ABSTRACT

Title of Document: VITAMIN D STATUS IN RELATION TO DIETARY INTAKE, SUN EXPOSURE OBESITY, LIFESTYLE FACTORS AND BONE HEALTH AMONG SAUDI PREMENPOUSAL WOMEN LIVING IN JEDDAH CITY.

Tahani Abdullah Zareef, Ph.D., 2016

Directed By: Professor Dr. Robert T. Jackson, Department of Nutrition and Food Science

Saudi women are at risk of vitamin D deficiency and low bone mass because traditional clothing and time spent indoors limit sun exposure. However, no information about other contributing individual, demographic, and cultural risk factors which may determine women's vitamin D status is available. Moreover, little is known about vitamin D intake, and the association between vitamin D and calcium intake and bone mineral density (BMD) in premenopausal Saudi women. The objectives of the study were to identify predictors strongly associated with vitamin D deficiency, identify dietary vitamin D sources, examine potential determinants of vitamin D intake, examine the association of vitamin D and calcium intake with BMD, and explore how the relationship between knowledge of vitamin D and attitudes about sun exposure are related to serum vitamin D levels.

This cross-sectional study was conducted in 257 women 20–50 years of age in Jeddah, Saudi Arabia, between December 2014 and April 2015. Data were obtained through pre-tested questionnaires. Serum 25(OH) D and serum parathyroid hormone (PTH) were measured. BMD was measured using double-energy X-ray absorptiometry in 102 participants. The prevalence of vitamin D deficiency was 77.6% (25(OH) D < 50 nmol/L). Vitamin D deficiency is associated with increased risk of elevated PTH levels. In the multiple regression analysis, serum 25(OH) D was not associated with body mass index or waist circumference. Predictors of vitamin D deficiency were low dietary and supplement vitamin D intake and younger age, explaining 41% of the variation in 25(OH) D serum concentrations ($p < 0.001$). Approximately 65% of the participants had vitamin D intakes below the U.S. Estimated Average Requirement (EAR) for vitamin D, and 61% fell below the EAR for calcium. Dairy products and supplements contributed the most to vitamin D intake. Older age was an independent determinant of sufficient vitamin D intake ($p < 0.001$). The prevalence of osteopenia was 33% in the lumbar spine and 30% in the femur neck. Education was a significant predictor of vitamin D knowledge ($p < 0.001$). Public health interventions offering early screenings of vitamin D status and improved nutrition in young Saudi women are recommended.

VITAMIN D STATUS IN RELATION TO DIETARY INTAKE, SUN EXPOSURE, OBESITY, LIFE STYLE FACTORS AND BONE HEALTH AMONG SAUDI PREMENOPAUSAL WOMEN LIVING IN JEDDAH CITY

By

Tahani Abdullah Zareef

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Advisory Committee:
Dr. Robert T. Jackson, Chair
Dr. David K. Y. Lei
Dr. Olivia Carter-Pokras
Dr. Mira Mehta
Dr. Hee-Jung Song

Dedication

I dedicate this dissertation to my beloved family, with special gratitude to my parents, Asma and Abdullah, who made all of this possible through their constant love, patience, support, and words of encouragement.

I also dedicate this work to my siblings, Hawazin, Mazin, Olfat, and Moayed, who truly believed in me and supported me and whose good examples have taught me to work hard with patience for what I aspire to achieve.

Last but not least, I dedicate this work to my lovely nieces and nephews and all the wonderful, special friends who supported me and encouraged my endeavors.

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Chapter 1: Introduction

Though vitamins play a pivotal role in preventing diseases, their ability to aid in maintaining good health was unknown for centuries and is still not fully comprehended in many parts of the world. Indeed, it was not until 1920 that a protracted search for a cure for rickets–a bone disease that is most common in children and infants and can lead to permanent deformities–yielded the discovery of vitamin D. The vitamin was found to be essential for calcium absorption, while a severe deficit of it decreases the efficiency and absorption of intestinal calcium and phosphorous, resulting in increased parathyroid hormone (PTH) concentrations. Secondary hyperparathyroidism due to low levels of 25 hydroxyvitamin D-25(OH) D may lead to increased bone turnover and loss. Thus, a severe vitamin D deficiency harms not only children, but it can also cause osteomalacia, osteopenia, osteoporosis, and an increased risk of fracture in adults (1-3).

