH)D level varies from minimal clinical significance (difference of 4.57 ng/ml) to a large difference (24.89 ng/ml).

In pregnant women, the quality of evidence was intermediate across all comparison. It was downgraded secondary to the high risk of bias in one trial, Soheilykhah et al.

The quality of evidence in children and adolescents was low in the intermediate versus placebo comparison, and very low in the low dose versus placebo comparison. The evidence derived from randomized controlled trials was downgraded because of high heterogeneity and imprecision of the results, in addition to high risk of bias in one study, Neyestani et al.

CHAPTER 4

DISCUSSION

4.1. Review of findings and comparison to western studies

Our systematic review shows that trials on vitamin D replacement in the MENA region are conducted in the Middle Eastern countries, while none were identified from North Africa. Most of these trials were conducted in adults and pregnant women and only few of them were implemented in the elderly, children/ adolescents and infants. More than half of the included studies administered a high dose of vitamin D (equivalent daily dose range: 3,333 - 7,140 IU/d), compared to placebo, or to a low vitamin D dose, and almost all administered vitamin D3 preparations.

As expected, we demonstrated a dose-dependent increase in 25(OH)D in our meta-analysis. Indeed, the 25(OH)D level achieved increased with increasing vitamin D supplementation doses. However, the increments per 100 IU/day vitamin D were lower as the total daily dose increased, suggesting a plateau in the dose response at higher doses.

In an elderly population from Lebanon, with mean baseline 25(OH)D level of 20 ng/ml (implying that around 50% of the population at target at study entry), reflecting vitamin D status in the general population (139), a vitamin D dose of 600 IU daily, close to the IOM RDA, that is 800 IU daily, allowed to 83% of participants to reach the 25(OH)D target level of 20 ng/ml. A high dose of 3,750 IU daily, which is more than 4 times the IOM RDA, brought the majority of the population (98%) to target. The increase in 25(OH)D level in response to supplementation was around 1 ng/ml per 100 IU/d vitamin D in the low dose and around 0.40 ng/ml per 100 IU/d vitamin D in the high dose group. Starting at a lower baseline 25(OH)D level (10.8-12.9 ng/ml)

in Israeli elderly, an intermediate dose of 1,000 IU administered for a period of 12 months increased 25(OH)D level by 5 ng/ml (increments equivalent to 2 ng/ml per 100 IU/d vitamin D) and allowed to only 40% of participants in the intermediate group to reach the target level of 20 ng/ml. Given that the we were not able to pool the result of the 2 studies conducted in elderly in a meta-analysis, we will compare their findings to studies from Western countries, that have assessed the increase in 25(OH)D level in response to escalating doses of vitamin D in elderly. Viljakainen et al used three low doses of vitamin D 200, 400 and 800 IU daily, compared to placebo, in Finnish elderly men and women with baseline 25(OH)D level of 18.5-18.9 ng/ml (146). The increase in 25(OH)D was parallel to the increase in the vitamin D dose, and ranged between 1.2-2.2 ng/ml per 100 IU/d vitamin D (146). Gallagher et al evaluated the effect of oral doses of vitamin D3, ranging from 400 to 4,800 IU/d in white elderly women from Omaha, Nebraska, with baseline 25(OH)D level around 15 ng/ml (84). The calculated increments per 100 IU/d vitamin D varied between 1.6 ng/ml for the lowest dose, and 0.6 ng/ml for the highest dose. In a multivariate analysis, the increment in 25(OH)D level per 100 IU/d vitamin D (ß-coefficient) was equivalent to 0.92 ng/ml and 25(OH)D level tended to reach a plateau at a dose ≥ 3,200 IU daily (84). In this latter study, a dose of 400-800 IU/d allowed to the majority of the population to reach the desirable level of 20 ng/ml. Findings from these trials, and similar to those from the MENA region, confirmed that increments in 25(OH)D level increased parallel to the dose at low to intermediate doses (increments > 1ng/ml per 100 IU/d). However, increments were lower at high doses (increments < 1ng/ml per 100 IU/d). On the other hand, while a low dose of 400-800 IU/d was enough to allow to 97.5% of American elderly men and women to reach the target level, such dose was not enough in elderly form our region.

In adults, meta-analyses were performed in two comparisons: intermediate dose versus placebo and high dose versus placebo. The baseline 25(OH)D level of participants ranged between 11 and 15 ng/ml. An intermediate vitamin D dose (weighted mean dose of 1,750 IU/d), compared to placebo, resulted in a WMD in the achieved 25(OH)D level of 14.7 ng/ml, while a high vitamin D dose (weighted mean dose of 4,851 IU/d, dose that is 2-3 folds the intermediate dose) versus placebo, resulted in only a slight further increment in the WMD of 25(OH)D level achieved, reaching 18.3 ng/ml. The achieved weighted mean 25(OH)D levels was 28.9 ng/ml in the intermediate dose and 38 ng/ml in the high dose, corresponding to 71% and 89% of the study subjects reaching the target level of 20 ng/ml in the aforementioned dose groups, respectively. The increment in 25(OH)D level was in the intermediate dose around 1 ng/ml for each 100 IU/d vitamin D, and in the high dose around 0.46 ng/ml for each 100 IU/d of vitamin D. A previous meta-analysis by Shab-bidar et al comparing various vitamin D doses, versus placebo, showed that the WMD in 25(OH)D level achieved was lower with doses >800 IU daily (13.7 ng/ml), compared to those at 800 IU daily (15.7 ng/ml). The meta-analysis by Cashman et al., aiming at informing European Guidelines in 2011, showed that the achieved 25(OH)D level increases minimally at doses ≥ 1,200 IU daily (88). Similarly, the IOM report showed that the response to vitamin D supplementation is blunted at high doses (68). All these results from our meta-analysis and others conducted in Western countries, unequivocally confirm that, in adults, the achieved 25(OH)D level increases in parallel to the increase in the vitamin D dose administered. However, the increments in 25(OH)D level, per 100 IU/d vitamin D follow a curvilinear pattern, suggesting a plateau in the 25(OH)D level at a certain dose threshold. In the low dose versus placebo comparison in adults, we identified only one small study (128). Starting at 8.6 ng/ml, the low dose group achieved a 25(OH)D level of 18.6 ng/ml, corresponding to 54% reaching the target

level of 20 ng/ml, and an increase in 25(OH)D level of 2.50 ng/ml per 100 IU/d of vitamin D. Cashman et al using low doses of vitamin D 200-600 IU daily versus placebo, in adults (age 20-40 years) form UK, showed that, starting at a 25(OH)D level of 28 ng/ml, the increase in 25(OH)D was around 1.96 ng/ml per 100 IU/d vitamin D (140). He demonstrated that a dose of 1,000-1,120 IU/d is needed to maintain 25(OH)D level > 20 ng/ml in 95-97.5% of the population (140). These findings show that, even in UK adults, who have relatively high 25(OH)D levels, an intermediate dose is needed to maintain the majority of the population vitamin D replete at 20 ng/ml, as it has been shown in the MENA region (Table 7).

In pregnancy, the weighted mean 25(OH)D level increased from 7.8 ng/ml to 26.5 ng/ml with an intermediate vitamin D dose (weighted mean dose of 1,832 IU/d); an increase equivalent to 1 ng/ml per 100 IU/d vitamin D. Starting at the same baseline level, the weighted mean 25(OH)D level reached 18.5 ng/ml with a weighted mean low dose of 300 IU/d; an increase equivalent to 3.57 ng/ml per 100 IU/d vitamin D. Starting at 11 ng/ml, a high dose (weighted mean dose 3,662 IU/d) increased the weighted mean 25(OH)D level to 35 ng/ml; an increase equivalent to 0.66 ng/ml per 100 IU/d vitamin D. The proportions of pregnant women reaching the target of 20 ng/ml were 41-43%, 73% and 90-94% in the low, intermediate and high dose, respectively. A study conducted in American pregnant women, with baseline 25(OH)D level of 23-24 ng/ml, comparing 3 doses of vitamin D (400, 2,000 and 4,000 IU/d), showed that, after 6 months of intervention, 25(OH)D levels at delivery were 31.6 (14.6), 39.4 (13.7), 44.5 (16.2) ng/ml, in the low, intermediate and high dose, respectively (104). The estimated proportion of women reaching the target level of 20 ng/ml were 78%, 92%, 93%, in the aforementioned doses, respectively. In another three-arm study (600, 1,200 versus 2,000 IU/d) from Turkey, starting at a lower baseline 25(OH)D of 9.9-11 ng/ml, the highest dose allowed to 80% of pregnant women

to reach the target level of 20 ng/ml, while the other doses allowed to < 50% of the participants to do so (141). These results show again that, even in western countries, a low dose of vitamin D does not allow to the majority of pregnant women to reach the IOM desirable level. On the other hand, a recent meta-analysis of RCTs in pregnancy, by Perez – Lopez et al., showed that vitamin D supplementation, compared to no supplementation, increases 25(OH)D level by 26.6 (26.5-26.7) ng/ml, using a fixed effect model. The equivalent daily vitamin D (D2 or D3) doses administered in the included studies were variable and ranged between 400 and 7,140 IU (63). Unfortunately, we could not compare these findings to ours, since our comparisons in pregnant women did not include a placebo arm.

In children and adolescents, an intermediate dose around 1,870 IU/d increased the weighted mean 25(OH)D level from 14 ng/ml to 31.6 ng/ml, an increase equivalent to 0.94 ng/ml per 100 IU/d vitamin D, that brought 73% of the participants to target. The effect of a low dose of 200 IU/d did not differ significantly from the placebo. However, in the latter comparison, the high quality trial by El Hajj Fuleihan showed that a low dose of 400 IU daily resulted in a significant increment in 25(OH)D level by 4 ng/ml (increment equivalent to 1 ng/ml per 100 IU/d vitamin D), allowing to 34% of children to reach the target level of 20 ng/ml. Data on the vitamin D dose response in children and adolescents from Europe and US are different, showing higher increments at low to intermediate doses, despite higher baseline 25(OH)D level in Western populations. The meta-analysis by McNally et al, compiling results from studies conducted in the US, Europe and Asia, showed that, in the pediatric and adolescents population, a cumulative vitamin D dose of 1,000 IU over 30 days increased 25(OH)D level by 3.6 ng/ml (92). Cashman et al pooled the results of 2 RCT from Finland and Denmark, conducted in adolescent girls with baseline 25(OH)D level of 22.7 ng/ml. and administering 2 low doses of

vitamin D, 200 and 400 IU daily, compared to placebo (142). He showed that the increment in 25(OH)D level parallels the increment in the vitamin D supplementation dose and is equivalent to 2.43 ng/ml for every 100 IU/d (142). Based on his findings, a vitamin D dose around 750 IU/d, 25% higher than the IOM RDA is needed to allow to 97.5% of adolescent girls to reach the target of 20 ng/ml (142). Interestingly, a study from the US, administering increasing doses of vitamin D 400 IU/d, 1,000 IU/d, 2,000 IU/d and 4,000 IU/d, compared to placebo, to white and black children (baseline 25(OH)D level 26.4-28 ng/ml), showed that, in white children, the low dose effect was not significantly different from placebo (143). In addition, the effect of the 2,000 IU/d dose allowed an increase in 25(OH)D level by 15 ng/ml (143), increment that is very close to our results of the intermediate dose versus placebo comparison WMD 15.77(8.68-22.87).

Noteworthy that the higher increments in 25(OH)D level in pregnant women (3.57ng/ml per100 IU/d vitamin D, from our data) and children (2.43 ng/ml per 100 IU/d, from Cashman data (142)), despite modest doses, may in part reflect better gastrointestinal absorption in these subgroups, that may in part be explained by accompanying hormonal changes with growth/puberty and pregnancy.

We have shown that, in adults, pregnant women and elderly from the MENA region, a low vitamin D dose of 300-600 IU/d increased 25(OH)D level by 1-3.57 ng/ml per 100 IU/d vitamin D and allowed to 40-54% of the population to reach the target level (Table 7). In adults, pregnant women and children/adolescents, an intermediate dose around 1,800 IU/d increased 25(OH)D level by 0.84-1 ng/ml per 100 IU/d vitamin D, and allowed to 71-73% to reach the target level. In elderly, adults and pregnant women, a high dose of 3,700 - 4,850 IU/d increased 25(OH)D level by 0.46-0.66 ng/ml per 100 IU/d vitamin D, and allowed to 89-98% of the population to reach the target level (Table 7). Therefore, the IOM RDA of 600-800 IU/d,

recommended for all age categories and pregnant women, does not seem enough to raise levels to target in the majority of individuals, in populations from our region. An intermediate dose, that is 2-3 folds the dose recommended by the IOM, allows to two-thirds of the population to reach the target level. Noteworthy, the WHO guidelines on vitamin D replacement, published in 2010, only addressed pregnant women to-date and have recognized the need for vitamin D supplementation in countries with a high prevalence of vitamin D deficiency, as is the case in the MENA region. However, the dose needed was not defined (144).

Indeed, an intermediate dose (800-2,000 IU/d) remains below the upper limit of intake set by the IOM at 4,000 IU/d and is not expected to be associated with any risk of vitamin D toxicity, at least as evident form studies using doses that extended over several months. Unfortunately, although more than half of the studies administered a high dose of vitamin D, adverse events were poorly reported, and therefore, we could not confirm the safety of high vitamin D doses in our region. In fact, across all comparisons and age groups, only 3 studies (one in children and adolescents (40) and 2 in elderly (105, 115)) reported on adverse events following supplementation. The other studies did not discuss adverse events at all, or reported "No" adverse events. Such results seem inaccurate, since an adverse event in clinical trials is defined as "any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related", as per the National Heart Blood and Lung Institute (NHLBI) (145). Accordingly, it is almost unrealistic not to have at least few adverse events with any intervention.

In addition to the vitamin D dose, variability in the baseline 25(OH)D level should not be overlooked. Based on the results from our meta-analysis (Table 7), an inverse relationship between the baseline vitamin D status and the achieved 25(OH)D level following intervention

may be suggested. Noteworthy that the effect of baseline 25(OH)D level depends on the outcome considered. It is a positively correlated with the achieved 25(OH)D level following supplementation (92), and negatively correlated with the change in 25(OH)D level, a lower baseline level resulting in further increments (89, 90).

Based on the results from the meta-analysis, the lack of consistent effects of vitamin D supplementation on increments in 25(OH)D levels across studies within each age group may reflect different baseline levels, different doses, differences in compliance, on which information was lacking, differences in dietary calcium and vitamin D intake, and possibly different vitamin D assays.

The meta-regression analysis confirmed statistically the results derived from the standard meta-analysis. It allowed to identify significant predictors of 25(OH)D level following intervention. Vitamin D dose (100 IU/d) and baseline 25(OH)D level (ng/ml) were the most powerful significant predictors, allowing to explain 87% of the variability in 25(OH)D level achieved following intervention. Based on the multivariate model (Table 5A), the increase in 25(OH)D level approximated 0.44 ng/ml per 100 IU/d vitamin D, and 0.77 ng/ml for each increase in baseline 25(OH)D level of 1 ng/ml. This increment was mostly driven by the effect of the high dose arms included and that constituted 65% of all the intervention arms. Indeed, this increment is very close to the increments estimated with high doses in adults and elderly in the standard meta-analysis, ranging between 0.38-0.40 ng/ml per 100 IU/d vitamin D (Table 4). The results of our meta-regression favored a linear model in both single variate (Figure 5) and multivariate analysis (Appendix 8). Although several papers showed that a curvilinear model, with natural logarithmic (Ln) transformation of the vitamin D dose, predicted better the increase in 25(OH)D level following supplementation (68, 88, 89), others have successfully used the

vitamin dose, rather than Ln dose, in their regression models (87, 90, 92). Seamans et al. pooled results from studies conducted at all age categories and showed that the increase in 25(OH)D was equivalent to 0.50 ng/ml per 100 IU/d vitamin D (87). Shab-bidar et al conducted his meta-regression in adults and elderly and found that the mean difference in 25(OH)D level in intervention versus placebo arms was around 0.24 ng/ml per 100 IU/d vitamin D (90). In the latter meta-regression, only 3 intervention arms fell into the high dose category (>2,000 IU/d). Results on other predictors, including baseline 25(OH)D level, age, BMI and concomitant calcium supplementation were also consistent with findings from Western reviews published on the topic (Table 2). Unfortunately, we were powered to demonstrate a significant effect of only one predictor, other than the vitamin D dose, that was the baseline 25(OH)D level. The vitamin D dose response curve in the MENA region may be close to the one characterized in Western countries, and the higher requirements may be driven by various factors, most importantly the lower baseline 25(OH)D levels, but also concomitant calcium supplementation and BMI. Unfortunately, a larger number of studies was needed to be able to confirm the significant impact of the latter factors.

The large variability in the vitamin D assays used in the identified studies is another factor significantly affecting 25(OH)D levels achieved. Only one study conducted in the elderly (105) and one study in children (135) used the highly accurate assay, HPLC. Quality assurance programs of vitamin D assays were described in only 2 trials from the same group (40,105,134). This is an important point in view of the high variability in accuracy and precision between assays (3), and the impact this may have on ultimate results obtained. Unfortunately, we could not, in light of relatively small number of studies, evaluate the impact of vitamin D assays on our results. Only in one comparison, intermediate dose versus placebo in adults, the Enzyme

Immuno-Assay (EIA) was used in the 2 studies that were included (125, 126). In other comparisons in elderly, adults, pregnant women, and children/adolescents various assays, including direct competitive chemiluminescence, radioimmunoassay, chemiluminescence immunoassay and enzyme-linked immunosorbent assay (ELISA) were used.

Subgroup analysis based on the intervention duration (3 months versus more than 3 months) showed significantly lower 25(OH)D levels with duration > 3 months. Several studies have showed that the duration may be a negative predictor and that the achieved 25(OH)D level decreases at the end of the study compared to levels achieved earlier at 3 to 6 months of the intervention (103, 104, 127, 146). In our case, studies of 3 months duration administered higher doses of vitamin D (dose range: 5,000-7,140 IU/d), compared to those that extended more than 3 months (dose range: 400-3,500 IU/d except one study administering 4,300 IU/d) (see Table 3). In addition, adherence to vitamin D supplementation may decrease with increased duration, and thus negatively affecting the 25(OH)D level achieved. Unfortunately, changes in compliance to study intervention cannot be confirmed given the scarce information reported on compliance in individual studies. Only three studies reported overall compliance. Only one study reported on compliance at each trial visit (see Table 3).

We could not demonstrate a significant effect of baseline 25(OH)D level (≤ 20 ng/ml versus > 20 ng/ml) and BMI on the achieved 25(OH)D level following intervention; this is most likely related to the limited number of studies allowing this comparison.

Data on the effect of vitamin D supplementation on various skeletal and extra-skeletal outcomes and surrogate markers was limited. No data on the vitamin D on fracture risk and BMD in the MENA, with the exception of one study in children/adolescents and one study in elderly, published by the same group, reporting the effect of vitamin D supplementation on BMD

(40, 105, 134). Vitamin D supplementation in adults, pregnant women and children/adolescents, including high doses, did not increase serum calcium level but reduced PTH level significantly in children. An intermediate dose, of around 1,800 IU daily, compared to placebo, resulted in a significant decrease in PTH level of 7 pg/ml, in school children boys and girls (40, 136). In adults, a high dose of vitamin D, compared to placebo, reduced systolic blood pressure significantly by 3.5 (0.76; 6.30) mmHg (117, 118, 121) and improved slightly insulin sensitivity by 1.21 (0.96; 1.46) (117, 120, 123). No effects were detected on lipid profile, HbA1c or body weight (116-118, 120-122).

4.2. Limitations and strengths

Our review has several limitations. A large number of studies come from one country, Iran. In adults and elderly, 9 out of 15 studies come from Iran. Therefore, the results may not be representative of all the MENA countries. In addition, several factors that could have affected the effect size of the intervention were poorly described. Dietary vitamin D and calcium intake was not taken into consideration, as it was infrequently reported in the individual studies. The season and the clothing style were not mentioned, except in few studies, and none of the studies quantified accurately sun exposure. Compliance to vitamin D supplementation was described only in 8 out of 25 studies. Furthermore, all the data described in adults are derived from studies conducted on individuals with diseases including diabetes mellitus, obesity, polycystic ovaries syndrome, and others. Only 1 study was conducted in healthy non obese subjects. The variability in vitamin D assays used in the included studies remains a major limitation of data pooling, as it would significantly affect the derived conclusions. In addition, the quality of several included studies is low and resulted in downgrading of the evidence derived from these trials. Finally, the

results of the meta-regression remain retrospective and observational, and do not allow to establish causality (108). Aggregate data, rather than individuals' data, are used in meta-regression, and therefore, the results are prone to aggregation bias or ecological fallacy (108).