In addition to its critical role in musculoskeletal health, vitamin D plays an important role in non-skeletal health. Vitamin D receptors have been found in many other tissues, from the prostate to the heart, blood vessels, muscles, and endocrine glands. Increasing evidence points to possible links between vitamin D insufficiency and a number of non-skeletal disorders, including cancer, heart disease, high blood pressure, diabetes, age-related cognitive decline, Parkinson’s disease, multiple sclerosis, and arthritis. Thus, vitamin D deficiency may have broader implications for public health than previously acknowledged (1,4,5).

Vitamin D is a particularly interesting micronutrient because it can be produced in the body. Vitamins are classically defined as organic chemicals that must be obtained through diet because they are not produced by the body (6). But vitamin D, while universally recognized as one of the four essential fat-soluble vitamins, is obtained through synthesis in the epidermis with exposure to ultraviolet (UVB) sunlight which converts 7-dehydrocholesterol to pre-vitamin D3.It is naturally present in very few foods, mainly vitamin D3 (cholecalciferol) from animal sources including oily fish, egg yolks, veal, beef, and liver (4,7,8).Vitamin D2 (ergocalciferol) comes from plant sources including sun-dried mushrooms (8).Vitamin D, whether obtained from an endogenous or exogenous source, must undergo hepatic hydroxylation to form 25 hydroxyvitamin D 25(OH) D, the chief circulating form, and further renal hydroxylation to convert 25 (OH) D to its active form of 1, 25-dihydroxyvitamin D, also known as calcitriol (4,8, 9-12)

Serum concentration of 25-hydroxyvitamin D [25(OH)D] is considered the best indicator of vitamin D nutritional status, as it reflects both vitamin D produced in the skin and that acquired from the diet. Levels are specified in both nanograms per milliliter (ng/mL) and nanomoles per liter (nmol/L)[1]. While the optimal 25(OH) D level is a topic of considerable debate, the general consensus is that a serum 25(OH)D concentration < 25 nmol/L may indicate vitamin D deficiency (11,13,14) As per the US Institute of Medicine's (IOM) 2011(12) conclusions, a serum 25(OH)D concentration < 30 nmol/L qualifies as "deficient" and has a known impact on skeletal health, calcium malabsorption, and secondary hyperparathyroidism, and leads

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[1] 1 nmol/L = 0.4 ng/mL

to rickets in children and increased bone resorption and osteomalacia in adults. Additionally, serum 25(OH)D in the range of 30–50 nmol/L may be “inadequate” in some people, while serum 25(OH)D > 50 nmol/L is “sufficient” for a majority (97.5%) of the population (4,12). However, the Endocrine Society released guidelines in 2011 stating that vitamin D deficiency is defined as blood serum of 25(OH) D < 50 nmol/L, while blood serum of 25(OH) D levels of 50–75 nmol/L indicate vitamin D insufficiency, and serum 25(OH)D concentration should be at least 75 nmol/L to maximize the effect of vitamin D on calcium, bone, and muscle metabolism (15). Given those thresholds, vitamin D deficiency (< 50 nmol/L) is prevalent in many populations worldwide.

In fact, Saudi Arabia reports some of the world’s highest rates of vitamin D deficiency (16,17) with Saudi women having a particularly high risk for the condition due to the traditional clothing limiting their exposure to sunlight (18). The most recent national study reported that the prevalence of vitamin D deficiency among both young and postmenopausal women is 72% and 85%, respectively (19). Additional studies (17, 20, 21) have highlighted concerns that Saudi women of reproductive age who have serum 25(OH)D of 10-45 nmol/L are at risk for suboptimal bone health, which can lead to osteomalacia, chronic back pain, and other bone related problems. Twenty-three Saudi adolescents with vitamin D deficiency (25(OH)D < 50 nmol/L) were diagnosed with osteomalacia in a retrospective hospital-based study in Riyadh from January 1990 to December 2014 (22). Low back pain was prevalent among Saudi patients, ranging from 53.2% to 79.17% in Saudi patients (23), and vitamin D deficiency was the major risk factor for chronic low back pain (17,23).