However, this meta-analysis and meta-regression fills an important knowledge gap on this public health topic in the region. Indeed, this is the first systematic review and meta-analysis in the MENA region assessing the dose response of vitamin D in this population specifically; it allows one to explore the applicability of the IOM recommendations in this region. The eligibility criteria were chosen to avoid bias and limitations of previously published systematic reviews, assessing the effect on vitamin D on various outcomes. Furthermore, the search methodology was very extensive, including 5 international databases, in addition to two other databases relevant to the region (search updated in July 2015), and clinical trials registries that were searched for potentially completed unpublished trials. This review sheds light on the availability and on the quality of trials addressing the effects of vitamin D supplementation on vitamin D levels, and therefore, identifies several knowledge gaps relevant to this topic, and allows one to draft priorities for future research agendas. In particular, the quality of the trials identified and of the vitamin D assays is not optimal, and the data on the effect of vitamin D supplementation of health outcomes, musculo-skeletal and others, is scarce.

4.3. Conclusion and recommendations

A rigorous assessment of the vitamin D dose-response is essential to allow one to set dietary recommendations and guidelines. The IOM vitamin D recommended doses, targeting Western countries, may not be sufficient to allow the majority of individuals from the MENA, to reach the desirable 25 (OH)D level of 20 ng/ml, as set by the IOM. Indeed, our analyses

demonstrate that doses that are at least two to three folds the IOM RDA may be needed. Indeed, findings from our meta-analysis will inform population specific recommendations on vitamin D doses in various age groups, as recommended by the IOM, but also by WHO and regional and national health authorities, and provide the needed information to formulate MENA specific vitamin D guidelines. Our findings set the stage for the formulation of a region specific research agenda on this important public health topic.

Figure 1: Flow diagram of the different phases of the systematic review

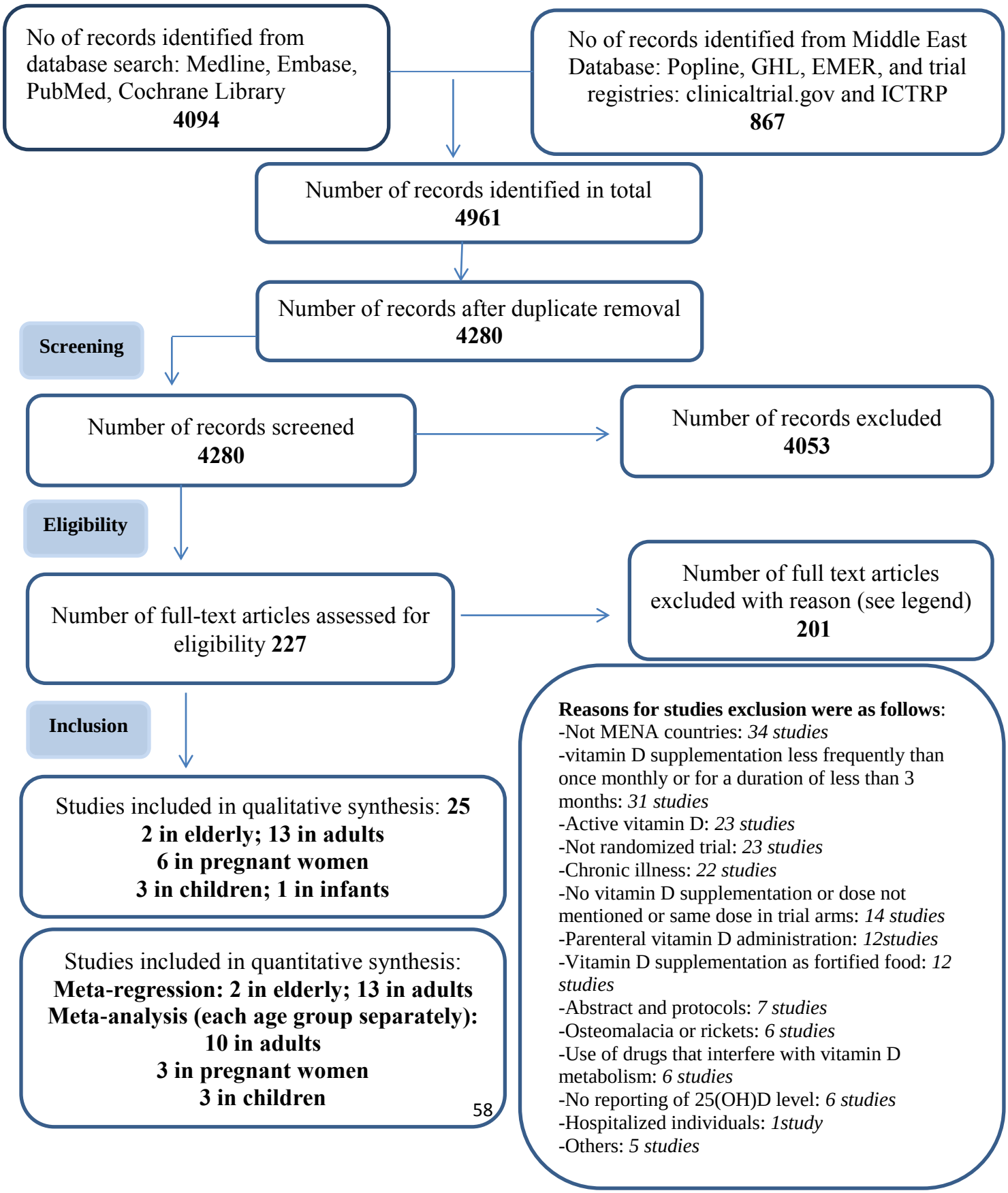
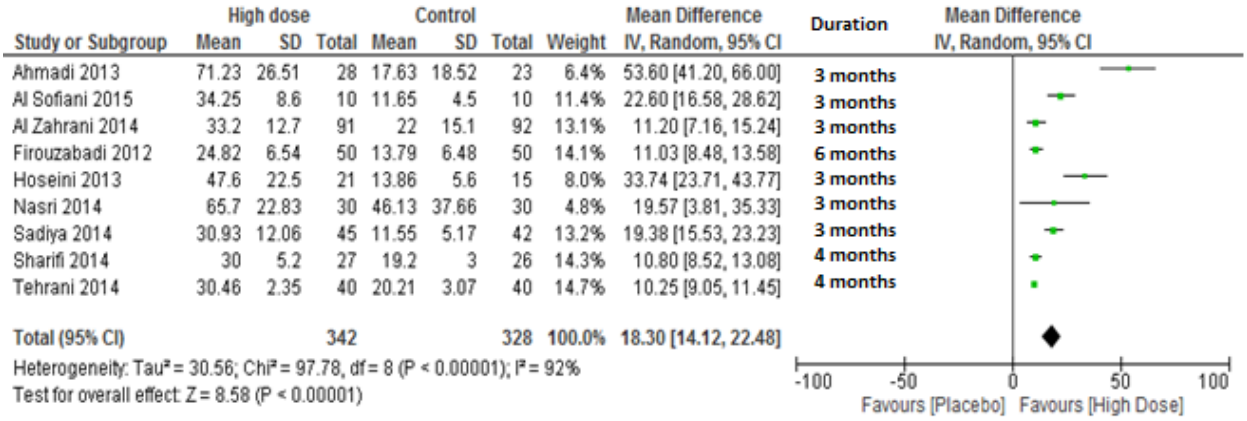
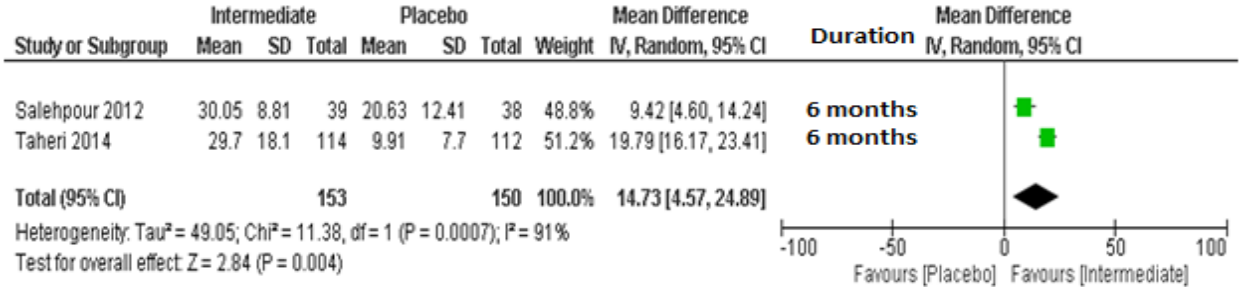


Figure 2 A: High dose (>2,000 IU/d) versus placebo comparison in adults



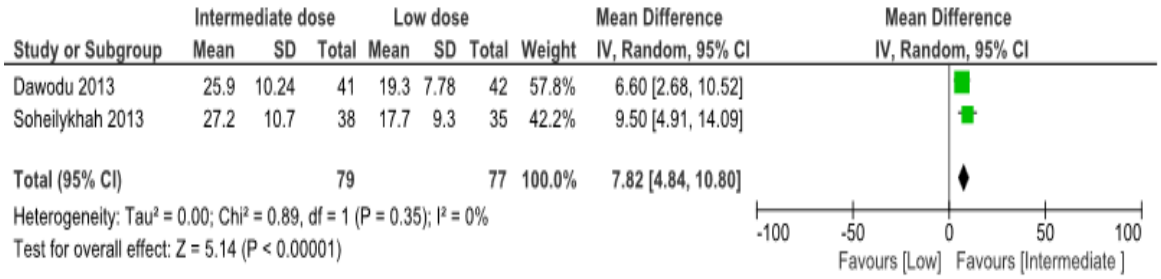
The vitamin D equivalent daily doses administered in the high dose group were as follows: Ahmadi 2013: 7,140 IU/d; Al Sofiani 2015: 5,000 IU/d; Al Zahrani 2014: 6,428 IU/d; Firouzabadi 2012: 3,333 IU/d; Hoseini 2013: 7,140 IU/d; Nasri 2014: 7,140 IU/d; Sadiya 2014: 6,000 IU/d; Sharifi 2014: 3,571 IU/d; Tehrani 2014: 3,571 IU/d

Figure 2 B: Intermediate dose (800-2,000 IU/d) versus placebo comparison in adults



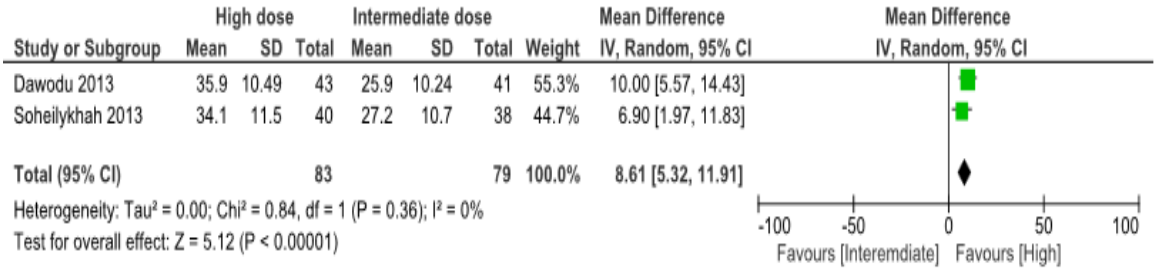
The vitamin D equivalent daily doses administered in the intermediate dose group were as follows: Salehpour 2012: 1,000 IU/d; Taheri 2014: 2,000 IU/d.

Figure 3A: Intermediate dose (800-2,000 IU/d) versus low dose (<800 IU/d) comparison in pregnant women



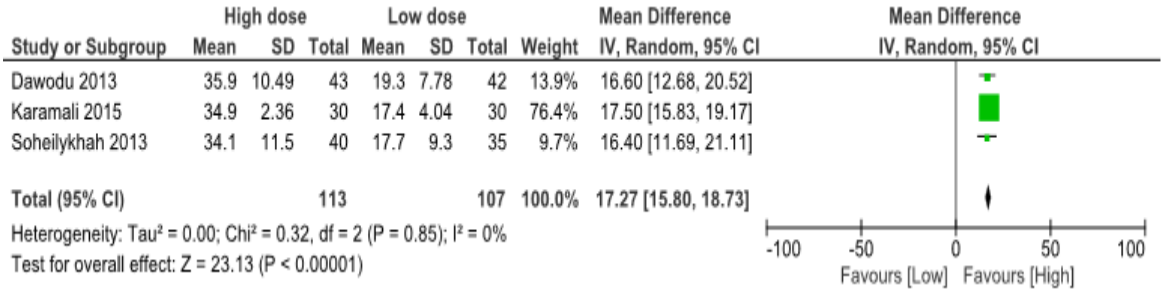
Vitamin D supplementation started early second trimester and continued until delivery.
Vitamin D equivalent daily doses were as follows: Dawodu 2013: 2,000 IU/d versus 400 IU/d; Soheilykhah 2013: 1,660 IU/d versus 200 IU/d.

Figure 3B: High dose (>2,000 IU/d) versus intermediate dose (800-2,000 IU/d) comparison in pregnant women



Vitamin D supplementation started early second trimester and continued until delivery.
Vitamin D equivalent daily doses were as follows: Dawodu 2013: 4,000 IU/d versus 2,000 IU/d; Soheilykhah 2013: 3,571 IU/d versus 1,667 IU/d.

Figure 3C: High dose (>2,000 IU/d) versus low dose (<800 IU/d) comparison in pregnant women



Vitamin D supplementation started early second trimester and continued until delivery.
Vitamin D equivalent daily doses were as follows: Dawodu 2013: 4,000 IU/d versus 400 IU/d; Soheilykhah 2013: 3,571 IU/d versus 200 IU/d

Figure 4: High versus intermediate dose comparison in venous cord

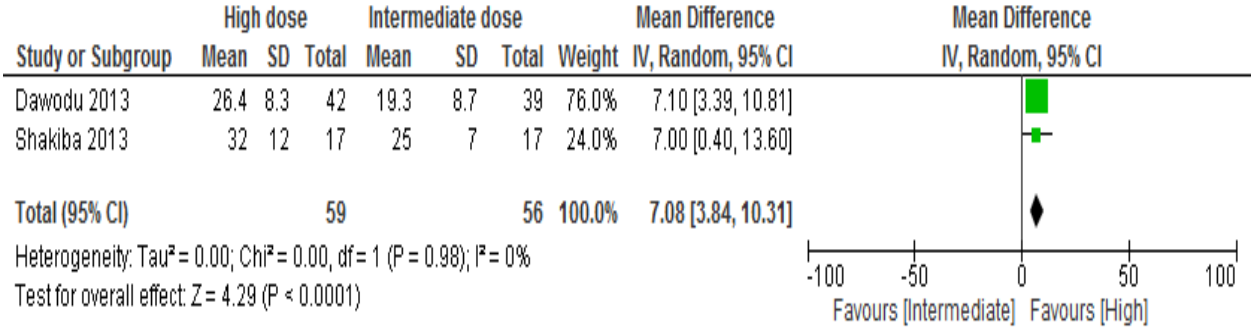
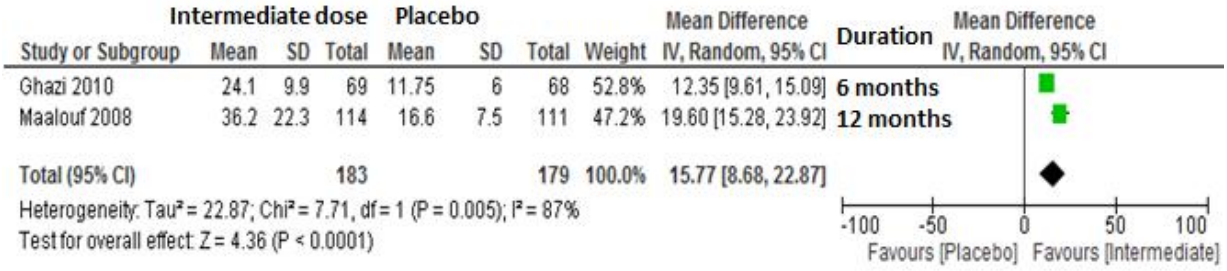
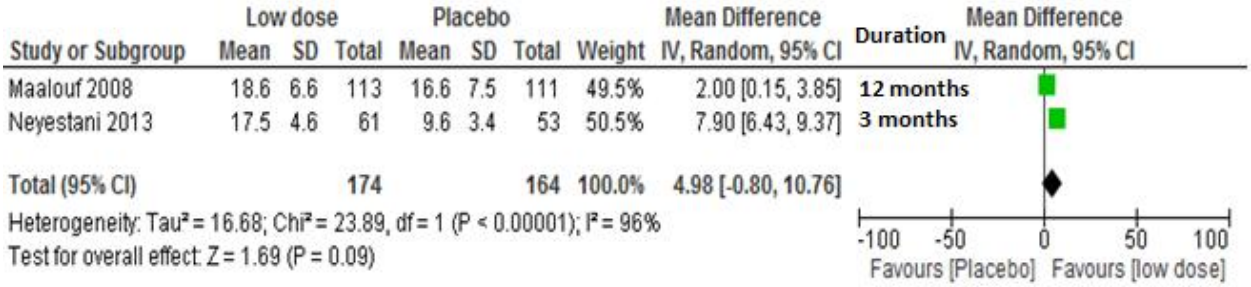


Figure 5A: Intermediate dose (800-2,000 IU/d) versus placebo comparison in children and adolescents



Vitamin D equivalent daily doses administered in the intermediate dose group were as follows: Maalouf 2008: 1,400 IU/d; Ghazi 2010: 1667 IU/d

Figure 5B: Low dose (<800 IU/d) versus placebo comparison in children and adolescents



Vitamin D equivalent daily doses administered in the intermediate dose group were as follows: Maalouf 2008: 200 IU/d; Neyestani 2013: 200 IU/d

Figure 6: Single variable random-effects meta-regression of the effect of (a) the vitamin D dose (IU/day) and (b) the natural log (ln) of the vitamin D dose (IU/d) of 25(OH)D level reached (ng/ml)

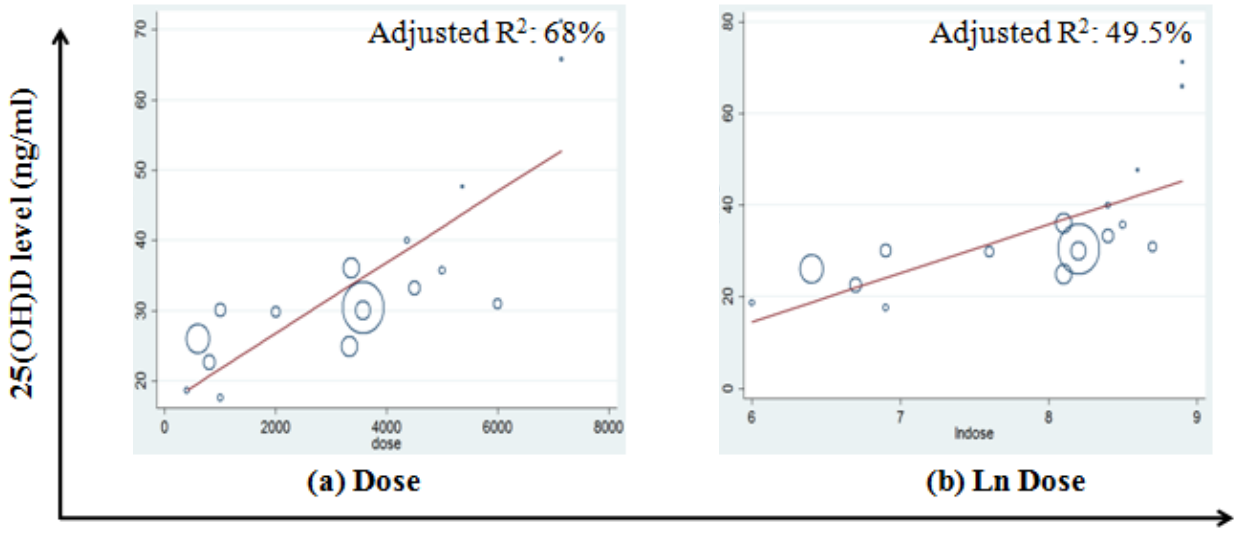
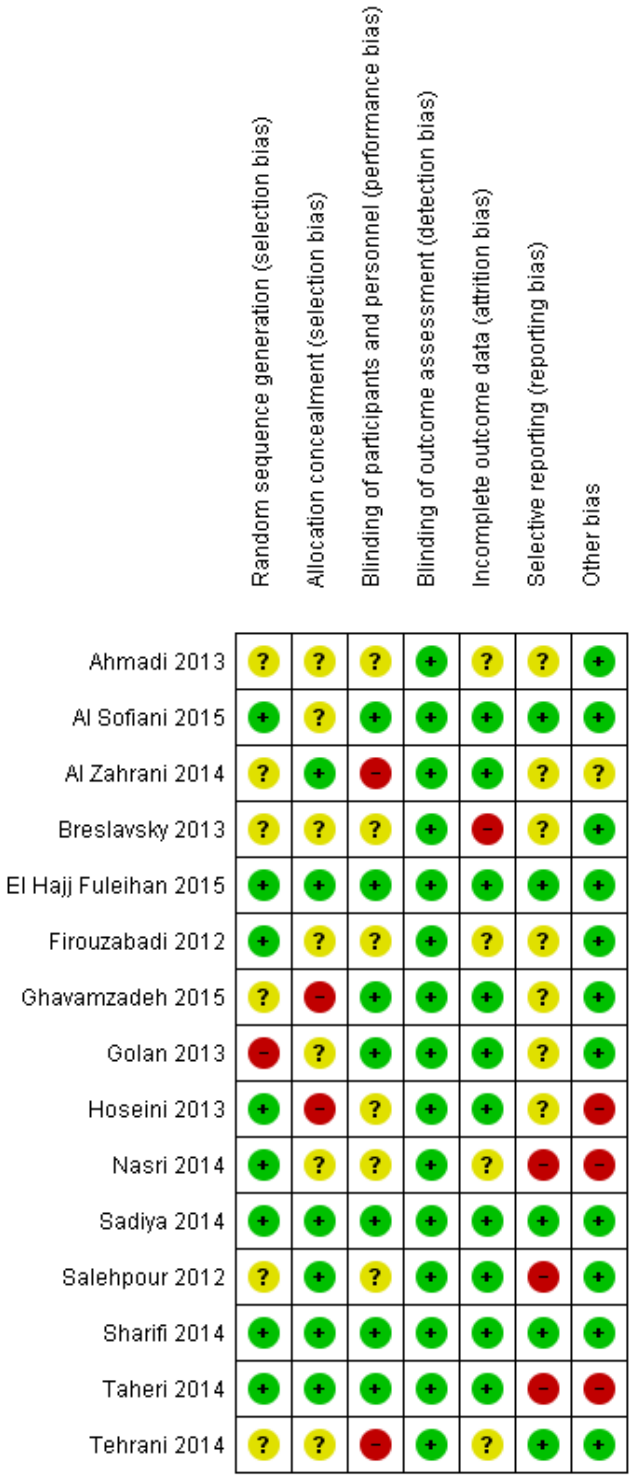
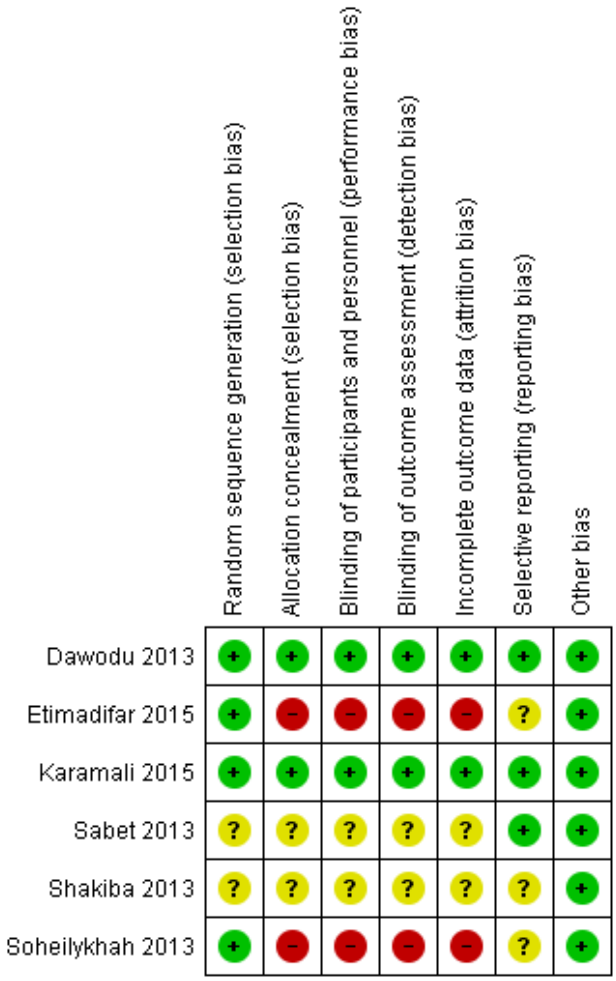


Figure 7A: Risk of bias assessment in studies conducted in adults and elderly



A green color and (+) sign represent a low risk of bias; A yellow color and (?) sign represent an unclear risk of bias; A red color and a (-) sign represent a high risk of bias.

Figure 7B: Risk of bias assessment in studies conducted in pregnant women



A green color and (+) sign represent a low risk of bias; A yellow color and (?) sign represent an unclear risk of bias; A red color and a (-) sign represent a high risk of bias.

Figure 7 C: Risk of bias assessment in studies conducted in children and adolescents

A green color and (+) sign represent a low risk of bias; A yellow color and (?) sign represent an unclear risk of bias; A red color and a (-) sign represent a high risk of bias.

Figure 8: Inverted funnel plot for trials in adults administering a high vitamin D dose versus placebo

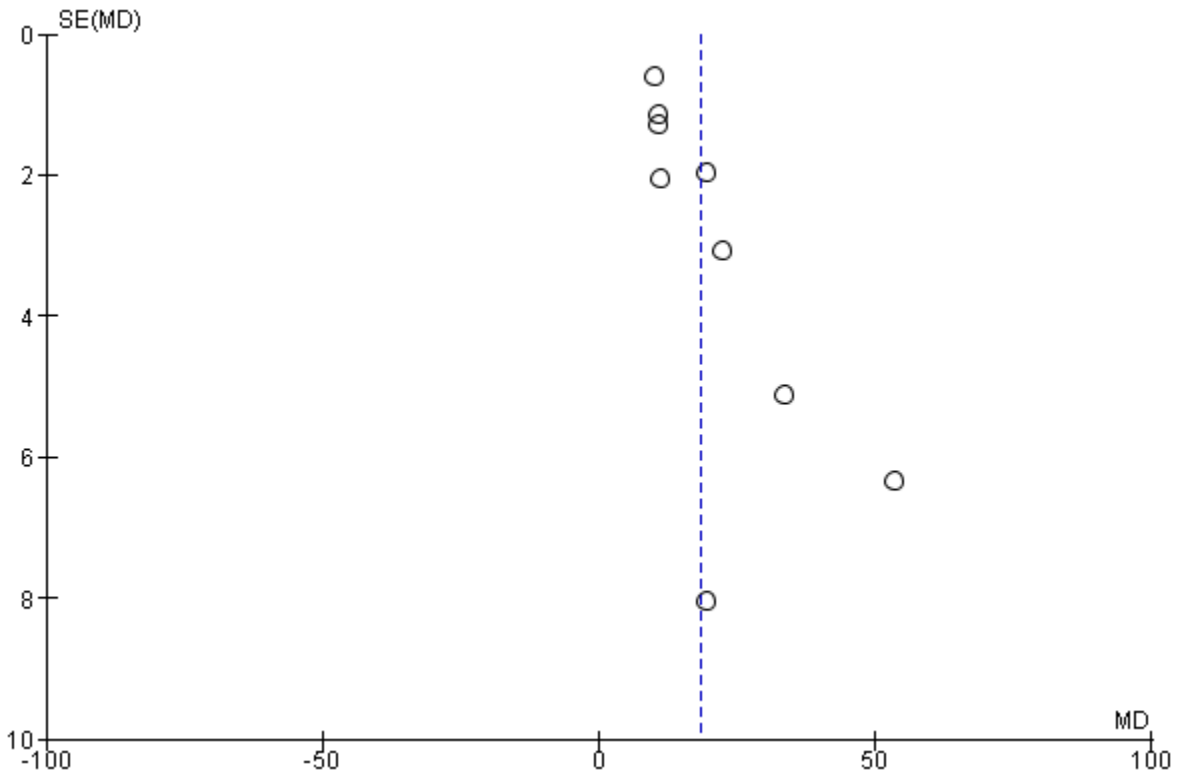


Table 1: Vitamin D systematic reviews and meta-analysis of randomized controlled trials summary (2010- July 2015)

Outcome Author year	Number of studies included	Vitamin D dose range IU/d[1]	Results	Absent pre and/or post intervention 25(OH)D level	25(OH)D before intervention >30 ng/ml or following intervention < 25 ng/ml	Duration of supplementation < 3 months	Parenteral/ high spaced vitamin D dose (more than one month between doses)	Active vitamin D	Risk of bias
I- Musculo-skeletal outcomes									
Fractures and BMD									
Fractures Bergman 2010 (33)	8	700-830	Significant decrease in non-vertebral fractures OR 0.77 (0.60, 0.93) and hip fractures OR 0.70 (0.53, 0.90). Non-significant effect on non-vertebral-non-hip fractures.	Levels not reported	Levels not reported	-	1	-	Not reported
Cancer and fractures Chung 2011 (same as 74)	19	300-1,000	Vitamin D alone: non-significant effect on mortality and cancer Combined Calcium and vitamin D reduced fracture risk RR 0.88 (0.78, 0.99); results on cancer were inconsistent.	8	1	1	1	-	Low to high
Fractures Bischoff 2012[2] (31)	11	400-2,000	At doses 792-2,000: Significant reduction in hip fractures HR 0.70 (0.58, 0.86) and non-vertebral fractures HR 0.86 (0.76, 0.96).	Levels not reported	Levels not reported	1	0	0	Not reported
Fractures and other outcomes Avenell 2014 (34)	53	400-2,285	Vitamin D and Calcium: Significant reduction in hip fracture RR 0.84 (0.74, 0.96), non-vertebral fracture and any type of fracture RR 0.95 (0.90, 0.99) Vitamin D alone: Non-significant effect.	19	3	2	7	22	Low to high
Fractures and other outcomes Bolland 2014 (43)	40	400-9,000	No significant effect on mortality, cancer, MI and ischemic heart disease, stroke or cerebro-vascular disease, total and hip fractures[3]	8	4	-	7	-	Not reported

Outcome Author year	Number of studies included	Vitamin D dose range IU/d[1]	Results	Absent pre and/or post intervention 25(OH)D level	25(OH)D before intervention >30 ng/ml or following intervention < 25 ng/ml	Duration of supplementation < 3 months	Parenteral/ high spaced vitamin D dose (more than one month between doses)	Active vitamin D	Risk of bias
Bone mineral density Reid 2014 (42)	23	100-7,140	Significant increase in femoral neck WMD: 0.8 (0.2, 1.4). No significant effect on other sites.	4	2	-	7	-	Low to high
Muscle strength and fall									
Gait and Imbalance Muir 2011 (39)	13	800-2,500	Significant postural sway reduction SMD -0.20 (-0.39, - 0.01). Significant decrease in time to complete the Timed Up and Go Test SMD -0.19 (-0.35,-0.02), no effect of lower extremity strength.	3	3	1	2	1	Low to high
Falls Murad 2011 (36)	26	400-1,430	Significant reduction in falls OR 0.86 (0.77, 0.96).	12	9	3	7	-	Moderate
Falls Bolland 2014 (43)	20	800-1,670	No significant decrease in falls risk	4	3	-	7	-	Low to high
Muscle strength Beaudart 2014 (38)	31	400-8,570	Significant increase in muscle strength SMD 0.7 (0.03-0.31) No significant effect on muscle mass and power	8	2	3	4	-	Moderate to high
Muscle Strength Tomlinson 2014 (37)	6	4,000-8,570	Significant increase in upper and lower limb muscle strength SMD 0.32 (0.10, 0.54) and 0.32 (0.01, 0.63) respectively.	2	-	2	-	-	Not reported
II- Other outcomes									
Glycemic control									
Glycemic control and insulin resistance George 2012 (47)	15	400-4000	Combining all studies: No significant improvement in fasting glucose, HbA1c or insulin resistance. For patients with diabetes or impaired glucose tolerance:	3	1	4	4	5	Low to moderate

Outcome Author year	Number of studies included	Vitamin D dose range IU/d[1]	Results	Absent pre and/or post intervention 25(OH)D level	25(OH)D before intervention >30 ng/ml or following intervention < 25 ng/ml	Duration of supplementation < 3 months	Parenteral/ high spaced vitamin D dose (more than one month between doses)	Active vitamin D	Risk of bias
			Small significant improvement in fasting glucose SMD -0.32 (-0.57, -0.07) mmol/l and in insulin resistance SMD -0.25 (-0.48, -0.03)						
Glucose homeostasis Seida 2014 (48)	35	125-8,570	No significant effect on insulin sensitivity, insulin secretion, glucose control.	Levels not reported	Levels not reported	3	4	-	Low to high
Glycemic control Haroon 2015 (49)	17	400-5,700	Improvement in short term studies; no significant effect in long term studies	Levels not reported	Levels not reported	3	5	1	Low to moderate
Dyslipidemia									
Dyslipidemia Wang 2012	10	300-8,570	Significant increase in LDL level MD 3.23 (0.55, 5.90) mg/dl. No significant effect on total cholesterol, HDL and Triglycerides.	-	2	3	2	2	Low to high
Body weight									
BMI Mora 2013 (50)	9	200-1,100	No significant effect on BMI.	Levels not reported	Levels not reported	1	-	-	Low to moderate
Body weight Pathak 2013 (51)	12	400-8,570	No significant effect on body weight.	2	7	3	-	1	Low
Asthma									
Asthma Pojsupap 2014 (52)	5	500-2,000	Significant reduction in asthma exacerbation RR 0.41 (0.27, 0.63).	3	-	1	-	-	Low to moderate
Asthma Fares 2015 (56)	4	Doses not reported	No significant effect on FEV1.	Levels not reported	Levels not reported	Not reported	Not reported	Not reported	Low to high

Outcome Author year	Number of studies included	Vitamin D dose range IU/d[1]	Results	Absent pre and/or post intervention 25(OH)D level	25(OH)D before intervention >30 ng/ml or following intervention < 25 ng/ml	Duration of supplementation < 3 months	Parenteral/ high spaced vitamin D dose (more than one month between doses)	Active vitamin D	Risk of bias
Infection and inflammation									
Respiratory tract infection Bergman 2013 (53)	11	300-4,000	Significant protective effect OR, 0.64 (0.49, 0.84).	4	5	-	1	-	Low to high
Respiratory tract infection Mao 2013(55)	7	300-6,800	No significant effect on respiratory infections.	Levels not reported	Levels not reported	1	1	-	Low to moderate
Respiratory tract infection Charan 2015 (54)	5	400-2,000	Significant reduction in respiratory infection OR 0.58 (0.42 – 0.81)	Levels not reported	Levels not reported	-	1	-	Low to high
CRP level Chen 2014	10	400-7,143	Significant decrease in hs-CRP by -1.08 (−2.13, −0.03) mg/L	1 Levels post intervention not reported	- Levels post not reported	2	-	-	Low to high
Cardiovascular									
Cardiovascular outcomes Mao 2013 (59)	11	400-1,000	Effect of calcium or vitamin D: No significant effect on major cardiovascular events, myocardial infarction, stroke	Levels not reported	Levels not reported	-	1	-	Low to high
Cardiovascular disease Ford 2014 (58)	21	400-17,600	No effect on cardiac failure, MI, and stroke	10	-	-	2	5	Low to moderate
Blood pressure Beveridge 2015 (57)	52	200-7,000	No significant effect on blood pressure	7	2	9	12	9	Low to high
Mortality									
Mortality Zheng 2013 (46)	42	400-28,571	Significant decrease in all-cause mortality with a duration of follow-up longer than 3 years with a RR	10 Levels post intervention not	1	-	6	-	Low to high

Outcome Author year	Number of studies included	Vitamin D dose range IU/d[1]	Results	Absent pre and/or post intervention 25(OH)D level	25(OH)D before intervention >30 ng/ml or following intervention < 25 ng/ml	Duration of supplementation < 3 months	Parenteral/ high spaced vitamin D dose (more than one month between doses)	Active vitamin D	Risk of bias
			0.94 (0.90, 0.98). No significant benefit in shorter follow-up periods	reported					
Mortality Bjelackovic 2014 (44)	56	200-9,000	Significant effect on mortality RR 0.97 (0.94, 0.99) D3 only studies: RR 0.88 (0.78, 0.98)	Levels not reported	Levels not reported	1	1	4	Low to moderate
Cancer and mortality Keum 2014 (45)	4	400-1,100	No significant effect on cancer Significant decrease in mortality: RR 0.88 (0.78, 0.98)	1	-	-	1	-	Not reported
Depression									
Depression Li 2014 (62)	6	1,500-7,140	No significant effect on depression scores	1	1	2	1	1	Moderate to high
Depression Spedding 2014 (60)	15	400-18,400	Studies without flaws: Significant improvement in depression score SMD +0.78 (+0.24, +1.27). Studies with biological flaws: Significant worsening in depression scores SMD −1.1 (−0.7, −1.5).	Levels not reported	Levels not reported	9	0	1	Low to moderate
Depression Gowda 2015 (61)	9	400-7,140	No significant effect on depression scores	1 Levels post intervention not reported	- Levels post intervention levels not reported	3	1	1	Low to high
Pregnancy and neonatal outcomes									
Pregnancy and neonatal outcomes Perez Lopez 2015 (63)	13	400-7,140	Significant improvement in birth WMD 107.6 (59.9–155.3) g and birth length MD 0.3 (CI 0.10–0.41) cm. No effect on the incidence of preeclampsia, gestational diabetes, small for gestational age, low birth	Levels not reported	Levels not reported	2	4	-	Low to moderate

Outcome Author year	Number of studies included	Vitamin D dose range IU/d[1]	Results	Absent pre and/or post intervention 25(OH)D level	25(OH)D before intervention >30 ng/ml or following intervention < 25 ng/ml	Duration of supplementation < 3 months	Parenteral/ high spaced vitamin D dose (more than one month between doses)	Active vitamin D	Risk of bias
			weight, preterm birth, and cesarean section						

BMD: bone mineral density; BMI: Body mass index; MD: mean difference; MI: Myocardial Infarction; SMD: Standardized mean difference; WMD: weighted mean difference

The studies included in Table 1 are the result of a search on PubMed, using the Mesh Term "Vitamin D" and limiting the results by article type: "systematic reviews" and "meta-analysis" and year of publication "2010-2015". The data included in the table are as reported in the papers, main text or appendices/supplements. In case of missing data, the item was labeled as "not reported" without retrieving the individual trials. Systematic reviews without meta-analysis were not included in this table. The meta-analysis by Junyu et al (2014) also was not included since the majority of the studies considered in that review used active vitamin D.

[1] Excluding once only doses.

[2] Participant-level data meta-analysis.

[3] Pre-defined significant result if decrease in mortality > 5% and improvement by > 15% in other outcomes.