Limited exposure to sunlight is a primary contributor to low 25(OH)D concentrations in Saudi Arabia and is a direct result of cultural practices (e.g., clothing that fully covers the skin), the hot climate that drives people to seek air-conditioned comfort indoors, and the pigmentation in dark skin that can reduce endogenous vitamin D production (16,17, 24). Besides limited exposure to sunlight, obesity also plays a role in the vitamin D deficiency observed in Saudi Arabia (19). It has been suggested that obesity may decrease the bioavailability of vitamin D obtained from food sources or cutaneous synthesis due to the sequestration of vitamin D, which is fat soluble, by adipose tissue (25). The sequestering of vitamin D in fat plays a major role in reducing the amount that can be presented to the liver for 25-hydroxylation (2). Therefore, dietary intake represents the primary means of attaining vitamin D sufficiency when environmental, cultural, or physiological factors prevent adequate skin exposure to sunlight (7, 26). However, because there are few vitamin D sources in the diet, fortification of staple foods (such as milk and margarine) with vitamin D has become standard practice worldwide to help populations meet its need.

In Saudi Arabia, many (but not all) Saudi dairy companies (approximately 11 out of 16) fortify milk and Laban (buttermilk) with vitamin D (400 IU/L). Fortification of other foods, such as cheese, cereal, and vegetable oils, is fairly low and variable in Saudi Arabia. A limited number of studies in Saudi Arabia have documented a lack of consumption of dairy products and products supplemented with vitamin D (19, 27). No data are available, however, examining dietary adequacy of vitamin D intake among premenopausal Saudi women. Furthermore, little is known about actual vitamin D intake in Saudi Arabia or the main food sources from which it is derived. No work has been done relating these intake levels to socio-demographic or lifestyle factors; therefore research in this area may help identify sub-populations at risk of inadequate vitamin D intake and potential vitamin D deficiency.

On the other hand, vitamin D and calcium intake are essential nutrients for maximizing peak bone and preventing bone loss, thus decreasing osteoporosis and the risk of fractures over the long term (28, 29), both of which are growing public health and economic issues in Saudi Arabia. The prevalence of lumbar and femur osteopenia ranges from 7–43.4% and osteoporosis from 2.5–46.7% (30-32). Most Saudi studies investigating the role of nutrition in bone health have utilized only postmenopausal women (30, 33), because these studies focus on osteoporosis prevalence and bone loss rather than bone maintenance. Bone mineral density (BMD) in premenopausal Saudi women has not been studied to a great extent. Young women are ideal candidates for investigating BMD research, as they can optimize their BMD and decrease their risk of fractures through dietary and lifestyle interventions while still young (34).

A comprehensive survey of the leading vitamin D studies (35) noted the need for future research that strengthens our understanding of vitamin D status in key subgroups worldwide. The subgroup of premenopausal women in Saudi Arabia is clearly one in which understanding of vitamin D status is extremely limited. Furthermore, there has been no systematic investigation of the subgroup-specific factors including individual, demographic, and cultural considerations that are associated with vitamin D deficiency.

One such factor hypothesized to play a key role is the presence or absence of knowledge regarding vitamin D nutrition and the importance of the micronutrient to overall health. Oudshoorn et al. (2011), Kung & Lee (2006), and Almutairi et al. (2012) all consider a lack of knowledge regarding vitamin D to be a contributing factor to vitamin D deficiency (36–38). The general lack of knowledge points to the need for quantitative research into the level of awareness Saudi women have regarding the importance of vitamin D intake, sun exposure, and how such things have impacted their serum 25(OH)D levels (39, 40). A better understanding of vitamin D, the prevailing attitudes about sun exposure, and the effect that these may have on vitamin D status is necessary before developing effective intervention programs.

Thus, this study focused on three areas. First, this research filled gaps in the understanding of potential predictors that are strongly associated with vitamin D status in healthy premenopausal women living in Jeddah. Second, the study sought to provide new, useful data on realistic vitamin D intake estimates based on vitamin D content from both food and supplements sources and evaluated the association between dietary calcium, vitamin D intake, and serum vitamin D levels with bone mineral density. Finally, this examination explored the prevailing attitudes and behaviors regarding exposure to sunlight, as well as knowledge about vitamin D among women.

To the best of our knowledge, this is the first study to assess vitamin D intake and examine the food sources that make the biggest contribution among premenopausal women in Jeddah and the subgroup most vulnerable to insufficient vitamin D intake. In addition, this study is the first assessment of dietary intake of vitamin D and calcium among premenopausal women in Jeddah and its association with the bone health parameter BMD. Moreover, this is the first study to examine the relationships between vitamin D knowledge, attitudes, and practices toward sun exposure and serum vitamin D levels among premenopausal women living in the city of Jeddah. It is hoped that the data generated by this study will prove instrumental both in the design of effective and appropriate intervention strategies for the prevention of vitamin D deficiency among premenopausal women in Jeddah and in the adoption of strong national fortification policies.