Table 2: Summary table of multivariate meta-regression analysis of previously published systematic reviews assessing the predictors of the vitamin D dose response

Author year	Number of included studies and countries	Age group	Vitamin D dose range (IU/d)	Dependent variable	Predictors assessed	Predictors included in the final model	Statistical software
Seamens 2009	32 studies US, UK, Europe, Middle East (one study form Lebanon[1])	All ages (including infants)	200-2,000	Achieved 25(OH)D level nmol/l	-Dose	-Dose (IU/d) (β 0.013) *(For every 100 IU/d vitamin D, 25(OH)D level increases by 1.3 nmol/l= 0.52 ng/ml)*	NA
Institute Of Medicine 2010	20 studies included in meta regression UK and Europe (Latitude >40 ^{0}N and conducted during winter)	All ages	Supplement and dietary 100-2,400	Achieved 25(OH)D level nmol/l	-Vitamin D intake -Age -Baseline 25(OH)D level -Latitude	Separate analysis based on Latitude <40 to <49.5^{0}N -Ln Vitamin D intake (IU/d) (β 12.3) >49.5^{0}N -Ln Vitamin D intake (IU/d) (β 9.9)	STATA
Cashman 2011	19 studies included in meta-regression UK and Europe (Latitude >40 ^{0}N and conducted during winter) (7 studies were common with IOM)	Mean age range: 9-74	Supplement and dietary 400-2,000	Achieved 25(OH)D level (nmol/l)	-Vitamin D intake -Age -Latitude	Separate analysis based on Latitude <40 to <49.5^{0}N -Ln Vitamin D intake (IU/d) (β 12.6) >49.5^{0}N -Ln Vitamin D intake (β 9.2) A linear model (without Ln transformation of vitamin D intake) was also tried	SPSS
Autier 2012	74 studies (98 intervention groups) UK, US, Australia and Europe	Adult >50 years	400-2,140 (three studies with higher doses were excluded from analysis)	Change in 25 (OH)D level per arm (ng/ml)	-Dose (log transformation) -Type of vitamin D -Calcium co-administration -25OH D baseline -Gender -Age -Type of population -Length of follow-up -Country -Publication year	- Ln Dose (mcg/d) (β 6.78) -Type of vitamin D (β-4.18(D2 compared to D3); -Ca supp (β -2.72, yes compared to no; not significant) -25OH D baseline (ng/ml) (β -0.12; not significant)	SAS
Shabbidar 2013	33 US, UK, Europe, one study from Africa	Mean age range: 22-84.9	200-5,000 IU daily	Pooled mean difference in achieved 25(OH)D level (nmol/l)	-Dose -Duration -Baseline 25(OH)D -Age	-Dose (IU/d) (β 0.006) *(For every 100 IU/d vitamin D, the pooled mean difference in 25(OH)D level increases by 6 nmol/l=2.4 ng/ml)* -Duration (months) (β 0.21) -Baseline 25(OH)D3 (nmol/L) (β _0.19) -Age (years) (β 0.42)	Comprehensive Meta-analysis and SPSS

Author year	Number of included studies and countries	Age group	Vitamin D dose range (IU/d)	Dependent variable	Predictors assessed	Predictors included in the final model	Statistical software
McNally 2014	88 studies (134 intervention groups) US, Europe, Middle East [3], Asia	Pediatrics including adolescents	>1,000	25(OH)D level post intervention(nmol/l)	-Baseline 25(OH)D level -Age -Disease status -Dosing regimen (dose, frequency, form, route) -Duration -Vitamin D assay -Study type -Study quality	-Baseline 25(OH)D level (nmol/l) (β 0.84) -Age (years) (β -0.54, not significant) -Disease status(β -19.5, diseased compared to healthy) -Cumulative dose (per 1,000 IU for 30 days) (β 0.27) -Loading dose (β 43.8, loading dose compared to other forms) -Duration (weeks) (β 0.02, not significant) (was a negative predictor in the model that didn't include study type, but not-significant) -Study type (β 34.95, non RCT compared to RCT)	SAS
Zitterman 2014	94 trials (144 intervention groups) US, Europe, one study from Africa	>10 years	> 0.1 mcg/kg/day	Change in 25(OH)D level (nmol/l)[3]	-Dose -Age -Ethnicity -Diseases -Frequency and duration of intake -Type of vitamin D Supplement -Baseline 25OHD level -Vitamin D producer -Vitamin D assay -Calcium co-administration	-Ln Dose (mcg/kg/day) (β 16.03) -Age (years) (β 0.22) -Type of vitamin D (β -20.19, D2 compared to D3) -Calcium supplementation (β -6.34, Yes compared to No) -Baseline 25(OH)D level (nmol/l) (β -0.13)	SPSS

[1] El Hajj Fuleihan 2006

[2] 45 intervention groups were from the Middle East.

[3] The reported adjusted R^2 of the model is 54%.

25(OH)D level conversion factor 1 ng/ml=2.496 nmol/l

Table 3: Characteristics of included studies in pre-specified age groups by treatment arm

Author Year	City Latitude Country	Sampling method/ setting	Intervention Duration	Ca supp	Nb of subject random-ized per arm	Nb of subject lost to follow up	Gender (% Male per arm)	Age Mean (SD) or median (range) (years)	BMI Mean (SD) or median (range) (kg/m^2)	Baseline mean (SD) or median (range) 25(OH)D (ng/ml)	Achieved mean (SD) or median (range) 25(OH)D (ng/ml)	Vitamin D assay	Co-morbidities	Compliance	Adverse events or serious adverse events
Adults and Elderly[1]															
Ahmadi J Res Med Sci 2013 (116)	Isfahan, Iran 32.6 ˚N	Isfahan endocrine and metabolism research center	I: D3 50.000 IU weekly (= 7,142 IU/d) C:PBO Duration: 3 months	No	I : 30 C: 30	I : 2 C: 7	I : 42.9 C: 30.4	I : 58.3 (11.1) C: 57.1 (10.7)	I : 28.4 (4.1) C: 29.4 (4.8)	I : 14.1 (7.8) C: 16.1 (6.1)	I : 71.2 (26.5) C: 17.6(18.5)	Direct competitive chemilumi-nescence	DM HTN DL, diabetic nephropathy	NA	No AE
Al-Sofiani Int J Endoc Metab 2015 (117)	Riyadh, Saudi Arabia 24.6 ˚N	Primary care clinic at King Khalid University Hospital	I: D3 5,000 IU/day C:PBO Duration: 3 months	No	I : 11 C : 11	I : 1 C: 1	75 Both arms	I : 54.8 (9.16) C: 55 (11.99)	I :28.8(26.7,30.88) C:33.3 (27.3,35.6)	I : 10.2 (8.9,11.6) C: 15.5 (9.5, 15.9)	I : 36.5 (29.8,39.8) C:11.8 (9.2, 13.7)	CLIA	All diabetics	Yes (97%)	NA
Al-Zahrani Int J Clin Exp Med 2014 (118)	Riyadh, Saudi Arabia 24.6 ˚N	Out-patient Diabetes Clinics King Abdul-Aziz Medical City	I: D3 45,000 IU weekly for 2 months and a single 45000 IU in the last month (=4,785 IU/d) C: control Duration: 3 months	No	I : 100 C : 100	I : 9 C: 8	I : 62 C: 36	I : 56.9 (9.4) C: 52.5 (8.1)	I : 31.3 (4.6) C: 32.0 (5.7)	I : 10.3 (6.33) C: 8.8 (6.1)	I: 33.2 (12.7) C: 22 (15.1)	Liaison DiaSorin USA.	DM (all) HTN DL	NA	NA
Breslavsky Clin Nut 2013 (115)	Wolfson, Israel 32.03 ˚N	HTN outpatient clinic at E. Wolfson Medical Center	I : D3 1000 IU/day C: PBO Duration: 12 months	No	I : 24 C: 23	I : 5 C: 10	I : 45.8 C: 47.8	I : 66.8 (9.2) C: 65.8 (9.7)	I : 27.9 (5.2) C: 30.6 (5.1)	I : 12.9 (10.7) C: 10.8 (6.6)	I : 17.6 (11.5) C: 14.0 (5.9)	Competitive protein-binding method	DM (all) HTN DL	NA	2 fractures (hip, radial) 1diarrhea 1 cholecyst-ectomy 1 weaknes, 1respiratory infection
El Hajj Fuleihan ASBMR abstracts 2015 (105)	Beirut, Lebanon 33.8 ˚N	Outpatient clinics AUB-MC, HDF, RHUH	I1: D3 3,750 IU/day I2: D3 600 IU/day Duration:12 months	Yes	I1 : 129 I2 : 128	I1 : 19 I2 : 16	I1 : 43 I2 : 46	I1 : 71.2 (4.8) I2 : 71(4.7)	I1 : 30.6 (4.4) I2 : 29.7 (4.6)	I1 : 20.6 (7.9) I2 : 20.1 (6.9)	I1 : 36.0 (9.7) I2 : 26.0 (6.9)	HPLC	CVD, CAD, CHF, HTN, DL	I1: 93.5 I2: 91.7	Various serious adverse events; see footnote[2]
Firouzabadi Compl Ther Clin Pract 2012 (119)	Yazd, Iran 31.8 ˚N	OB- GYN, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Science	I: D3 100,000 IU/month (=3,333 IU/d) C: control Duration: 6 months	Yes	I : 50 C: 50	NA	0	I : 27.9 (4.1) C: 28.5 (4.2)	I : 26.89 (2.1) C: 26.91 (2.3)	I : 13.2 (6.6) C: 13.5 (6.4)	I : 24.8 (6.5) C: 13.8 (6.5)	RIA	PCOS (all)	NA	NA

Author Year	City Latitude Country	Sampling method/ setting	Intervention Duration	Ca supp	Nb of subject random -ized per arm	Nb of subject lost to follow up	Gender (% Male per arm)	Age Mean (SD) or median (range) (years)	BMI Mean (SD) or median (range) (kg/m^2)	Baseline mean (SD) or median (range) 25(OH)D (ng/ml)	Achieved mean (SD) or median (range) 25(OH)D (ng/ml)	Vitamin D assay	Co-morbidities	Compliance	Adverse events or serious adverse events
Ghavam -zadeh Int J Prev Med 2014 (128)	Urmia, Iran 37.5 ˚N	Diabetes clinic of Taleqani hospital	I : D3 400 IU/day C: PBO Dietary vitamin D: I :120 IU/d C:118 IU/d Duration: 3.5 months	No	I : 60 C : 60	I: 33 C: 34	41.2 (both arms)	I : 52.3 (10.6) C: 49.28 (10)	I : 28.9 (0.86) C: 27.9 (0.93)	I : 8.6 (9.5) C: 8.9(10.6)	I : 18.6 (14) C: 8.4 (14.5)	CLIA	DM (all)	NA	NA
Golan Brain Behav Immun 2013 (127)	Haifa, Israel, 32.8° N	MS clinic	I1: D3 75,000 IU every 3 weeks plus 800 IU daily (= 4,370 IU/d). I2: D3 800 IU daily Duration: 12 months	No	I1: 19 I2: 21	I1: 6 I2: 8	I1: 23 I2: 15.4	I1: 47.7 (11.6) I2: 46.3 (9.2)	I1: 25.2 (6.2) I2: 26.2 (7.4)	I1:20.0 (10-28.8) I2: 20.0 (6.9-28.8)	I1: 40 (22.6-63.8) I2: 22.6 (12-30.4)	CLIA	MS (on INFβ) (all)	NA	NA
Hoseini J Res Med Sci 2013 (120)	Isfahan, Iran 32.6 ˚N	Pre diabetics at Isfahan Endocrine and Metabolism Research Center, Isfahan University of Medical Sciences	I: D 50,000 IU weekly or every other week if serum 25(OH)D less or more than 30 ng/ml respectively. (average 5,300 IU/d) C: Control Duration: 3 months	Yes	I : 22 C: 16	I : 1 C: 1	I : 19 C: 47	I : 46.3 (6.5) C: 48.9 (6.1)	I : 30.4(4.3) C: 28.6 (2.6)	I : 31 (15.7) C: 17.9 (7.33)	I : 47.6 (22.5) C: 13.9 (5.6)	Direct competitive CLIA	Pre-DM (all)	NA	NA.
Nasri J Ren Inj Prev 2014 (121) [3]	Shahrekord, Iran 32.3 ˚N	Endo clinic at Shahrekord University of Medical Sciences	I: D3 50,000 IU weekly (=7,142 IU/d) C: Placebo Duration: 3 months	No	I : 30 C: 30	NA	28.3 in both arms	55 (10.7) both arms	I : 29.3 (4.4) C: 28.8 (4.5)	I : 33.6 (20.8) C: 42.3 (25.6)	I : 65.7 (22.8) C: 46.4 (37.7)	ELISA	DM (all)	NA	NA
Sadiya Clin Nut 2015 (122)	Ajman, UAE 25.3 ˚N	Rashid Centre for Diabetes and Research, a tertiary outpatient diabetes care clinic	I : D3 6,000 IU daily C: Placebo Duration: 3 months	No	I : 45 C: 42	0	I : 20 C: 16.7	I : 49 (8) C: 48 (8)	I : 38.0(6.1) C:37.6(7.8)	I : 11.4(3.7) C: 12.2 (4.5)	I : 30.9(12.1) C: 11.5(5.2)	Chemilumi-nescence method	DM and obese (all)	NA	None
Sharifi Endoc 2014 (123)	Ahvaz, Iran 31.3 ˚N	Outpatient clinic of Jundishapur University of Medical Sciences	I: D3 50,000 IU every 14 days (3,571 IU/d) C: Placebo Duration: 4 months	No	I : 30 C: 30	I : 3 C: 4	I : 48 C: 50	I : 40.3 (8.6) C: 43.9 (9.5)	I : 31.3 (28.6, 32.5) C: 29.3 (26.8, 31.9)	I : 11.5 (8.8, 28.4) C:16.8 (11.7, 24.8)	I : 30.0 (25.8, 46.6) C:19.2 (14.7, 26.7)	RIA	NAFLD (all)	I : 94.4 C: 92.2	None

Author Year	City Latitude Country	Sampling method/ setting	Intervention Duration	Ca supp	Nb of subject random-ized per arm	Nb of subject lost to follow up	Gender (% Male per arm)	Age Mean (SD) or median (range) (years)	BMI Mean (SD) or median (range) (kg/m^2)	Baseline mean (SD) or median (range) 25(OH)D (ng/ml)	Achieved mean (SD) or median (range) 25(OH)D (ng/ml)	Vitamin D assay	Co-morbidities	Compliance	Adverse events or serious adverse events
Salehpour Nutr J 2012 (125)	Tehran, Iran 35.6 ˚N	Heart and Vascular Lab in Tehran University of Medical Sciences	I : D3 1,000 IU/d C: Placebo Dietary vitamin D: I : 21 IU/d C: 15 IU/d Duration: 3 months	No	I : 42 C: 43	I : 3 C: 5	0	I : 38(7) C: 37(8)	I : 30.1 (3.9) C: 29.5 (4.4)	I : 14.7 (12) C: 18.8 (12.8)	I : 30 (8.8) C: 20.6 (12.4)	EIA	Obese	I : 87.1% C: 87.4%	NA
Taheri Iran Red Cresc Med J 2014 (126)	Tehran, Iran 35.6 ˚N	Gynecology clinic of Tehran Imam-Khomeini hospital	I: D 2,000 IU/d C: Placebo Duration: 3.5 months	No	I : 116 C: 113	3 from the whole study	0	I : 29.43 (5) C: 29.8 (4.4)	I : 25.9(4.8) C:26.2 (4.6)	I : 10.1 (7.4) C: 9.3(6.4)	I : 29.7 (18.1) C: 9.9(7.7)	EIA	-	NA	NA
Tehrani J Res Med Sci 2014 (124)	Isfahan, Iran 32.6 ˚N	obstetrics and gynecology clinic of Alzahra hospital	D3 50,000IU every 2 weeks (=3,571 IU/d) 2 arms received vitamin D: I1:Ca+D I2:Ca+D+MTF I3:MTF I4: Placebo Duration: 4 months	Yes in 2 arms	I1: 20 I2: 20 I3: 20 I4: 20	NA	0	I1: 31.3 (4.6) I2: 28.7 (4.5) I3: 27.4 (2.2) I4: 27.2 (6.5)	I1: 26.3 (2.5) I2: 27.8 (3.3) I3: 26.8 (2.2) I4: 27.3 (1.3)	I1: 19.5 (3.2) I2: 18.7 (2.7) I3: 20.1 (3.2) I4: 20.0 (2.9)	I3: 20.1 (3.2) I2: 29.4 (2.3) I1: 31.5 (2.4) I4: 20.0 (2.9)	ELISA	PCOS	NA	NA
Pregnancy															
Dawodu JCEM 2013 (103)	United Arab Emirates, Al Ain 24.2 ˚N	Primary health care clinics, affiliated with Tawam Hospital Pregnant women	I1: D3 3,600 IU/ d I2: D3 1,600 IU/ d C: Placebo All received also 400 IU daily as prenatal vitamins Duration: 12-16 weeks GA till delivery	No	I1: 63 I2: 65 C : 64	I1 : 8 I2:13 C : 9	0	I1: 25.6 (5.5) I2: 27.3 (4.9) C : 27.5 (5.5)	I1: 26.3 (5.4) I2: 26.3 (6.4) C : 25.8 (6.3)	I1:7.84(3.08 I2:8.2(4.76) C: 8.6(5.2)	I1:35.9 (12.12) I2:25.9 (12.23) C :19.3 (19.27)	RIA (DiaSorin, Stillwater, Minnesota)	None	I1:86% I2:87% C :82%	None
Shakiba Sing Med J 2013 (129)	Yazd, Iran 31.8 ˚N	Two primary care clinics	I1: D3 50,000 IU/month (=1,667 IU/d) I2: 50,000 IU every two weeks (=3,571 IU/d) I3: D3 50,000 IU/week for four weeks, then 50,000 IU/month (=2,579 IU/d)[4] Duration: second	No	I1: 17 I2: 17 I3: 17	No lost to follow up	0	25 (3) (all arms)	NA	I1:16 (7.4) I2: 18 (7.8) I3: 7 (3.0)	In neonates: I1: 25 (7) I2: 32 (12) I3: 35 (8)	Chemi-luminesc-ence immuno-assay	None	NA	NA

Author Year	City Latitude Country	Sampling method/ setting	Intervention Duration	Ca supp	Nb of subject random-ized per arm	Nb of subject lost to follow up	Gender (% Male per arm)	Age Mean (SD) or median (range) (years)	BMI Mean (SD) or median (range) (kg/m^2)	Baseline mean (SD) or median (range) 25(OH)D (ng/ml)	Achieved mean (SD) or median (range) 25(OH)D (ng/ml)	Vitamin D assay	Co-morbidities	Compliance	Adverse events or serious adverse events
			trimester until delivery												
Soheilykhah Gynecol Endocrinol 2013 (130)	Yazd, Iran 31.8 ˚N	Two prenatal clinics (Mojibian Hospital and Shahid Sadoughi Hospital)	I1: D2 200 IU/d I2: D2 50,000 IU/ month (=1,666 IU/d) I3: D2 50,000 IU every 2 weeks (=3,571 IU/d). Duration: 12 weeks until delivery	No	I1: 40 I2: 40 I3: 40	I1: 5 I2: 2 I3: 0	0	I1: 25 (4.3) I2: 26.5 (4.5) I3: 26.3 (4.8)	I1: 26.2 (4.5) I2: 25 (3.8) I3: 25.9 (4.6)	I1: 8.3 (7.8) I2: 7.3 (5.3) I3: 7.3 (5.9)	I1: 17.7 (9.3) I2: 27.2 (10.7) I3: 34.1 (11.5)	Chemi-luminesce-ence assay	-	NA	None
Sabet Acta Endocrinol 2012 (133)	Tehran, Iran 35.6 ˚N	Mahdieh Hospital	I:D3 100,000 IU/ months (=3,333 IU/d) C: Placebo Duration: 27 weeks until delivery	No	I : 25 C: 25	NA	0	I : 26.6 (4.7) C: 26 (6.2)	Weight I : 72 (10) C: 70 (9)	I : 33.5 (21.4) C: 38.3 (23.2)	Maternal I : 61.45 (30) C: 29.4 (16) Venous cord I : 52 (40.5) C: 26 (21.3)	EIA (Immune diagnostic system Ltd, Bolden, UK	-	NA	NA
Karamali Horm Metabol Res 2015 (132)	Arak, Iran 34.1° N	Not detailed	I: D3 50,000 IU every 14 days (3,571 IU/d) C: Placebo Duration: 3 months	were on multi vit	60	0	0	27.4 (5.2) both arms	25.9 (4.6) both arms	I:17 (1.4) C: 17.1(2.2)	I : 34.9 (2.4) C:17.4 (4.0)	ELISA kit (IDS, Boldon, UK).	at risk for pre-eclampsia	100	NA
Etemadifar Iran J Neurol 2015 (131)	Isfahan, Iran 32.6˚N	MS outpatient clinics of Isfahan University of Medical Sciences	I: D3 50,000 IU/ week (7,142 IU/d) C: Placebo Duration: form 12 to 16 weeks GA till delivery	No	I : 21 C: 22	I : 15 C: 13	0	I : 27.7 (2.4) C: 30.0 (3.9)	NA	I : 15.3 (2.9) C: 18.3 (1.9)	I: 33.7 (15.2) C: 14.6 (1.3)	Radioimmu noassay kit (DiaSorin, Stillwater, MN, USA).	MS	Patient who failed to be compliant were excluded	None
Children and adolescents															
El Hajj Fuleihan JCEM 2006[5] (40)	Beirut, Lebanon 33.8 ˚N	School children and adolescents	I1: D3 1,400 IU/week (= 200 IU/d) I2: D3 14,000 IU/week (=2,000 IU/d) C: Placebo Duration: 12 months	No	Girls C:55 I1:58 I2 55 Boys C: 56 I1: 56 I2: 60	Girls I1: 4 I2: 4 C : 3 Boys I1: 3 I2: 4 C : 5	I1: 49 I2: 52 C : 50	Girls 13.2 (2.1) Boys 13.0 (1.9)	Girls 20.1 (3.6) Boys 21.1 (4.2)	Girls I1: 14 (9) I2: 13 (8) C: 14 (7) Boys: I1: 16 (7) I2: 16 (7) C: 16 (6)	Girls: I1: 17 (6) I2: 38 (31) C: 16 (8) Boys: I1: 20 (7) I2: 35 (9) C: 17 (6)	DiaSorin RIA (Diasorin, Incstar, Sallugia, Italy)	Healthy	I1: 97.5 (3) I2: 97 (3) C: 98 (2.6)	High 25(OH)D level in 4 cases. High Ca in 5 cases
Ghazi Eur J Clni Nutr 2010	Taleghan, Tehran Iran 36.5 ˚N	School children (no other details)	I1: D3 50,000U / month (=1,667 IU/d) I2: D3 50,000 IU every other month[6] C: placebo monthly	No	I1 Girls 35 Boys 35 I2 Girls 35 Boys 34	3 from the whole study	I1: 50 I2: 49 C : 49	I1 Girls 16.0 (1.0) Boys 16.5 (1.4) I2	I1 Girls 21.8 (3.1) Boys 22.3 (4.6) I2 Girls 21.7 (3.6) Boys 20.7 (2.7)	I1 Girls 20.5 (22.5) Boys 43.75 (14) I2 Girls 17.5 (6) Boys 39 (12)	Girls at 5 mo I1: 48 (23.5) I2: 33.7 (23) C: 20 (14) Boys at 6 mo I1: 72.5 (26.2)	ELISA kits (Immunodia gnostic Systems, Boldon, UK)	Healthy	Observed administra-tion of intervention	None

Author Year	City Latitude Country	Sampling method/ setting	Intervention Duration	Ca supp	Nb of subject random -ized per arm	Nb of subject lost to follow up	Gender (% Male per arm)	Age Mean (SD) or median (range) (years)	BMI Mean (SD) or median (range) (kg/m^2)	Baseline mean (SD) or median (range) 25(OH)D (ng/ml)	Achieved mean (SD) or median (range) 25(OH)D (ng/ml)	Vitamin D assay	Co-morbidities	Compliance	Adverse events or serious adverse events
			Duration: 6 months in boys and 5 months in girls		C Girls 35 Boys 34			Girls 16.4 (1.2) Boys 16.1 (1.4) C Girls 16.2(1.2) Boys 16.6(1.4)	C Girls 20.7 (2.9) Boys 21.9 (4.4)	C Girls 19.7 (14.7) Boys 38.7 (16)	I2: 57.7 (19) C: 39 (16)				
Neyestani J Hum Nutr Diet 2013 (135)	Tehran, Iran 35.6°N	6 primary schools	I : D3 200 IU/d C: PBO Duration: 3 months	Yes	I : 67 C: 60	I : 6 C: 7	I : 24 C: 32	I : 10.4 (0.6) C: 9.8 (0.8)	I : 18.2 (3.3) C: 18.7(3.9)	I : 9.5 (4.6) C: 10.1 (4.3)	I : 17.5 (4.6) C: 9.6 (3.4)	HPLC	Healthy	NA	NA
Infants															
Samadpour Eur J Clin Nutr 2011 (137)	Hashtgerd, a regional urban area of Iran	Three urban health centres and two health posts	I1: 200 IU/d (Foodlet) I2: 200 IU/d (Sprinkles) I3: D3 400 IU/d (Drops) Duration: 4 months	No	I1: 121 I2: 120 I3: 121	I1: 28 I2: 16 I3: 17	I1: 59.2 I2: 58.7 I3: 55.4	Months I1: 12.2(3.6) I2: 12 (3.8) I3: 12.4(3.3)	Weight (kg) I1: 9.2 (1.3) I2: 9.2(1.4) I3: 9.4(1.3) Height (cm) I1: 74.3(4.6) I2: 74 (5.3) I3: 74.7(4.9)	I1: 86.7 (27.8) I2: 82.0 (28.5) I3: 88.9 (31.1)	I1: 88.6 (28.4) I2: 87.4 (32.0) I3: 96.4(32.1)	RIA (BioSource Europe S.A., Belgium	Healthy	No difference in compliance between groups but no further details	NA

AUB-MC: American University of Lebanon –Medical Center; CAD: Coronary Artery Disease; CLIA: chemiluminescence immunoassay CVD: Cerebrovascular Disease; CHF: Congestive Heart Failure; DL: Dyslipidemia; DM: Diabetes Mellitus; HDF: Hotel Dieu de France; HTN: Hypertension; INF-ß: Interferon-ß; MTF: Metformin; MS: Multiple Sclerosis; NAFLD: Non-alcoholic fatty liver disease; PBO: Placebo; PCOS: Polycystic Ovary Syndrome; RHUH: Rafic Hariri University Hospital; RIA: radioimmunoassay; UAE: United Arab Emirates

I1: intervention 1 group; I2: Intervention 2 group; C: control group; NA: not available

[1]A trial is considered conducted in elderly if >50 % of participants are >65 years old.

[2] In the low dose group: death, stroke, thrombophlebitis, hemorrhoids, glaucoma, disc disease; in the high dose group: death, kidney stone, hypertensive crisis, retinal detachment, knee arthroplasty.

[3] Same trial in Behradmanesh 2013.

[4] Arm excluded from analysis as it did not include randomized participants but those who are vitamin D deficient.

[5] Same trial in Maalouf 2008 and Al-Shaar 2014.

[6] Arm excluded from analysis as vitamin D was given less frequently than once monthly.

Table 4: Summary of results across all age groups

Age category (number of studies)	Dose category	Number of subjects by dose category	Baseline 25(OH)D ng/ml	Increase per 100 IU vitamin D (ng/ml)	Proportion ≥ 20 ng/ml (%)
Elderly (2 studies)	Low (600 IU/d)	110[1]	20	1	83
	Intermediate (1,000 IU/d)	19[2]	10.8-12.9	2	40
	High (3,700 IU/d)	112[1]	20	0.40	98
Adults (13 studies)	Low (400 IU/d)	27[3]	8.6	2.50	54
	Intermediate (1,750 IU/d)	153	11	1	71
	High (4,850 IU/d)	342	15	0.46	89
Pregnancy (6 studies)	Low (300 IU/d)	107	7.8	3.57	41-43
	Intermediate (1,800 IU/d)	79	7.8	1	73
	High (3,700 IU/d)	113	11	0.66	90-94
Children (3 studies)	Intermediate (1,870 IU/d)	183	14	0.94	73

[1] Results from a single randomized controlled trial El Hajj Fuleihan 2015.

[2] Results from a single randomized controlled trial Breslavsky 2013.

[3] Results from a single randomized controlled trial Ghavamzadeh 2014.

Table 5: Single variable random-effect meta-regression of different variables on 25(OH)D level achieved at the end of the intervention

Independent variable	β	p-value	Adjusted R^2
Dose (100 IU/d)	**0.50**	**<0.001**	**75.9**
Duration [1] (months)	-0.34	0.675	-3.5
Duration [2] (3 months vs >3 months)	**-9.30**	**0.088**	**6.6**
Latitude	-.04	0.96	-4
Calcium supplementation (Yes vs No)	-0.74	0.9	-4
Age (years)	.22	0.31	-0.03
BMI (Kg/m^2)	-.33	0.73	-3.6
Baseline 25(OH)D (ng/ml)	**1.09**	**0.002**	**29.8**
Risk of bias (low vs unclear and high risk)	2.7	0.66	-3.48

[1] continuous variable; [2] categorical variable.

Variables in bold are those that are statistically significant at p-value of 0.1

Table 6A: Multivariate analysis including variables significantly associated with the 25(OH)D level achieved at the end of the intervention at p-value 0.1

Independent variable	β	p-value	Model characteristics
Constant	6.40	0.068	p-value < 0.001 Tau^2 26.7 I^2 res 92.4 Adjusted R^2 87.1
Dose (100 IU/d)	0.44	<0.001	
Duration (3 months vs >3 months)	-1.5	0.514	
Baseline 25(OH)D (ng/ml)	0.76	<0.001	

Table 6B: Multivariate analysis same as in Table 6A after removing the duration.

Independent variable	β	p-value	Model characteristics
Constant	5.30	0.077	p-value < 0.001 Tau^2 25.6 I^2 res 91.8 Adjusted R^2 87.6
Dose (100 IU/d)	0.44	<0.001	
Baseline 25(OH)D (ng/ml)	0.77	<0.001	

Table 7: Sensitivity analysis multivariate model, forcing in the model variables that are clinically relevant: age, BMI and presence or absence of concomitant calcium supplementation.

Independent variable	**β**	**p-value**	**Model characteristics**
Constant	19.10	0.125	Tau^2: 23.3 I^2 res: 89.8 Adjusted R^2: 88.6
Dose (100 IU/d)	0.44	<0.001	
Baseline 25(OH)D (ng/ml)	.81	<0.001	
Age (years)	.08	0.351	
Ca supplementation (Yes versus No)	-4.00	0.088	
BMI (kg/m^2)	-0.57	0.139	

Appendix 1: Vitamin D Guidelines Comparison on desirable level, who to screen and maintenance dose.

	Desirable level (ng/ml)	Who to screen	Maintenance	Comments on vitamin D assay
Institute of Medicine (IOM) 2010	20	-	RDA: 1-18 years : 600 IU/d 19-70 years : 600IU /d >70 years : 800 IU/d Pregnant women: 600 IU/d	"Concerns about inaccurate or imprecise serum 25OHD measurements are being overcome by methodological advances, frequent quality assessments, and accurate calibration tools."
International Osteoporosis Foundation (IOF) 2010	30	-	-Vitamin D requirement for older adults (>60-65 years): 800-1,000 IU/d. -Higher doses in high risk individuals: "obese, and in those with osteoporosis, limited sun exposure (institutionalized, homebound), and malabsorption, and in non-European populations known to be at high risk for vitamin D deficiency such as those in the Middle East and South Asia, or immigrants from such regions living in Europe."	"Assay variability should be addressed by the use of standard reference material such as the NIST standards and participation in the DEQAS quality control program."
Osteoporosis Canada 2010	30	"25(OH)D should be measured only if deficiency is suspected or would affect the person's response to therapy (e.g., in cases of impaired intestinal absorption, such as celiac disease, or osteoporosis requiring pharmacologic therapy), in patients taking daily doses above tolerable upper intake level, individuals with recurrent fractures, bone loss despite osteoporosis treatment or comorbid conditions that affect vitamin D absorption or action."	"For most healthy adults, regardless of age, the recommended vitamin D3 intake is 800–1,000 IU/d. For individuals at high risk for vitamin D deficiency, supplementation at doses between 800 – 2,000 IU/d is recommended, with potential for higher doses."	"Clinical laboratories participation in external laboratory proficiency testing programs, such as the Vitamin D External Quality Assessment Scheme, and this should be mandatory for accreditation."
Endocrine Society 2011	30	"Rickets; Osteomalacia; Osteoporosis; Chronic kidney disease; Hepatic failure; Malabsorption syndromes; Cystic fibrosis, Inflammatory bowel disease; Bariatric surgery; Radiation enteritis; Hyperparathyroidism; Medications; African-American and Hispanic children and adults Pregnant and lactating women; Older adults with history of falls; Older adults with history of non-traumatic fractures; Obese children and adults (BMI ≥ 30 kg/m2); Granuloma-forming disorders"	0-1 year : 400-1,000 IU/d 1-18 years : 600-1,000 IU/d 19-70 years : 1,500-2,000 IU/d >70 years : 1,500-2,000 IU/d Pregnant women: 14-18 years : 600-1,000 IU/d 19-50 years : 1,500-2,000 IU/d	"Using the serum circulating 25(OH)D level, measured by a reliable assay, to evaluate vitamin D status in patients who are at risk for vitamin D deficiency."

	Desirable level (ng/ml)	Who to screen	Maintenance	Comments on vitamin D assay
Swiss 2012	General population : 20 Elderly: 30	"Bone disorders, older adults, obese, granulomatous disease, medication, liver or renal disease, malabsorptive disorders, pregnant and lactating women, Children and adults with a dark skin tone, athletes of all ages"	0-1 year : 400 IU/d 1-60 years : 600 IU/d >60 years : 800 IU/d Pregnant women : 600 IU/d	"High quality assay are needed for screening"
USPTF 2013	-	"Persons with low vitamin D intake, decreased vitamin D absorption, and little or no sun exposure (for example, due to the winter season, high latitude, or physical sun avoidance) may be at increased risk for vitamin D deficiency. Obesity and darker skin pigmentation may be associated with low levels of serum 25(OH)D level but it is not clear whether low levels in these populations reflect vitamin D deficiency or are associated with adverse clinical outcomes."	-Insufficient evidence to recommend vitamin D for fracture prevention. - Vitamin D supplementation (the median dose of vitamin D in available studies was 800 IU) to prevent falls in community-dwelling adults aged 65 years and older who are at increased risk for falls because of a history of recent falls or vitamin D deficiency (http://www.uspreventiveservicestaskforce.org/Page/Document/RecommendationStatementFinal/vitamin-d-and-calcium-to-prevent-fractures-preventive-medication)	"Numerous testing methods to measure serum 25(OH)D are available. However, their accuracy is difficult to determine because of the lack of studies that use an internationally recognized reference standard and the lack of consensus on the laboratory values that define vitamin D deficiency."
National Osteoporosis Society (NOF) 2013	20	**"-**Patients with bone diseases that may be improved with vitamin D treatment or where correcting vitamin D deficiency prior to specific treatment would be appropriate -Patients with musculoskeletal symptoms that could be attributed to vitamin D deficiency."	-Pregnant and breastfeeding women: 400 IU/d -People aged 65 years and over and people who are not exposed to much sun: 400 IU daily	"Assay used should have the ability to recognize all forms of 25OHD (D2 or D3) equally. In practice, this means that it should use either HPLC or, more likely, tandem MS"
National Institute for Health and Care Excellence (NICE) 2014	-	-	Infants up to 6 months : 340 IU/d 6 months-6 years : 280 IU/d Pregnant and breastfeeding women: 400 IU/d Elderly > 65 years : 400 IU/d	-

25(OH)D: 25-hydroxyvitamin D; RDA: Recommended Dietary Allowance; National Osteoporosis Foundation: endorsed by Bone research society, IOF, British orthopedic Association, UK clinical pharmacology association, Society of Endocrinology, Royal College of nursing, Royal pharma-society, the primary care rheumatology society, Paget's association.

Appendix 2: Studies cited in the Institute Of Medicine and Endocrine Society guidelines, to define recommended vitamin D dose in each age category

Age category	Institute Of Medicine 2010	Endocrine society 2011
0-1 year	400 IU/d	400-1,000 IU/d
	Aim is to get optimal D level. **No RDA but AI:** Intake of vitamin D 400 IU/day appears to maintain a serum 25OHD level generally above 50 nmol/L in infants. Ala-Houhala et al (1985), Finland RCT Placebo versus vitamin D 400IU daily versus vitamin D 1,000 IU daily ➔Conclusion: no rickets in vitamin D groups Greer et al (1989),Wisconsin RCT Placebo versus vitamin D 400 IU daily ➔Conclusion: supplemented group only reached 25(OH)D level ≥ 21 ng/ml	Feliciano et al (1994), China RCT Vitamin D 100 versus vitamin D 200 versus vitamin D 400 IU daily ➔No effect on weight and height at 6 months Formon et al (1966), Iowa RCT Vitamin D 350-550 versus vitamin D 1,380- 2,170 IU daily ➔no difference in growth rate Specker et al (1992),China RCT Vitamin D 100 versus vitamin D 200 versus vitamin D 400 IU daily. ➔Increased 25(OH)D level with increasing dose, none had rickets and supplementation with vitamin D 400 IU is prudent. Markestad and Elzouki (1991), Norway Review article ➔ vitamin D 300 IU daily is required to get 25(OH)D of 11 ng/ml Hyponen et al (2001), Finland Observational Vitamin D 2,000 IU daily decreased the incidence of DM type 1 by 88%

Age category	Institute Of Medicine 2010	Endocrine society 2011
1-18 years	600IU/d	600-1,000 IU/d
	Aim is to ensure normal, healthy bone accretion is central to the DRI values. Ala-Houhala et al (1988), Finland RCT Vitamin D 400 IU daily versus Placebo ➔ 25(OH)D level increased from 46 nmol/l to 71 nmol/l in D group. Schou et al (2003), Denmark RCT Vitamin D 600 IU daily versus placebo ➔25(OH)D level reached 50 nmol/l in D group only Viljakainen et al(2006), Finland RCT Placebo versus vitamin D 200 IU versus vitamin D 400 IU daily ➔ 25(OH)D level reached 42 vs 51 vs 58.8 nmol/l. Rajakumar et al (2008), Pittsburgh Observational, all received vitamin D 400 IU daily and compared obese versus non obese	Urashima et al (2010), Japan RCT Vitamin D 1,200 IU daily versus placebo ➔1200 IU daily decreased the incidence of influenza A by 42% Dong et al (2010), Richmond (African American) RCT Vitamin D 200 IU versus vitamin D 400 IU daily ➔Higher D levels and lower arterial stiffness Aksnes et al (1982), abstract only Observational Dietary vitamin D 100-400 IU to maintain 25(OH)D above 11 ng/ml Gultekin et al (1987), Turkey Observational ➔ Vitamin D intake <100 IU daily leads to 25(OH)D level <11 ng/ml Maalouf et al (2008), Lebanon RCT in boys Placebo versus vitamin D3 1400 IU versus vitamin D3 14,000 IU weekly ➔25(OH)D level increased from 15 to 19 ng/ml in low dose group and from 15 to 36 ng/ml in high dose group; no toxicity El Hajj Fuleihan et al (2006), Lebanon RCT in girls Vitamin D3 1400 IU weekly versus vitamin D3 14,000 IU weekly ➔25(OH)D level increased from 14 to 17 ng/ml in low dose group and from 14 to 38 ng/ml in high dose group; no toxicity

Age category	Institute Of Medicine 2010	Endocrine society 2011
19-50 years	600 IU/d	1500-2,000 IU/d
	Aim is bone maintenance. Cashman et al(2009), Ireland RCT Placebo versus vitamin D 200 versus vitamin D 400 versus vitamin D 600 IU daily ➔25(OH)D level >25 nmol/l in ≥ 97% of the population requires 7.9-42.8 mcg daily depending on sun exposure Smith et al (2009), Antarctica vitamin D 400 versus vitamin D 1,000 versus vitamin D 2,000 IU daily ➔25(OH)D level increased from 45 nmol/l to 55, 63 and 71 nmol/l Valjakainen et al(2009),Finland RCT Placebo vs vitamin D 400 versus vitamin D 700IU daily ➔baseline 60 nmo/l➔drop in placebo,75 nmol/l , 90 nmol/l Biancuzzo et al(2010), Boston RCT Placebo versus vitamin D2 1,000 IU versus vitamin D3 1,000 IU daily➔25(OH)D levels reached 45, 70, 58 nmol/l Harris (2002), Boston RCT Placebo versus vitamin D 800 25(OH)D levels reached 48 to 53 vs 59 to 82 nmol/l	Bischoff Ferrari et al (2004), NHANES Observational Highest quintile(25(OH)D>39 ng/ml in white and >31 ng/ml in African American) had a higher mean BMD Holick et al (2008), Boston RCT Placebo versus vitamin D2 1000 IU daily versus vitamin D3 1,000I U daily versus vitamin D2 500 IU + D3 500 IU daily ➔in vitamin D groups, 25(OH)D level increased from 19-30 ng/ml (in the deficient group ,none reached a level > 30 ng/ml) Pietras et al (2010),US Retrospective Vitamin D 5,0000 IU every other week for 6 years ➔Mean 25(OH)D level reached was 46 ng/ml ➔No toxicity

Age category	Institute Of Medicine 2010	Endocrine society 2011
	Heany et a l(2003), Omaha RCT Placebo vs D 1000 vs 5000 vs 10000IU daily ➔ 25(OH)D levels reached 52, 77,150 ,212 nmol/l Holick et al (2008), Boston RCT Placebo versus vitamin D2 1000 IU versus vitamin D3 1000IU versus vitamin D2 500IU + D3 500IU ➔in D groups, 25(OH)D level increased from 19-30 ng/ml (in the deficient group ,none reached a level > 30 ng/ml) Li-Ng et al (2009), Long Island RCT Placebo versus vitamin D 2000 IU daily ➔Baseline mean 25(OH)D >60 nmol/l, reached mean 25(OH)D level 88 nmol/l in D group Nelson et al (2009), Bangor RCT Placebo versus vitamin D 800 ➔baseline mean 25(OH)D >60 nmol/l and reached mean 25(OH)D level 97nmol/l in the vitamin D group	
50-70 years	600 IU/d	1,500-2,000 IU/d
>70 years	800 IU/d	1,500-2,000 IU/d
	50-70 years: *Aim is to reduce peri-menopausal bone loss* >70 years: *Aim is to reduce fracture risk*	*Target: Bone health and fractures* Greene Finestone (2011), Canada RCT Placebo vs D 400 IU daily ➔ Vitamin D > 400 IU daily is needed to keep 25(OH)D> 50 nmol/l