I. Research Objectives

1. To investigate and comprehensively evaluate important predictors of vitamin D deficiency including dietary intake, sunlight exposure, skin color, anthropometric measures, socio-demographic, and life style related behaviors in a sample of healthy premenopausal women living in the city of Jeddah, Saudi Arabia.
2. To assess the adequacy of vitamin D intake among premenopausal women in Jeddah as compared to the U.S Estimated Average Requirement (EAR) of 400 IU/day, to assess the sources of vitamin D in their diets, and to examine potential determinants of vitamin D intake.
3. To assess bone health and the association of dietary calcium, vitamin D intake, serum vitamin D levels, and serum parathyroid hormone levels with bone mineral density among premenopausal women in Jeddah.
4. To examine the knowledge of vitamin D and attitudes toward sun exposure among women in Jeddah, and to explore associations between knowledge and attitudes with serum vitamin D levels.

II. Research Questions

1. Based on the U.S Estimated Average Requirement EARs of 400 IU (10µg/day), is vitamin D intake adequate among premenopausal women in Jeddah? What are the main sources of vitamin D intake (dietary, supplemental) in this study's population?

2. What is the association between sociodemographic factors (*e.g.* age, income level, education level) and total vitamin D intake (diet + supplement)?

3. Is there a significant difference in calcium intake, vitamin D intake, serum vitamin D levels, and serum parathyroid hormone levels between women with normal bone density and women with low bone density in this study population?

4. What knowledge do women in Jeddah possess of vitamin D and its association with their actual intakes and serum vitamin D levels?

5. What are the existing attitudes and behaviors (*e.g.* time spent in direct sunlight, sunscreen use) toward sun exposure among women in Jeddah and its association with their serum vitamin D levels?

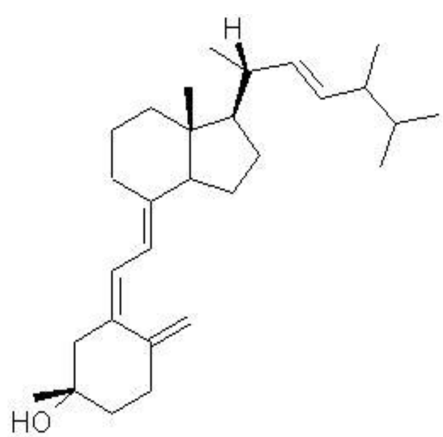
6. Which factors (vitamin D intake, sunlight exposure index, skin color, sun screen use, anthropometric measures, sociodemographic factors, and life style factors) correlate most closely with serum 25 (OH) D levels in this specific sample?

Chapter 2: Literature Review

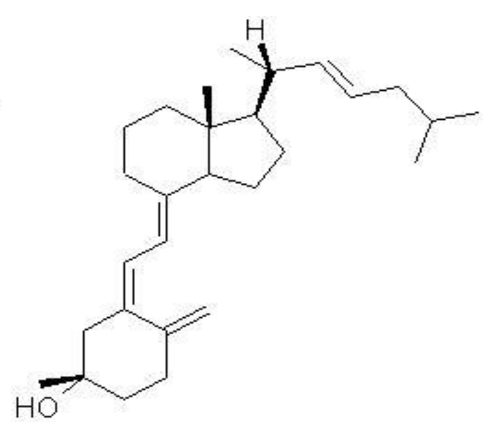
Forms of Vitamin D

Vitamin D is an essential fat soluble vitamin that has a structure close to that of the classical steroid hormones. There are two chemical forms of vitamin D, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_3 is formed photochemically in the skin when 7 dehydrocholesterol is exposed to sunlight or ultraviolet B (UVB) radiation (wave length 290- 310 nm). Dietary sources of vitamin D_3 include food naturally containing or fortified with vitamin D. Vitamin D_2 is manufactured from ergosterol by yeast in response to ultraviolet irradiation (1,41). The only difference between D_2 and D_3 forms is the structure of their side chain to the sterol skeleton **(Figure1).** This difference does not affect metabolism and both function as prohormones (42).

Figure1. Structures of vitamin D2 and D3



Vitamin D2 (ergocalciferol)



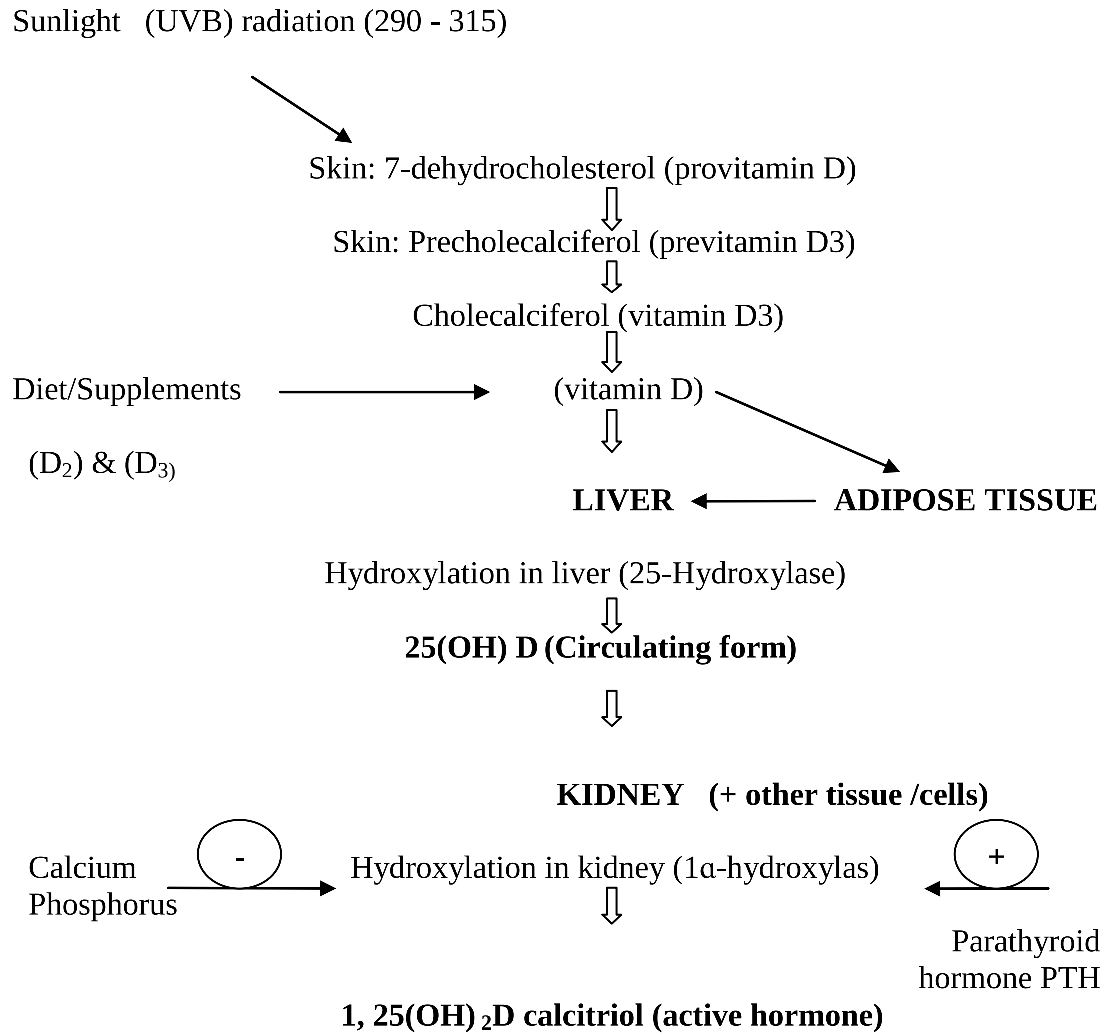
Vitamin D3 (cholecalciferol)