Age category	Institute Of Medicine 2010	Endocrine society 2011
	Cashman et al (2009), Ireland RCT Placebo versus vitamin D 200 versus vitamin 400 versus vitamin D 600 IU daily ➔baseline 25(OH)D level >50 nmol/l Levels reached are 41, 53, 69 and 73 nmol/l, respectively in treatment arms. Honkanen et al (1990), Finland RCT Placebo versus vitamin D 1800 IU daily ➔25(OH)D level increased from 40 to 80 nmol/l in D group Van Der Kils et al (1996), Netherlands RCT Placebo versus vitamin D 400 versus vitamin 800 IU daily. ➔25(OH)D level increased similarly in vitamin D 400 and 800 groups from 60 to 87.9 nmol/l Dawson Hughes et al (1991),Boston RCT Placebo versus vitamin D 400 IU daily ➔increasing D intake by 400 IU daily improves bone density in post-menopausal women Harris et al (2002), Boston RCT Placebo versus vitamin D 800 IU daily ➔ vitamin D group increased from 61 to 83 nmol/l	Dawson-Hughes et al (1991), US RCT Placebo versus vitamin D 400 IU daily ➔Increasing vitamin D intake by 400 IU is needed to increase bone density in post-menopausal women Lips et al (1988),US RCT: placebo versus vitamin D 400 IU daily ➔ Vitamin 400 IU is needed to increase 1,25D level and decrease PTH Chapuy et al (1992), France RCT Placebo vs D 800 IU daily ➔800 IU decreases hip and non-vertebral fractures Dawson Hughes (1997),US RCT Placebo versus vitamin D 700IU daily ➔ Vitamin D decreases bone loss and reduces non vertebral fracture Bischoff Ferrari 2005 and 2009 Meta-analysis Vitamin D700-800 IU daily is required to reach 25(OH)D level ≥ 30 ng/ml Vitamin D 480-770 IU daily is required to decrease non vertebral fractures <u>Muscle:</u> Pfeifer et al (2000), Germany Ca + vitamin D 800 IU daily versus Calcium only D 800 IU daily improves body sway and decreases falls

Age category	Institute Of Medicine 2010	Endocrine society 2011
		Murad et al (2011), Meta-analysis Vitamin D supplementation is associated with fall reduction (OR 0.79); dose response was not assessed and high heterogeneity. Pfeifer et al (2009), Austria and Germany RCT Calcium vs Calcium + vitamin D 400 IU daily ➔ Vitamin D 400 IU daily decreases falls Broe et al (2007), US RCT Vitamin D 200 versus vitamin D 400 versus vitamin D 600 versus vitamin D 800 IU daily ➔ Vitamin D 800 IU daily decreases fall by 72 % Graafmans et al (1996), Netherlands RCT Vitamin D 400 IU vs placebo ➔ Vitamin D was not related to falls or recurrent falls

Appendix 3: PRISMA Checklist of items to include when reporting a systematic review or meta-analysis, adapetd from the PRISMA statement [1](page 18)

Section/topic	Item number	Checklist item
Title		
Title	1	Identify the report as a systematic review, meta-analysis, or both
Abstract		
Structured summary	2	Provide a structured summary including, as applicable, background, objectives, data sources, study eligibility criteria, participants, interventions, study appraisal and synthesis methods, results, limitations, conclusions and implications of key findings, systematic review registration number
Introduction		
Rationale	3	Describe the rationale for the review in the context of what is already known
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS)
Methods		
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (such as web address), and, if available, provide registration information including registration number
Eligibility criteria	6	Specify study characteristics (such as PICOS, length of follow-up) and report characteristics (such as years considered, language, publication status) used as criteria for eligibility, giving rationale
Information sources	7	Describe all information sources (such as databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated
Study selection	9	State the process for selecting studies (that is, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis)
Data collection process	10	Describe method of data extraction from reports (such as piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators
Data items	11	List and define all variables for which data were sought (such as PICOS, funding sources) and any assumptions and simplifications made
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis
Summary measures	13	State the principal summary measures (such as risk ratio, difference in means).
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (such as I2) for each meta-

Section/topic	Item number	Checklist item
		analysis
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (such as publication bias, selective reporting within studies)
Additional analyses	16	Describe methods of additional analyses (such as sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified
Results		
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram
Study characteristics	18	For each study, present characteristics for which data were extracted (such as study size, PICOS, follow-up period) and provide the citations
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see item 12).
Results of individual studies	20	For all outcomes considered (benefits or harms), present for each study (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see item 15)
Additional analysis	23	Give results of additional analyses, if done (such as sensitivity or subgroup analyses, meta-regression [see item 16])
Discussion		
Summary of evidence	24	Summarise the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (such as health care providers, users, and policy makers)
Limitations	25	Discuss limitations at study and outcome level (such as risk of bias), and at review level (such as incomplete retrieval of identified research, reporting bias)
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research
Funding		
Funding	27	Describe sources of funding for the systematic review and other support (such as supply of data) and role of funders for the systematic review

[1] http://www.prisma-statement.org/

Appendix 4: Search strategy

Medline search:

1. exp Vitamin D/
2. Vitamin D Deficiency/
3. ((avitamin* or hypovitamin* Hypervitamin* or plivit or glycol or davitamon or chemovit or arthrin* or crivit or vita* or vitasan or vio or idro* or inovitan or vitastab* or vatin* or difvitamin or uvesterol or wandervit or vitavel or oleovit or oleovitamin or min* or vitamin* or hydroxyvitamin* or (hydroxy adj vitamin*) or dihydroxyvitamin* or (dihydroxy adj vitamin*)) adj3 (d or d2 or d3)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
4. (vitamind* or cholecalciferol* or calciol* or calcitriol* or hydroxycholecalciferol* or (hydroxy adj cholecalciferol*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
5. (dihydroxycholecalciferol* or (dihydroxy adj cholecalciferol*) or ergocalciferol* or calcifediol* or calcidiol* or calderol* or dedrogyl* or calciferol* or hidroferol* or calcijex or sitriol* or silkis or osteotriol* or soltriol* or decostriol*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
6. (renatriol* or rocaltrol* or tirocal* or dihydrotachysterol* or (dihydro adj tachysterol*) or tachystin* or calcamin* or dihydrotachysterin* or (dihydro adj tachysterin*) or ercalcidiol*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
7. (bocatriol* or alphacalcidiol* or (alpha adj calcidiol*) or alfacalcidiol* or (alfa adj calcidiol*) or colecalciferol* or ercalcitriol* or sterogyl* or (euro adj d) or hydroxyergocalciferol* or (hydroxy adj ergocalciferol*) or hydroxycalciferol* or (hydroxy adj calciferol*) or calcitriolnefro* or (calcitriol adj nefro*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
8. (secoergosta or secocholesta or Diol* or delakmin* or didrogyl or dydrogil or alcovit* or aldevit* or bentavit or calciferovit* or drisdol or ergosterol* or devaron or duphafral or dupharinterfran or irradia or irradian or ostoforte or uvedose* or vigantol or vigorsan or viosterol* or arachitol or calciol or condol or davitan or davitin or sterogyl*OR vitaplex or osteovit* or sterosol or ercalciol or didrol or desyn* or diferol* or drisdol* or ergosteri* or fortedol or fortodyl* or sterodin* or ostelin or vitasterol or feroxyl* or shockferol* or infron* or vitadit or vid*OR sterobiol or kalciferol* or raquiferol* or sterovit or vioster*OR vitaminol or vitasterin or mukostin or radiamon or radiostol or radsterin* or radsterin* or delta* or derad* or diergin* or calciosterina or osteod*OR osteovit* or ertron* or steramin* or vitasterin* or mulsiferol* or oldevit or dergosten or deeosterol or deratol or detalup or deterap* or devit* or diactol or disterin* or ostergil or ergorone or feroxyl* or ostelin*OR infad* or steral or dekristol or activatum or diviturto or idrosol).ti,ab.
9. or/1-8

10. exp Middle East/
11. exp Africa, Northern/ or exp Djibouti/
12. (gaza or (west* adj2 bank)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
13. ((Middle adj2 east*) or mid-east* or (mid adj east*) or arab* or orient* or (near adj2 east*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
14. (MENA or leban* or syri* or yemen* or Iraq* or irak* or KSA or UAE or saudi* or Kuwait* or gulf* or bahrain* or transjordan* or jordan* or qatar* or quatar* or katar* or Israel* or palestin* or djibout* or persia* or iran* or malt* or oman* or byzanti* or fertile cresent or (islamic adj republic*) or (united adj arab* adj (emirat* or republic))).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
15. (beirut* or beyrouth or damascus or sana* or baghdad or riyadh* or dubai or (trucial adj stat*) or (abu adj dhabi) or manama or amman or doha or ghaza or (tel adj aviv) or haifa or jerusalem or ramallah or tehran or muscat or valetta).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
16. ((north* adj2 africa*) or (french adj speaking adj africa*) or aden or maghrib* or maghreb* or sahara or algeri* or algier* or egypt* or mediterranean* or cairo or liby* or libi* or tripoli or morocc* or rabat or tunisi* or tunesi* or ifni).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
17. or/10-16
18. randomized controlled trial.pt.
19. controlled clinical trial.pt.
20. randomized.ab.
21. placebo.ab.
22. placebo.ab.
23. clinical trials as topic.sh.
24. randomly.ab.
25. trial.ti.
26. or/18-25
27. exp animals/ not humans.sh.
28. 26 not 27
29. 9 and 17 and 28

PubMed Search:

((((((((((((((((randomized controlled trial[pt]) OR controlled clinical trial[pt]) OR randomized[tiab]) OR drug therapy[sh]) OR randomly[tiab]) OR trial[tiab]) OR groups[tiab]) OR placebo[tiab]) NOT (animals[mh] NOT humans[mh])))))) AND ((((((((((((((((middle east) OR middle east) OR north africa) OR djibouti) OR (middle east* OR mid-east* OR mid east* OR arab OR arabian OR arabic OR orient* OR near east*)) OR ((MENA OR leban* OR syri* OR yemen* OR Iraq* OR irak* OR KSA))) OR ((UAE OR saudi* OR Kuwait* OR gulf* OR bahrain* OR transjordan* OR jordan* OR qatar* OR quatar* OR katar* OR Israel* OR palestin* OR djibout* OR persia* OR iran* OR malta OR oman* OR byzanti*))) OR (united arab* AND emirat*)) OR united arab* AND republic*) OR gaza) OR (((west OR western) AND bank))) OR ((beirut* OR beyrouth OR damascus OR sanaa OR baghdad OR riyadh* OR dubai OR abu dhabi OR manama OR amman OR doha OR ghaza))) OR ((tel aviv OR haifa OR jerusalem OR ramallah OR tehran OR muscat OR aden OR maghrib* OR maghreb* OR sahar* OR algeri* OR algier* OR egypt* OR mediterranean* OR cairo OR liby* OR libi* OR tripoli OR morocc* OR rabat OR tunisi* OR tunesi* OR ifni*))) OR (((north* OR french) AND africa*)))))) AND ((((((((((vitamin d) OR cholecalciferol) OR vitamin d deficiency) OR ((vitamind* OR cholecalciferol* OR calciol* OR calcitriol* OR hydroxycholecalciferol* OR (hydroxy cholecalciferol*)))) OR ((avitamin* OR vitamin* OR Hypervitamin* OR glycol OR davaamon OR arthrin* OR vita OR vio OR idro* OR uvesterol OR oleovit OR oleovitamin OR mina OR hypovitamin* OR hydroxyvitamin* OR (hydroxy vitamin*) OR dihydroxyvitamin* OR (dihydroxy vitamin*)) AND (d OR d2 OR d3))) OR ((dihydroxycholecalciferol* OR (dihydroxy cholecalciferol*) OR ergocalciferol* OR calcifediol* OR calcidiol* OR calderol* OR dedrogyl* OR calciferol* OR hidroferol* OR calcijex OR soltriol* OR rocaltrol* OR tirocal* OR dihydrotachysterol* OR (dihydro tachysterol*) OR tachystin* OR calcamin* OR dihydrotachysterin* OR ercalcidiol*))) OR ((alphacalcidiol* OR (alpha calcidiol*) OR alfacalcidiol* OR (alfa calcidiol*) OR colecalciferol* OR ercalcitriol* OR sterogyl* OR (euro d) OR hydroxyergocalciferol* OR (hydroxy ergocalciferol*) OR hydroxycalciferol* OR (hydroxy calciferol*)))) OR ((secoergosta[tiab] OR secocholesta[tiab])))))))))

Embase Search:

#1.30 #1.13 AND #1.23 AND #1.29
#1.29 #1.24 OR #1.25 OR #1.26 OR #1.27 OR #1.28
#1.28 random* OR factorial* OR crossover* OR cross NEAR/2 over* OR placebo* OR doubl* NEAR/2 blind* OR singl* NEAR/2 blind* OR assign* OR allocat* OR volunteer*
#1.27 'single blind procedure'/exp
#1.26 'randomized controlled trial (topic)'/exp
#1.25 'double blind procedure'/exp
#1.24 'crossover procedure'/exp
#1.23 #1.14 OR #1.15 OR #1.16 OR #1.17 OR #1.18 OR #1.19 OR #1.20 OR #1.21 OR #1.22
#1.22 north* NEAR/2 africa* OR (french AND speaking AND africa*) OR aden OR maghrib* OR maghreb* OR sahara OR algeri* OR algier* OR egypt* OR mediterranean* OR cairo OR liby* OR tripoli OR morocc* OR rabat OR tunisi* OR tunesi* OR libi* OR ifni
#1.21 beirut* OR beyrouth OR damascus OR sana* OR baghdad OR riyadh* OR trucial NEAR/2 stat* OR abu NEAR/2 dhabi OR manama OR amman OR doha OR ghaza OR tel NEAR/2 aviv OR haifa OR jerusalem OR ramallah OR tehran OR muscat OR valetta OR dubai OR byzanti* OR transjordan* OR persia* OR islamic NEAR/2 republic* OR fertile NEAR/2 crescent
#1.20 united AND arab* NEAR/2 (emirat* OR republic*)
#1.19 middle NEAR/2 east* OR mid NEAR/2 east* OR arab* OR orient* OR near NEAR/2 east*
#1.18 'malta'/exp
#1.17 'yemen'/exp
#1.16 'djibouti'/exp
#1.15 'north africa'/exp
#1.14 'middle east'/exp
#1.13 #1.1 OR #1.2 OR #1.3 OR #1.4 OR #1.5 OR #1.6 OR #1.7 OR #1.8 OR #1.9 OR #1.10 OR #1.11 OR #1.12
#1.12 secoergosta:ab,ti OR secocholesta:ab,ti
#1.11 bocatriol* OR alphacalcidiol* OR alpha NEAR/2 calcidiol* OR alfacalcidiol* OR alfa NEAR/2 calcidiol* OR colecalciferol* OR ercalcitriol* OR sterogyl* OR euro NEAR/2 d OR hydroxyergocalciferol* OR hydroxy NEAR/2 ergocalciferol* OR hydroxycalciferol* OR hydroxy NEAR/2 calciferol* OR calcitriolnefro* OR calcitriol NEAR/2 nefro*
#1.10 dihydroxycholecalciferol* OR dihydroxy NEAR/2 cholecalciferol* OR ergocalciferol* OR calcifediol* OR calcidiol* OR calderol* OR dedrogyl* OR calciferol* OR hidroferol* OR calcijex OR sitriol* OR silkis OR osteotriol* OR soltriol* OR decostriol*
#1.9 renatriol* OR rocaltrol* OR tirocal* OR dihydrotachysterol* OR dihydro NEAR/2 tachysterol* OR tachystin* OR calcamin* OR dihydrotachysterin* OR dihydro NEAR/2 tachysterin* OR ercalcidiol*
#1.8 cholecalciferol* OR diol* OR delakmin* OR didrogyl OR dydrogil OR alcovit* OR aldevit* OR bentavit OR calciferovit* OR drisdol OR ergosterol* OR devaron OR duphafral OR dupharinterfran OR irradia OR irradian OR ostoforte OR uvedose* OR vigantol OR vigorsan OR viosterol* OR arachitol OR calciol OR condol OR davitan OR davitin OR sterogyl* OR vitaplex OR sterosol OR ercalciol OR didrol OR desyn* OR diferol* OR drisdol* OR ergosteri* OR fortedol OR fortodyl* OR sterodin* OR ostelin OR vitasterol OR shockferol* OR infron* OR

vitadit OR vid* OR sterobiol OR kalciferol* OR raquiferol* OR sterovit OR vioster* OR vitaminol OR vitasterin OR mukostin OR radiamon OR radiostol OR radsterin* OR delta* OR derad* OR diergin* OR calciosterina OR osteod* OR osteovit* OR ertron* OR steramin* OR vitasterin* OR mulsiferol* OR oldevit OR dergosten OR deeosterol OR deratol OR detalup OR deterap* OR devit* OR diactol OR disterin* OR ostergil OR ergorone OR feroxyl* OR ostelin* OR infad* OR steral OR dekristol OR activatum OR diviturto OR idrosol OR calciol* OR calcitriol* OR hydroxycholecalciferol* OR hydroxy NEAR/2 cholecalciferol*
#1.7 (dihydroxyvitamin OR avitamin* OR hypoviatmin* OR hypervitamin* OR plivit OR glycol OR davitamon OR chemovit OR arthrin* OR crivit OR vita* OR vitasan OR vio OR idro* OR inovitan OR vitastab* OR vatin* OR difvitamin OR uvesterol OR wandervit OR vitavel OR oleovit OR oleovitamin OR min*) NEAR/2 (d OR d2 OR d3)
#1.6 dihydroxy AND vitamin NEAR/2 (d OR d2 OR d3) #1.5
hydroxy AND vitamin NEAR/2 (d3 OR d2 OR d)
#1.4 vitamind* OR (vitamin* OR hydroxyvitamin*) NEAR/2 (d3 OR d2 OR d)
#1.3 'vitamin d intoxication'/exp
#1.2 'vitamin d deficiency'/exp
#1.1 'vitamin d'/exp

Cochrane Library Search:

Search Hits
#1 MeSH descriptor: [Vitamin D] explode all trees
#2 MeSH descriptor: [Vitamin D Deficiency] explode all trees
#3 (avitamin* or hypovitamin* or vitamin* or hydroxyvitamin* or hydroxy near/2 vitamin*or dihydroxyvitamin* or dihydroxy near/2 vitamin*) near/3 (d or d2 or d3)
#4 vitamind* or cholecalciferol* or calciol* or calcitriol* or hydroxycholecalciferol* or hydroxy near/2 cholecalciferol*
#5 dihydroxycholecalciferol* or dihydroxy near/2 cholecalciferol* or ergocalciferol* or calcifediol* or calcidiol* or calderol* or dedrogyl* or calciferol* or hidroferol* or calcijex or sitriol* or silkis or osteotriol* or soltriol* or decostriol*
#6 renatriol* or rocaltrol* or tirocal* or dihydrotachysterol* or (dihydro near/2 tachysterol*) or tachystin* or calcamin* or dihydrotachysterin* or (dihydro near/2 tachysterin*) or ercalcidiol*
#7 bocatriol* or alphacalcidiol* or alpha near/2 calcidiol* or alfacalcidiol* or alfa near/2 calcidiol* or colecalciferol* or ercalcitriol* or sterogyl* or euro near/2 d or hydroxyergocalciferol* or hydroxy near/2 ergocalciferol* or hydroxycalciferol* or hydroxy near/2 calciferol* or calcitriolnefro* or calcitriol near/2 nefro*
#8 secoergosta or secocholesta 0
#9 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8
#10 MeSH descriptor: [Middle East] explode all trees
#11 MeSH descriptor: [Africa, Northern] explode all trees
#12 MeSH descriptor: [Djibouti] explode all trees
#13 gaza or (west* near/2 bank)
#14 Middle near/2 east* or mid east*
#15 mid near/2 east* or arab*
#16 orient* or east*
#17 MENA or leban* or syri* or yemen* or Iraq* or irak* or KSA or UAE or saudi* or Kuwait* or gulf* or bahrain* or transjordan* or jordan* or qatar* or quatar* or katar* or Israel* or palestin* or djibout* or persia* or iran* or malt* or oman* or byzanti* or fertile cresent
#18 islamic near/2 republic* or (united near/2 arab* near/2 (emirat* or republic))
#19 beirut* or beyrouth or damascus or sana* or baghdad or riyadh* or dubai or (trucial near/2 stat*) or (abu near/2 dhabi) or manama or amman or doha or ghaza or (tel near/2 aviv) or haifa or jerusalem or ramallah or tehran or muscat or valetta
#20 (north* near/2 africa*) or (french near/2 speaking near/2 africa*) or aden or maghrib* or maghreb* or sahara or algeri* or algier* or egypt* or mediterranean* or cairo or liby* or libi* or tripoli or morocc* or rabat or tunisi* or tunesi* or ifni
#21 #10 or #11 or #12 or #13 #14 or #15 or #16 or #17 or #18 or #19 or #20
#22 #9 and #21