Metabolism of Vitamin D

Dietary vitamin D (either D_2 or D_3) once ingested is absorbed with other dietary fats in the small intestine. Within the intestinal wall, vitamin D is packaged together with cholesterol, triglycerides, lipoproteins and other lipids into chylomicron fraction, absorbed through the lymphatic system with subsequent entry into venous circulation (41, 43). Dietary and endogenously synthesized vitamin D_2 and D_3 are transported by vitamin D-binding protein (VDBP) and are metabolized first in the liver by the enzyme 25-hydoxylase to 25(OH)D (calcidiol). Most of 25(OH) D synthesized in the liver is secreted in the blood and transported by VDBP. Storage sites for vitamin D included blood (the largest storage site) and muscle for 25(OH) D and adipose tissue for cholecalciferol (44). Following hydroxylation in the liver, further conversions of 25(OH)D occur in the kidney (functioning as an endocrine gland) by the enzyme 25(OH)D-1α-hydroxylase to produce the biology active hormone, 1,25$(OH)_2$D (calcitriol).The production of 1, 25$(OH)_2$D is regulated by the parathyroid hormone PTH levels and serum calcium and phosphate levels **(Figure 2).**

In the circulation, 1, 25$(OH)_2$ D is bound to VDBP, which has a higher affinity for 25(OH)D,1,25$(OH)_2$D and has a high homology with albumin. The 1,25$(OH)_2$D transport to target tissue (bone, intestine, and kidney) where it interacts with a specific nuclear receptor known as the vitamin D receptor (VDR) in the intestine to enhance absorption of calcium and phosphate, and in bone to induce bone resorption (1, 11, 41, 44, 45).

Figure 2.Vitamin D Synthesis, intake, and activation (13 , 46)



Function of Vitamin D

The vitamin D hormonal form 1, 25 $(OH)_2$ D works to increase blood serum calcium concentrations in three separate ways. First, 1,25 $(OH)_2$ D induces proteins involved in active intestinal absorption of calcium and enhances active intestinal phosphate absorption. Second, 1, 25$(OH)_2$ D together with PTH, stimulates osteoblasts to produce Receptor Activator of Nuclear Factor-Kappa B Ligand

(RANKL), which in turn stimulates osteoclastogenesis and activates resting osteoclasts for bone resorption. Third, 1, 25(OH) $_2$ D and PTH stimulate the renal distal tubule to reabsorb filtered calcium (2, 41). Renal production of 1, 25(OH)$_2$ D is tightly regulated through the action of PTH. Calcium-sensing proteins in the parathyroid gland stimulate the secretion of a parathyroid hormone when calcium concentrations decrease below normal levels. PTH promotes 1, 25(OH) $_2$ D synthesis in the kidney, which by itself stimulates intestinal calcium absorption or together with the PTH, at higher concentrations, stimulates bone calcium mobilization and calcium renal reabsorption. As serum calcium concentrations exceed the set point of the calcium sensing system, shutting down the parathyroid gland generates a cascade of events. If serum calcium concentrations became too high, then the C-cells of the thyroid gland secrete calcitonin, which blocks calcium resorption from bone and helps to maintain calcium levels within normal range (2, 41).

The active form of vitamin D 1, 25(OH) $_2$D, binds with a vitamin D receptor (VDR). This receptor acts through nuclear vitamin D-responsive elements, which are involved in the regulation of gene transcription. This receptor VDR is expressed in cells involved in calcium homeostasis such as enterocytes, osteoblasts, parathyroid and distal renal tubule cells, but is also present in cells that are unrelated to calcium homeostasis. VDR is present in the small intestine, colon, and other organs in the body including brain, heart, prostate, breast, and skin. The 1α-hydroxylase, which converts 25(OH)D to 1,25(OH)$_2$D, has been found in many extrarenal tissues, including osteoclasts, skin, macrophages, placenta, colon, brain, prostate, endothelium and parathyroid glands (13, 47). Extrarenal production of 1, 25(OH) $_2$D appears to play an essential role in cell differentiation, proliferation and immune function. The vitamin D system may, therefore, be involved in other physiological processes beyond bone. However, the relationship between the extrarenal expression of 1 α hydroxylase and actual local synthesis of 1, 25(OH) 2D3 remains unclear (41, 47).

Assessment of Vitamin D Status

Although calcitriol 1, $25(OH)_2D$ can be measured in circulation, the most important vitamin D status from cutaneous synthesis and total intake is provided by measuring serum 25(OH)D concentration. Because circulating 25(OH)D can arise from hydroxylation of either vitamin D_2 or vitamin D_3, measurement of total 25(OH)D is necessary for accurate assessment of vitamin D status. Serum 25(OH)D has a long serum half-life (approximately 3 weeks) and the 25-hydroxylation step is unregulated, thus reflecting substrate availability (2). In contrast to 25(OH)D, calcitriol 1, 25(OH) $_2$D does not reflect vitamin D nutritional status for several reasons. It has a short half-life (hours), and serum concentrations levels are regulated by parathyroid hormone, calcium, and phosphate (43, 48).

There is no consensus regarding serum concentrations of 25(OH) D being associated with deficiency, sufficiency for bone health, and/or optimal overall health. As per the US Institute of Medicine's (IOM) 2011 recommendations for vitamin D (12), a serum 25(OH) D concentrations <30 nmol/L (<12 ng/mL) reflect a vitamin D deficiency, and serum levels ranging from 30–50 nmol/L (12–20 ng/mL) reflect inadequacy with sufficiency at levels ≥ 50 nmol/L **(** ≥ 20 ng/mL). The IOM committee stated that 50 nmol/L is the serum 25(OH) D level that covers the needs of the majority of the population (97.5%)(12). However, the Endocrine Society released guidelines in 2011 stating that vitamin D deficiency is defined as a blood serum of 25(OH)D < 50 nmol/L, while a blood serum of 25(OH)D levels of 50–75 nmol/L indicate vitamin D insufficiency, and serum 25(OH)D levels ≥ 75 nmol/L indicate sufficiency to maximize the effect of vitamin D on calcium, bone. and muscle metabolism (15).

The estimation of optimal serum concentration of 25(OH)D with respect to bone health is that bone mineral density (BMD) may be a better endpoint than serum PTH for the majority of the population including younger and older adults and non-White ethnic groups **(**49)**.** A threshold for optimal serum 25(OH)D and hip BMD concentrations was investigated among 13,432 participants in the third National Health and Nutrition Examination Survey (NHANES III), which included younger (20–49 years) and older (≥ 50 years) adults with different ethnic-backgrounds (50). Participants in the highest 25(OH)D quintile had a mean BMD that was 4.1% higher in younger European Americans, 4.8% higher in older European Americans, 1.8% higher in younger Mexican Americans, 3.6% higher in older Mexican Americans, 1.2% higher in younger African Americans, and 2.5% higher in older African Americans compared to participants in the lowest 25(OH)D quintile. Higher serum 25(OH) D concentrations were associated with higher BMD levels throughout the reference range of 22.5 to 94 nmol/L in all study groups. This study concluded that serum 25(OH) D concentrations at the upper end of the reference range are preferable to lower 25(OH) D concentrations for better BMD.

Methods for Assessing 25(OH)D Concentrations

There are several methods for vitamin D determination, including ones based on competitive protein binding assay (CPBA), enzyme-linked immunoassay (EIA, ELISA), radioimmunoassay (RIA), random access automated assay using chemiluminescence technology (RAAA), high performance liquid chromatography (HPLC) (51). Liquid chromatography-mass spectrometry (LC-MS/MS) is considered the gold standard for measuring 25(OH)D. Its advantages comprise separation of 25(OH) D_2 and 25(OH) D_3 and other serum lipids by their unique molecular masses and mass fragments, and a rather small bias compared with other assay methods (2). Because there is a lack of agreement in assay results obtained by different methods, some assays also differ in their detection of 25(OH) D_3 and 25(OH)D_2, it is important to exercise caution when comparing studies conducted in different laboratories or using different methods and when interpreting results against the universal threshold value.

However, laboratories participation in one of the Proficiency Testing programs such as the Vitamin D External Quality Assessment Scheme (DEQAS), the United Kingdom Proficiency Testing, or Accuracy Based vitamin D (ABVD) Surveys of the College of American Pathologists (PABVD)**,** or NIST-NIH Vitamin D Metabolites Quality Assurance Program (VitDQAP) is useful in evaluating different methodologies. The aim of these programs is to insure the accuracy and precision of 25(OH)D and 1, 25$(OH)_2$ D assays by regularly distributing serum samples to participants throughout the world and thus establishing performance targets (52, 53). Samples submitted by these programs are quantified by each participating laboratory in the program, and all the results are provided to determine the "all laboratory trimmed mean" (ALTM) (52).