Appendix 5: Comparisons of proportions above a certain cutoff of 25(OH)D level reported in papers versus proportions calculated assuming normality

Dawodu et al 2013:

a-Maternal vitamin D levels:

Arms	**400 IU/d (N=42)**	**2,000 IU/d (N=41)**	**4,000 IU/d (N=43)**
Mean 25(OH)D (SD)	19.27(7.96)	25.95(10.23)	35.92(12.12)
Proportion (%) above 32 ng/ml reported in paper A	10	24	65
Proportion (%) above 32 ng/ml calculated assuming normality B	5.6%	27.7	62.5
Difference(%) between the 2 values A-B	4.4	-3.7	2.5

b-Neonatal vitamin D levels:

Arms	**400 IU/d (N=41)**	**2,000 IU/d (N=39)**	**4,000 IU/d (N=42)**
Mean 25(OH)D (SD)	14.76(7.12)	19.32(8.68)	26.44(8.32)
Proportion (%) above 20 ng/ml reported in paper A	22	47	75
Proportion (%) above 20 ng/ml calculated assuming normality B	23	47	78
Difference(%) between the 2 values A-B	-1	0	-3

Hollis et al 2011:

Maternal 25(OH)D	**400 IU/d (N=111)**	**2,000 IU/d (N=122)**	**4,000 IU/d(N=117)**
Mean 25(OH)D (SD)	31.6(14.6)	39.4(13.7)	44.5(16.2)
Proportion (%) above 32 ng/ml reported in paper A	50	73.9	82
Proportion (%) above 32 ng/ml calculated assuming normality B	49	70.5	78
Difference(%) between the 2 values A-B	1	3.4	4

El Hajj Fuleihan et al 2015:

Arms	**600 IU/d (N=112)**	**3,740 IU/d(N=110)**
Mean 25(OH)D (SD)	31.6(14.6)	39.4(13.7)
Proportion (%) above 32 ng/ml reported in paper A	83	98
Proportion (%) above 32 ng/ml calculated assuming normality B	81	95
Difference(%) between the 2 values A-B	2	3

Appendix 6: Excluded studies with the specific reason for exclusion

Author year	Reason for exclusion
1. Abdel Kader 1972	Rickets
2. Abdel Salam 1973	Rickets
3. Abdel Maksoud 2013	Not RCT
4. Abou Raya 2013	Steroids
5. Abu Raya 2015	Abstract only
6. Abu Faraj 2003	Not RCT
7. Abu-Much 2010	Hepatitis C patients
8. Abushama 2003	Not RCT
9. Achiron 2015	Active vitamin D
10. Aflatonian 2014	Duration 6-8 weeks
11. Aghaei 2010	Published only as abstract
12. Al Afarj 2003	Not RCT
13. Al Asmari 2014	Not humans
14. Albenali 2012	Duration 2 months
15. Al Daghri 2011	Not RCT
16. Al Faraj 2003	Not RCT
17. Al Hilali 2008	Active vitamin D and Hemodialysis patients
18. Al Humaidi 2013	Not RCT
19. Al Jawad 2008	No Vitamin D
20. Alizade 2006	Duration 9 weeks
21. Alrefai 2014	Protocol only
22. Al Shaar 2013	Same as El Hajj Fuleihan
23. Aluisio 2013	Afghanistan, not MENA
24. Amin 2013	Not RCT
25. Arab 2012	Vitamin D as fortified milk
26. Arabi 2009	Same as El Hajj Fuleihan
27. Arshi 2014	Inhaled steroids
28. Arvold 2009	Not MENA
29. Assadi 2014	IM vitamin D
30. Azemi 2013	Duration 6 weeks
31. Ataie-Jafari 2013	Active D
32. Badsha 2011	Abstract only
33. Bar Yoseph 2015	Duration 6 weeks
34. Barak 2014	Active vitamin D
35. Baziar 2014	Duration 6 weeks
36. Bevenuti 2009	Abstract only
37. Behradmanesh 2013	Same as Nasri 2014 (Persian)
38. Beigi 2012	25(OH)D levels not mentioned
39. Benchimol 2007	Not MENA
40. Ben-Ezer 1991	Vitamin D analogues dialysis patients

Author year	Reason for exclusion
41. Bhat 2001	Vitamin D as fortified milk
42. Bilenko 2010	25(OH)D levels not mentioned
43. Bloomer 2015	Not MENA
44. Blumberg 1980	Active vitamin D
45. Bonakdaran 2008	Active vitamin D
46. Bugrul 2013	Turkey, not MENA
47. Chagnac 1999	Active vitamin D
48. Chen 1996	Active vitamin D
49. Colacuri 2011	Same vitamin D dose in both groups
50. Connell 2013	Not MENA, protocol without results
51. Dadaei 2015	Inflammatory Bowel Disease
52. Dahifar 2007	Vitamin D for 20 days
53. Darabi 2013	Inhaled steroids
54. Duweb 2003	Topical calcipotriol
55. Duweb 2005	Topical calcipotriol
56. Duweb 2005	Topical calcipotriol
57. Duweb 2000	Topical calcipotriol
58. Duweb 2001	Topical calcipotriol
59. Eastwood 1971	Renal osteomalacia
60. El-Agroudy 2003	Steroids in renal transplant
61. El-Agroudy 2005	Steroids , active D, renal transplant
62. El-Husseini 2004	Active vitamin D in renal transplant
63. El-Husseini 2004	Active vitamin D in renal transplant
64. El Reshaid 1997	Injectable D versus active D
65. El Shafey 2011	Active vitamin D
66. Esmat 2015	Chronic Hepatitis C
67. Eftekhari 2014	Active vitamin D
68. Farvid 2005	no vitamin D
69. Feldman 2013	Topical vitamin D
70. Fisk 2012	Vitamin D as fortified milk and for 4 weeks
71. Foroughi 2014	Duration 10 weeks
72. Gendelman 2015	No vitamin D level post intervention
73. Grau 2003	Not MENA
74. Gholami 2015	Single IM dose of vitamin D
75. Groleau 2013	Philadelphia, not MENA
76. Guingnard 1971	Rachitic and hospitalized neonates
77. Haddad 2004	Hemodialysis
78. Hamdy 1995	Active D and not MENA
79. Hamidieh 2015	Active vitamin D
80. Hashemipour 2014	Duration 2 weeks
81. Hellstrom 1988	Sweden, not MENA
82. Heravifard 2013	Yogurt fortified with D

Author year	Reason for exclusion
83. Heshmat 2012	Single IM dose
84. Helou 2013	Duration 8 weeks
85. Holick 2010	US, not MENA
86. Hosseinzad 2012	Single IM D dose
87. Hosseinzadeh 2012	IM vitamin D
88. Ibrahim 2013	Active vitamin D
89. Iraj 2012	IM vitamin D
90. Irct..Shaygannejad 2011	Active vitamin D
91. Ish-Shalom 2008	Duration 8 weeks
92. Javanbakht 2009	Duration 60 days
93. Josse 2010	no vitamin D
94. Jovanovic 1993	Active vitamin D
95. Jozanikohan 2015	Duration 10 weeks
96. Kaviani 2012	Not RCT
97. Kalai 2008	not RCT, prospective with 2 arms
98. Karmali 2015	Duration 6 weeks
99. Kardegari 2010	Inconsistency of data between persian article and English abstract
100. Kelishadi 2014	Duration 4 weeks apart.
101. Keshtkar 2015	Vitamin D as fortified milk
102. Kermack 2014	Not MENA
103. Khadilkar 2014	Not MENA (India)
104. Khajehdehi 2000	Hemodialysis
105. Khajehdehi 2003	Hemodialysis
106. Khajehi 2009	Duration 2 months
107. Khan 2011	Not RCT
108. Khoraminya 2013	8 weeks duration
109. Knusten 2014	Immigrants from MENA
110. Lamb 2011	US, not MENA
111. Levi 1998	Not RCT
112. Llach 1998	Hemodialysis
113. Lips 2001	Not RCT
114. Lubani 1989	Rickets
115. Maalouf 2008	Same as El Hajj Fuleihan 2006
116. Madar 2011	No vitamin D supplementation
117. Maguire 2014	Not MENA
118. Manaseki-Holland 2010	Afghanistan, not MENA
119. Manaseki-Holland 2012	Afghanistan, not MENA
120. Mann 2015	Not MENA
121. Marwaha 2011	Rickets
122. Masole 2010	Vitamin D dose not mentioned
123. Mazahery 2015	MENA immigrants

Author year		Reason for exclusion
124.	Mehdi 2013	Vitamin D as fortified milk
125.	Melhem 2015	Duration < 3 months
126.	Memmos 1998	Active D in hemodialysis
127.	Menczel 1994	Active D
128.	Mikati 2006	Anticonvulasnts
129.	Mirghafourvand 2014	Duration 6 days
130.	Moghassemi 2014	No vitamin D level post intervention
131.	Mottaghi 2014	Duration 10 weeks
132.	Mozaffari-Khosravi 2015	Duration 6 weeks
133.	Mustafar 2014	Chronic kidney disease
134.	Moe 2008	Hemodialysis
135.	Morcos 1998	Duration 8 weeks
136.	Mosayebi 2011	IM vitamin D
137.	Mozaffari 2013	IM vitamin D
138.	Mucci 2006	Not MENA
139.	Mutlu 2014	Turkey, not MENA
140.	Nadi 2015	Same dose of vitamin D in both arms
141.	Neyestani 2012	Vitamin D as fortified yogurt
142.	Nguema-Asseko 2005	Gabon, not MENA
143.	Nikooyeh 2013	Vitamin D as fortified food
144.	O'Connell 2013	Not MENA
145.	Osborne 2011	Not RCT
146.	Pasalic 2014	Not MENA
147.	Patel 2010	Not RCT
148.	Przedlacki 1993	CKD
149.	Puel 2011	Vitamin D Fortified milk
150.	Rajah 2010	Active D and rickets
151.	Rajakumar 2005	Not RCT
152.	Ramnath 2013	Active D
153.	Rashidi 2009	25(OH)D levels not mentioned
154.	Rassoul 1995	Active D
155.	Rees 2013	Not MENA
156.	Rees 2001	Not MENA
157.	Rizoli 2012	Not RCT
158.	Rothberg 1982	Not MENA
159.	Rowaily 2009	Not RCT
160.	Saad 2015	Duration in hours
161.	Saadi 2009	Same vitamin D dose
162.	Saadi 2007	Same vitamin D dose
163.	Sabry 2015	Chronic Hepatitis C
164.	Sakalli 2012	Turkey, not MENA

Author year		Reason for exclusion
165.	Salahuddin 2013	IM vitamin D
166.	Salesi 2011	Steroids
167.	Segal 2003	Same vitamin D dose
168.	Sedighi 2014	Protocol only
169.	Shab-bidar 2011	Vitamin D fortified yogurt
170.	Shab-bidar 2012	Vitamin D fortified yogurt
171.	Shab-bidar 2014	Vitamin D fortified yogurt
172.	Shabbidar 2015	Vitamin D fortified dough
173.	Shahidi 2012	Renal transplant
174.	Shajari 2009	Duration <3 mo
175.	Shaker 2010	Not RCT
176.	Shakiba 2011	Not RCT
177.	Shakiba 2011	vitamin D given every 3 mo
178.	Shakinba 2011	Vitamin D given every 3 mo
179.	Shams 2014	No vitamin D levels
180.	Shedeed 2012	Children with heart failure
181.	Shirvani 2015	Not RCT
182.	Siafarikas 2009	Immigrants from MENA
183.	Siafarikas 2011	Not MENA
184.	Soliman 2011	Not RCT
185.	Srour 2013	No Vitamin D supplementation
186.	Salahuddin 2013	IM vitamin D
187.	Tabesh 2014	Duration 8 weeks
188.	Taghizadeh 2014	Same dose of D in both arms
189.	Tapola 2004	No Vitamin D supplementation
190.	Tarrass 2006	End stage renal disease
191.	Tavakoli 2011	Active D
192.	Teramato 2006	Not MENA
193.	Ueda Yasus 2004	Hemodialysis
194.	Wang 2014	Not MENA
195.	Whiting 2005	Not RCT
196.	Yousefi 2014	Duration 2 months
197.	Yu 2009	Immigrants from MENA
198.	Zabihiyeganeh 2012	IM vitamin D
199.	Zabihiyeganeh 2015	Duration 8 weeks
200.	Zanghene 2014	Abstract only
201.	Zeitoun 1967	Single high dose vitamin D

MENA: Middle East and North Africa; RCT: Randomized Controlled Trial; IM: Intra-muscular

Appendix 7: Effect of vitamin D supplementation on other outcomes

A- Adults

High dose (>2,000 IU/d) versus placebo comparison

Outcome	Result	Studies included
Calcium level mg/dl	0.08 [-0.11, 0.28]	Ahmadi 2013 Sadiya 2014 Sharifi 2014 Tehrani 2014 Al-Sofiani 2015
BMI kg/m^2	-0.65 [-1.36, 0.07]	Firouzabadi 2012 Nasri 2014 Sadiya 2014
HbA1c %	0.11 [-0.33, 0.55]	Ahmadi 2013 Hosseini 2013 Nasri 2014 Al-Zahrani 2014 Al-Sofiani 2015
HOMA-IR	0.96 [0.32, 1.61]*	Hoseini 2013 Sharifi 2014 Al-Sofiani 2015
Triglycerides mg/dl	19.67 [-0.59, 39.93]	Behradmanesh 2011/Nasri 2014 Al-Zahrani 2014
LDL mg/dl	-3.51 [-10.93, 3.91]	Behradmanesh 2011/Nasri 2014 Al-Zahrani 2014
HDL mg/dl	-0.85 [-2.99, 1.29]	Behradmanesh 2011/Nasri 2014 Al-Zahrani 2014
Diastolic blood pressure mmHg	0.84 [-3.07, 4.75]	Nasri 2014 Al-Zahrani 2014 Al-Sofiani 2015
Systolic blood Pressure mmHg	-3.53 [-6.30, -0.76]*	Nasri 2014 Al-Zahrani 2014 Al-Sofiani 2015

*significant results.

B-Pregnant women:

Intermediate (800-2,000 IU/d) versus low dose (< 800 IU/d)

Outcome	Result	Studies included
Calcium level mg/dl	0.06 [-0.06, 0.18]	Dawodu 2013 Soheilykhah 2013

High (>2,000 IU/d) versus intermediate dose (800-2,000 IU/d)

Outcome	Result	Studies included
Calcium level mg/dl	-0.05 [-0.41, 0.30]	Dawodu 2013 Soheilykhah 2013

High (>2,000 IU/d) versus low dose (<800 IU/d)

Outcome	Result	Studies included
Calcium level mg/dl	0.01 [-0.28, 0.31]	Dawodu 2013 Soheilykhah 2013

C- Children

Intermediate dose (800-2,000 IU/d) versus placebo

Outcome	Result	Studies included
Calcium level mg/dl	0.01 [-0.28, 0.31]	Maalouf 2008 Ghazi 2010
PTH pg/ml	-7.00 [-7.38, -6.62]*	Maalouf 2008 Ghazi 2010

*significant results.

Low dose (<800 IU/d) versus placebo

Outcome	Result	Studies included
PTH pg/ml	-8.18 [-22.68, 6.32]	Maalouf 2008 Neyestani 2013

Appendix 8: Comparison of multivariate analysis including Vitamin Dose (1,000 IU/d) versus Ln Dose (IU/d), as covariates

a- Analysis using vitamin D dose as a covariate

Multivariate analysis at p-value 0.1➔ model includes dose, duration and baseline level

Independent variable	β	p-value	Model characteristics
Constant	6.4	0.068	p-value < 0.001 Tau^2 26.7 I^2 res 92.4 Adjusted R^2 87.1
Dose (1,000 IU/d)	4.4	<0.001	
Duration category (3 months vs > 3 months)	-1.5	0.514	
Baseline 25(OH)D (ng/ml)	0.76	<0.001	

Removing duration category from the model

Independent variable	β	p-value	Model characteristics
Constant	5.3	0.077	p-value < 0.001 Tau^2 25.6 I^2 res 91.8 Adjusted R^2 87.6
Dose (1,000 IU/d)	4.45	<0.001	
Baseline 25(OH)D (ng/ml)	0.77	<0.001	

Sensitivity analysis forcing in the model Age, BMI and Ca supplementation

Independent variable	β	p-value	Model characteristics
Constant	19.1	0.125	Tau^2: 23.3 I^2 res: 89.8 Adjusted R^2: 88.6
Dose	4.45	<0.001	
Baseline 25(OH)D (ng/ml)	.81	<0.001	
Age (years)	.08	0.351	
Ca supplementation (yes versus no)	-4	0.088	
BMI (kg/m^2)	-0.57	0.139	

b- Sensitivity analysis replacing Dose by Lndose as a covariate (and using only the intervention arms)

Model including Ln dose, duration and baseline level

Independent variable	β	p-value	Model characteristics
Constant	-26.6	0.263	p-value = 0.0016 Tau^2 55.9 I^2 res 91 Adjusted R^2 70.4
Ln Dose (IU/d)	6.7	0.029	
Duration category (3 months vs > 3 months)	-8.11	0.12	
Baseline 25(OH)D (ng/ml)	0.78	0.029	

Model including Ln dose and baseline level

Independent variable	**β**	**p-value**	**Model characteristics**
Constant	-49.3	0.025	p-value = 0.0013 Tau^2 67.6 I^2 res 93 Adjusted R^2 64.2
Ln Dose (IU/d)	8.9	0.004	
Baseline 25(OH)D (ng/ml)	0.77	0.039	

Model including Ln dose and baseline level, and forcing in the model Age, BMI, Ca supplementation

Independent variable	**β**	**p-value**	**Model characteristics**
Constant	-46.3	0.113	p-value: 0.001 Tau^2: 65.3 I^2 res: 90.5 Adjusted R^2: 65.4
Ln Dose (IU/d)	9.79	0.004	
Baseline 25(OH)D (ng/ml)	0.85	0.048	
Age (years)	0.19	0.317	
Ca supplementation (yes versus no)	-7.5	0.172	
BMI (kg/m^2)	-0.6	0.491	

Appendix 9A: Risk of bias assessment in studies conducted in adults

Author Year	Sequence generation (Selection bias)	Allocation concealment (Selection bias)	Blinding of participants and personnel (Performance bias)	Blinding of outcome assessment (Detection bias)	Incomplete outcome data (Attrition bias)	Selective outcome reporting (Reporting bias)	Other sources of bias	Summary assessment
Ahmadi 2013	*"Permuted random blocks"*but no further details ***Unclear***	Not mentioned ***Unclear probably not done***	*"Double blind RCT", no further information* ***Unclear probably blinding was done***	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	7 in placebo group lost to follow up and 2 in vitamin D group lost to follow up, but no further details. ***Unclear***	No published protocol ***Unclear***	They did not mention any of the predictors of low vitamin D such as physical activity, veiling, sun exposure or season ***Low risk***	***Unclear***
Al Sofiani 2015	*"simple computer-generated program randomization"* ***Low risk***	Not mentioned ***Unclear probably not done***	*"Randomized, placebo-controlled, double-blind trial.The placebo was matchedto the D capsules for shape, size and color(Bio-Tech-Pharmacal, Fayetteville, AR, USA)."* ***Low risk***	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	*"Two patients left the study due to moving overseas (n = 1) or personal reasons (n = 1)."* ***Low risk***	*"Registered at the Australian NewZealand Clinical Trial Registry (ACTRN12612000714886)"* ***Low risk***	*None of the predictors of vitamin D level were mentioned* ***Low risk***	***Unclear***
Al-Zahrani 2014	*No details* ***Unclear***	*"Sequentially numbered, opaque sealed envelopes; randomization done by one clinical nurse, not in direct contact with patients or physicians."* ***Low risk***	Blinding not discussed. Since the control group received education on how to increase vitamin D using non-pharmacologic ways, blinding of participants was not done. ***High risk***	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	*"Out of the 200 subjects who started, 183 subjects (N = 91 treated, N = 92 control) were able to complete the intervention."* Lost to follow up are evenly distributed between the 2 arms ***Low risk***	*No published protocol* ***Unclear***	Some of the predictors of vitamin D level were collected. Several covariates were not evenly distributed between the 2 arms: age, gender, OAD ***Unclear***	***High risk***
Breslavsky 2013	*No details* ***Unclear***	*No details* ***Unclear probably not done***	*No available info* ***Unclear probably not done***	Blinding of outcome assessor not mentionedbut the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	*15 lost to follow up* *5 in vitamin D group and 10 in control group* *1 patient from Vitamin D group withdrew because diarrhea and 1 because weakness. In control. Group 2 patients discontinued follow up because of prolonged hospitalization for respiratory infection and elective hospitalization for cholecystectomy. Two patients, both of them women, discontinued*	*No published protocol* ***Unclear***	None of the predictors of vitamin D level were mentioned. ***Low risk***	***High risk***

Author Year	Sequence generation (Selection bias)	Allocation concealment (Selection bias)	Blinding of participants and personnel (Performance bias)	Blinding of outcome assessment (Detection bias)	Incomplete outcome data (Attrition bias)	Selective outcome reporting (Reporting bias)	Other sources of bias	Summary assessment
					follow-up because fracture (hip and radial). The reason for the rest of dropouts was loss to follow-up. *High proportion lost to follow up and not evenly distributed between the 2 arms* ***high risk***			
El Hajj Fuleihan 2015	Stratified randomization by center and gender with treatment assignment based on matching subjects' baseline randomization ID number with treatment code ***Low risk***	*Allocation was done by pharmacy* ***Low risk***	*Researchers and subjects were blinded to the study intervention, intervention and placebo tabs were exactly similar* ***Low risk***	All research team was blinded to the study intervention ***Low risk***	Participants lost to follow up were evenly distributed in both groups, ***Low risk***	Predefined outcomes and published protocol on clinical trials.gov NCT01315366 ***Low risk***	***Low risk***	***Low risk***
Firouzabadi 2012	*Patientswere divided into two groups of 50 patients based ona random number table* ***Low risk***	*Not discussed* ***Unclear*** ***probably not done***	*Not discussed* ***Unclear*** ***probably not done***	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	Not discussed ***Unclear***	No published protocol ***Unclear***	**Low risk**	***Unclear***
Ghavamzadeh 2015	Not discussed ***Unclear***	*"Investigator assistant did the randomization"* ***High risk***	*"Randomized double-blind placebo-controlled. Vitamin D and placebo tabs were similar"* ***Low risk***	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	*Loss to follow up of > 50% in each treatment arm (32 in D group and 33 in the control group); the reason was the same in both groups* ***Low risk***	No published protocol ***Unclear***	***Low risk***	***High risk***
Golan 2013	*"Assignment to groups was randomly set in advance, accordingto recruitment order"* ***High risk***	*Not discussed* ***Unclear*** ***probably not done***	*"Double-blind in this study – both participants, physicians and investigators were unaware of the ingredients of the solution bottles".* ***Low risk***	*"Double-blind in this study – both participants, physicians and investigators were unaware of the ingredients of the solution bottles".* ***Low risk***	6 in the high dose group and 8 in the low dose group were lost to follow up. *"Censored patients in the high dose group were closer to the time of MS diagnosis. Censored patients in the low dose group were mainly males whereas those with complete follow up were mainly females.*	No published protocol ***Unclear***	***Low risk***	***High risk***

Author Year	Sequence generation (Selection bias)	Allocation concealment (Selection bias)	Blinding of participants and personnel (Performance bias)	Blinding of outcome assessment (Detection bias)	Incomplete outcome data (Attrition bias)	Selective outcome reporting (Reporting bias)	Other sources of bias	Summary assessment
					Apart from these, no other significant differences in baselinecharacteristics were noted between the censored and uncensoredpatients in the two intervention groups." Low risk			
Hoseini 2013	*"Pre-diabetic patients from the previous project had been randomized by using a random number table into three treated groups"* ***Low risk***	*Not discussed* ***Unclear probably not done***	*"Double blind RCT", no further information* ***Unclear probably blinding was done***	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	One in the control group, because of journey, and two refused to do OGTT (one in the control group and one in oral vitamin D treated group). ***Low risk***	No published protocol ***Unclear***	Baseline characteristics were not evenly distributed between arms. ***High risk***	***High risk***
Nasri 2014	*"computer-generated randomly permutated codes (prepared by WHO/Geneva)."* ***Low risk***	*Not discussed* ***Unclear probably not done***	*"Double blind RCT", no further information* ***Unclear probably blinding was done***	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	*Not discussed and the number of participants who completed the study is not mentioned* ***Unclear***	Published protocol IRCT201011185191N6, but protocol outcomes and outcomes pre-specified in manuscript are glucose control but reported outcomes are SBP and DBP ***High risk***	*Difference in baseline vitamin D level 83.9(52) in intervention group vs 105.7(64) nmol/l in vitamin D group;* ***High risk***	***High risk***
Sadiya 2014	*"computer-generated random number sequence into one of the two groups"* ***Low risk***	*Vitamin D3 (Solgar, Leonia, NJ, USA) and placebo (starch) (Compound Pharmacy, Dubai, UAE) were in capsule form and identical in appearance. They were pre-packed in bottles and consecutively numbered to be dispensed by an independent pharmacist according to the randomization list.* ***Low risk***	*"Participants and the research team remained blinded to treatment allocation until after the final analysis was completed."* ***Low risk***	*"Participants and the research team remained blinded to treatment allocation until after the final analysis was completed."* ***Low risk***	Lost to follow up adequately distributed between the 2 arms, reason for loss to follow up was mentioned. ***Low risk***	Trial predefined Outcomes registered on clinicaltrial.gov NCT02101151 ***Low risk***	***Low risk***	**Low risk**

Author Year	Sequence generation (Selection bias)	Allocation concealment (Selection bias)	Blinding of participants and personnel (Performance bias)	Blinding of outcome assessment (Detection bias)	Incomplete outcome data (Attrition bias)	Selective outcome reporting (Reporting bias)	Other sources of bias	Summary assessment
Salehpour 2012	*"Individuals were randomly allocated in a double-blind parallel manner from randomized number in an 85-person list"* ***Unclear***	*To remain blinded, one research assistant who was notinvolved in data collection coordinated the supplement assignment schedule* ***Low risk***	***"Double blind trial", no further information*** ***Unclear probably was done***	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	*"In the placebo group, 4subjects were unwilling to continue the 12-week intervention for personal reasons and another subject usedOCP. In the vitamin D group, one subject followed a weight reduction program, one got pregnantand one was unwilling to continue the 12-weekintervention for personal reasons."* ***Low risk***	Registered on ClinicalTrial.gov NCT01344161. Iranian Registry of Clinical Trial (registration no. IRCT138809092709N2) Outcomes in protocol: change in glucose concentration and change in insulin concentration were not reported ***High risk***	***Low risk***	***High risk***
Sharifi 2014	*"Participants were randomly assigned to intervention or control group (1:1 ratio)in the random blocks of 6 subjects based on the blocked randomization method. The sequence of permuted blocks was generated with a computer random number generator"* ***Low risk***	*"An investigator with no clinical involvement in the trial packed the Supplements and placebos in numbered bottles based on the random list. The other person, who was notinvolved in the trial and not aware of random sequences, assigned the patients to the numbered bottles of pearls"* ***Low risk***	*"Randomized, double-blind, placebo-controlled trial with parallel design"; "Randomization and allocation were concealed from the researchers and participants until the statistical analysis was completed"* ***Low risk***	*"Randomization and allocation were concealed from the researchers and participants until the statistical analysis was completed"* ***Low risk***	4/30 in placebo and 3/30 in D were lost to follow up, reasons specified and evenly distributed between the 2 arms. ***Low risk***	The trial was registered at IRCT.ir (IRCT 2012071810333N1), published data consistent with the protocol. ***Low risk***	***Low risk***	**Low risk**
Taheri 2014	*"block randomization methodcomputer-generated randomization and based on it, each women with unique identification number was assigned to study groups. Vitamin D and placebo solutions were labeled as A and B by the pharmacist."* ***Low risk***	*"Oily vitamin D solution and same amounts of placebo with the similar color, smell, taste and appearance (both solutions were made by pharmacist of relevant university)"* ***Low risk***	*"researchers and participants didn't have any information about real contains of these solutions."* ***Low risk***	*"researchers and participants didn't have any information about real contains of these solutions."* ***Low risk***	*"Only 3 women were not eager to continue the study and were replaced"* ***Low risk***	**Published protocol** IRCT201105096284N2 Vitamin D level is secondary outcome, Bacterial vaginosis is primary outcome. ***High risk***	Variability in baseline characteristics, including baseline 25(OH)D level and oral supplement intake, affected the 25(OH)D level reached after the intervention. ***High risk***	**High risk**
Tehrani 2014	Not discussed ***Unclear***	Not discussed ***Unclear probably not done***	"double-blind trial" However, blindingcould not have been securedsince vitamin D groupwas receiving weekly tabs and	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor	Patients who have reported diarrhea, vomiting and severe abdominal distention during intervention were excluded from the study,	*Iranian Registry of Clinical Trials (IRCT), IRCT registration number (201308037513 N 3*	***Low risk***	**High risk**

Author Year	Sequence generation (Selection bias)	Allocation concealment (Selection bias)	Blinding of participants and personnel (Performance bias)	Blinding of outcome assessment (Detection bias)	Incomplete outcome data (Attrition bias)	Selective outcome reporting (Reporting bias)	Other sources of bias	Summary assessment
			placebo groups were taking only daily tabs. ***High risk***	blinding. ***Low risk***	but no further details The number of participants in the results was not mentioned ***Unclear***	***Low risk***		

Appendix 9B: Risk of bias assessment in studies conducted in pregnant women

Author Year	Sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (Attrition bias)	Selective outcome reporting (Reporting bias)	Other sources of bias	Summary assessment
Dawodu 2013	*"The randomization list was computer generated by the statistician"* ***Low risk***	*"A secretary not involved in the project allocated and kept a list of the randomization code of the enrolled patients.* *No details on concealment"* ***Low risk***	*"The investigators, patients, health care providers, and the laboratory staff performing the biochemical tests were blinded to the treatment."* ***Low risk***	*"The investigators, patients, health care providers, and the laboratory staff performing the biochemical tests were blinded to the treatment."* ***Low risk***	*An intention-to-treat analysis was followed. The women who exited the study before delivery had similar baseline characteristics as thosewhowere followed up to delivery except for lower vitaminD intake.* *Lost to F/U* *H=8 I=13 L=9* *(reasons for missing data not mentioned)* ***Low risk***	*(clinicaltrials.gov, number NCT00610688, protocol addition to IND 66346)* *Published protocol, predefined outcomes reported* ***Low risk***	***Low risk***	***Low risk***
Etimadifar 2015	*"List produced by a computer program"* ***Low risk***	Open label ***High risk***	Open label ***High risk***	Open label trial but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	15 lost to follow up in the vitamin D group. 13 lost to follow up in the control group. Reasons for loss to follow up not mentioned ***High risk***	No published protocol ***Unclear***	***Low risk***	***High risk***
Karamali 2015	*"computer random number generator"* ***Low risk***	An investigator with no clinical involvement in our study packed cholecalciferol and placebos in numbered bottles based on the random list.	Double blind Placebo pearls were similar in color, shape, size, and package to the vitamin D3 ones and contained edible paraffin.	Randomization and allocation were hidden from the researchers and pregnant women until the statistical analysis was completed	No loss to follow up ***Low risk***	Published protocol IRCT201410035623N27 Outcomes predefined ***Low risk***	***Low risk***	***Low risk***

Author Year	Sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (Attrition bias)	Selective outcome reporting (Reporting bias)	Other sources of bias	Summary assessment
		Randomization and allocation were hidden from the researchers and pregnant women until the statistical analysis was completed ***Low risk***	***Low risk***	***Low risk***				
Sabet 2013	Not mentioned ***Unclear probably not done***	Not mentioned ***Unclear probably not done***	Not mentioned ***Unclear probably not done***	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	Not mentioned ***Unclear probably not done***	Registered protocol IRCT201104306335N1 ***Low risk***	***Low risk***	***Unclear***
Shakiba 2013	Not mentioned ***Unclear probably not done***	Not mentioned ***Unclear probably not done***	Not mentioned ***Unclear probably not done***	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	Not mentioned ***Unclear probably not done***	No published protocol ***Unclear***	***Low risk***	***Unclear***
Soheilykhah 2013	*"Computer-generated random number lists were drawn up by an independent researcher"* ***Low risk***	Pregnant women and researchers were not blind to treatment assignment ***High risk***	Pregnant women and researchers were not blind to treatment assignment ***High risk***	Researchers were not blind to treatment assignment. However, the outcome - vitamin D level- assessment is not influenced by the	2 in the intermediate dose and 5 in the low dose were lost to follow up. The reason for loss to follow up not mentioned and numbers lost to follow up not	No published protocol ***Unclear***	***Low risk***	***High risk***

Author Year	Sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (Attrition bias)	Selective outcome reporting (Reporting bias)	Other sources of bias	Summary assessment
				assessor blinding. ***Low risk***	evenly distributed between arms ***High risk***			

Appendix 9C: Assessment of risk of bias in studies conducted in children and adolescents

Author Year	Sequence generation (Selection bias)	Allocation concealment (Selection bias)	Blinding of participants and personnel (Performance bias)	Blinding of outcome assessment (Detection bias)	Incomplete outcome data (Attrition bias)	Selective outcome reporting (Reporting bias)	Other sources of bias	Summary assessment
Ghazi 2010	*"Computer-generated random number table"* ***Low risk***	*"Randomization was performed with the use of sealed Envelopes"* ***Low risk***	*"Neither the research team nor the subjects was informed about type of intervention in the study."* ***Low risk***	*"Neither the research team nor the subjects was informed about type of intervention in the study."* ***Low risk***	*Not mentioned* ***Unclear***	*No published protocol* ***Unclear***	***Low risk***	***Unclear***
El Hajj Fuleihan 2006 Maalouf 2008	*"Randomization sequence, stratified by socioeconomic status, was generated by a computer at Merck headquarters, mailed to the study center"* ***Low risk***	*"Administered by a senior pharmacist"* ***Low risk***	*"Subjects were randomly assigned in a double-blind manner"* ***Low risk***	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding. ***Low risk***	12 boys (6.5%) and 11 girls (9.8%) There were no differences in dropout rates by treatment group in either sex. The reasons for dropout included being afraid of needle pricks, unable to make appointments, not liking the taste of the medication, and changing their mind about the study. ***Low risk***	*The study protocol is not available (trial published before the era of trials registry) but it is clear that the published reports include all expected outcomes, including those that were pre-specified* ***Low risk***	***Low risk***	***Low risk***
Neyestani 2013	*"Simple randomization (looks like tossing a coin which works with large sample size)* ***Low risk***	*"Test products and their related placebos had similar appearances and so they were coded in a way that was known only to the main*	*Test products and their related placebos had similar appearances and so they were coded in a way that was known only to the main*	Blinding of outcome assessor not mentioned but the outcome - vitamin D level- assessment is not influenced by the assessor blinding.	*The attrition rate was <8% (but my calculations in our groups of interest show 8.9% (6/67) loss to f/u in SP group and 11%(7/60) in the PBO group). The*	*No published protocol* ***Unclear***	***Low risk***	***High risk***

Author Year	Sequence generation (Selection bias)	Allocation concealment (Selection bias)	Blinding of participants and personnel (Performance bias)	Blinding of outcome assessment (Detection bias)	Incomplete outcome data (Attrition bias)	Selective outcome reporting (Reporting bias)	Other sources of bias	Summary assessment
		researchers. ***High risk***	*researchers."* ➔the researcher was not blinded ***High risk***	***Low risk***	*major cause of discontinuation was an unwillingness to donate blood for the second time (n = 28). Five children were absent in the second call on day as a result of a cold. No adverse effect of either of the interventions was reported.* ***Low risk***			

Appendix 10: Evaluation of the level of evidence on mean difference in 25(OH)D level reached using GRADE

A-Adults

High dose Vitamin D compared to Placebo for adults MENA population

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	High dose Vitamin D	Placebo	Relative (95% CI)	Absolute (95% CI)		
25-hydroxyvitamin D level (follow up: range 3-6 months to)												
9	randomized trials	serious [1]	serious [2]	not serious	not serious		332	318	-	MD **17.59 higher** (13.29 higher to 21.84 higher)	Low	IMPORTANT

MD – mean difference, RR – relative risk

1. Risk of bias was moderate to high; only two studies were at low risk of bias
2. High heterogeneity between studies, related to variability in the dose, duration and baseline 25(OH)D level

Intermediate dose Vitamin D compared to Placebo for adult MENA population

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Intermediate dose Vitamin D	Placebo	Relative (95% CI)	Absolute (95% CI)		
25-hydroxyvitamin D level (follow up: range 3-3.5 months to)												
2	randomized trials	serious [1]	serious [2]	not serious	serious [3]		153	150	-	MD **14.73 more** (4.57 more to 24.89 more)	very Low	IMPORTANT

MD – mean difference, RR – relative risk

1. Both studies were at high risk of bias
2. High heterogeneity
3. Wide confidence interval; the increase in 25(OH)D level can increase from 4.57 ng/ml to 24.89 ng/ml.

B-Pregnancy

Intermediate dose compared to low dose Vitamin D for pregnant women in the MENA region

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Intermediate dose	low dose Vitamin D	Relative (95% CI)	Absolute (95% CI)		
25-hydroxyvitamin D level (follow up: range 5-6 months to)												
2	randomized trials	serious [1]	not serious	not serious	not serious		79	77	-	MD **7.84 more** (4.84 more to 10.8 more)	moderate	IMPORTANT

MD – mean difference, RR – relative risk

1. Soheilykhah et al: high risk of bias in allocation concealment and blinding of participants, personnel and outcome assessors

High dose compared to Intermediate dose Vitamin D for pregnant women in the MENA region

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	High dose	Intermediate dose Vitamin D	Relative (95% CI)	Absolute (95% CI)		
25-hydroxyvitamin D level (follow up: range 5-6 months to)												
2	randomized trials	serious [1]	not serious	not serious	not serious		83	79	-	MD **8.61 more** (5.32 more to 11.91 more)	moderate	IMPORTANT

MD – mean difference, RR – relative risk

1. soheilykhah et al.: high risk of bias in allocation concealment and blinding of participants, personnel and outcome assessors

High dose compared to low dose Vitamin D for pregnant women in the MENA region

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	High dose	low dose Vitamin D	Relative (95% CI)	Absolute (95% CI)		
25-hydroxyvitamin D level (follow up: range 5-6 months to)												
3	randomized trials	serious [1]	not serious	not serious	not serious		113	107	-	MD **16.52 more** (13.5 more to 19.53 more)	moderate	IMPORTANT

MD – mean difference, RR – relative risk

1. Soheilykhah et al: high risk of bias in allocation concealment and blinding of participants, personnel and outcome assessors

Children

Intermediate dose vitamin D compared to Placebo for children in the MENA region

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Intermediate dose vitamin D	Placebo	Relative (95% CI)	Absolute (95% CI)		
25-hydroxyvitamin D level (follow up: range 5-12 months to)												
2	randomised trials	not serious	serious [1]	not serious	serious [2]		179	183	-	MD **15.77 more** (8.68 more to 22.87 more)	low	IMPORTANT

MD – mean difference, RR – relative risk

1. High heterogeneity
2. Wide confidence interval; 25(OH)D can increase from 8.68 ng/ml to 22.87 ng/ml

Low dose Vitamin D compared to Placebo for children in the MENA region

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Low dose Vitamin D	Placebo	Relative (95% CI)	Absolute (95% CI)		
25-hydroxyvitamin D level (follow up: range 3-12 months to)												
2	randomised trials	serious [1]	serious [2]	not serious	serious [3]		174	164	-	MD **4.98 more** (0.8 fewer to 10.76 more)	VERY LOW	IMPORTANT

MD – mean difference, RR – relative risk

1. Neyestani et al: High risk of bias
2. High heterogeneity
3. wide confidence interval including negligible effect

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