Sources of Vitamin D

Dietary Intake

There are few foods that naturally contain vitamin D. Fatty fish (5-10 µg/100g) such as salmon, tuna, sardines, mackerel, and cod liver oils are among the rich sources. Small amounts of vitamin D are also found in egg yolks, veal, beef, liver, cheese, and mushrooms, but the amount of vitamin D depends on sun exposure and the feed given to the animals providing these products (13, 54). Because there are few vitamin D sources in the diet, the number of vitamin D fortified products has increased in the past few years. In the United States and a number of other Western countries, milk, yogurt, margarine and butter, ready-to-eat breakfast cereals, breads, and orange juice are fortified with vitamin D (11). Supplemental vitamin D is available in two forms, ergocalciferol (D_2) or cholecalciferol (D_3). Both forms effectively raise serum 25(OH) D levels. However, a recent meta-analysis (55) indicated that cholecalciferol increases serum 25(OH) D levels more efficiently than does ergocalciferol when taken in large single doses; thus, vitamin D3 could be the preferred choice for vitamin D supplementation, however, this effect was lost with small doses (1000-4000 IU) of daily supplements.

Serum 25(OH) D levels increase in response to increased vitamin D intake (food and supplements). A cohort study of dietary and lifestyle predictors of vitamin D deficiency among British adults found that serum 25(OH)D was higher in subjects who consumed vitamin D supplements (200 IU) or oily fish than in those who did not

(56). However, comparing vitamin D intake estimates from foods and supplements to serum 25(OH)D levels is problematic because sun exposure influences vitamin D status ; thus, serum 25(OH)D levels are greater than what would be predicted based on vitamin D intake alone (2,48).

Sun Exposure

Most people obtain the majority of their vitamin D needs by exposure of skin to sunlight (13). The skin has a large capacity to produce vitamin D and exposure of about 20% of the body's surface to either direct sunlight or ultraviolet (UVB) radiation with a wavelength of (290–320 nanometers) increases the plasma concentration of 25(OH)D in both young and older adults (13,57). The factors affecting the cutaneous synthesis of vitamin D and studies to date regarding the amount of sun exposure required to maintain sufficient vitamin D levels make it difficult to provide general recommendations. It has been suggested by some vitamin D researchers that exposure to sunlight for 5–30 minutes (depending on time of day, season, latitude, and skin pigmentation) during the peak hours of 10 am to 3 pm at least twice a week to the face, arms, legs, or back without sunscreen protection usually leads to sufficient vitamin D synthesis, and the moderate use of tanning beds that emit 2%–6% UVB radiation is also effective (1, 48).

Factors Affecting the Cutaneous Synthesis of Vitamin D

Ultraviolet sunlight is the primary source of vitamin D therefore any factors that interfere with the penetration of ultraviolet B radiation into the skin will affect the cutaneous synthesis of vitamin D. These factors include the winter season, lower latitudes, time of the day, darker skin pigmentation, sun screen use, skin covering and the avoidance of direct sun exposure. Age and gender, overweight and obesity also affect cutaneous synthesis of vitamin D (11, 51).

Seasons, Latitude, and Time of Day

Seasons, geographic latitude, time of day affect ultraviolet B radiation exposure and vitamin D synthesis (58). The amount of ultraviolet B radiation in the geographical location depends on length of the sun's rays travelling through the atmosphere, where it is more or less absorbed. Countries at latitudes nearer the poles receive less sunlight yearly compared to the countries near to the equator. Seasons influence the degree of possible solar radiation a population may receive; the northern hemispheres is tilted towards the sun from April to September, while the southern hemisphere is tilted towards the sun from October to March. The studies have provided strong evidence that vitamin D deficiency is greater in winter months for all age groups (10). The time spent outdoors is the primary factor for determining vitamin D status; 60% of the effective UVB radiation occurs between 11:00 am and 3:00 pm (13). The households and people who have few opportunities to spend time outdoors are at higher risk of vitamin D deficiency (51). Other transient factors such as air pollution are also affect the amount of UVB radiation reaching the earth and consequently influencing the cutaneous synthesis of Vitamin D (59).

Skin Pigmentation

Skin pigmentation affects vitamin D production (60). Melanin is efficient in absorbing UVB radiation in the 290-320 nm range and functions as a light filter determining the amount of UV radiation available for cutaneous synthesis of vitamin D (13,61). Increased skin pigmentation is associated with reducing vitamin D synthesis (11). Individuals with darker skin produce less cholecalciferol in the skin for the same exposure to UVB light compared to lighter-skin individuals, and they need longer exposure to achieve the same plasma 25 (OH) D concentrations (51,57, 61). Serum 25-hydroxyvitamin D levels in the U.S. population showed that among the three ethnic groups, non-Hispanic Blacks and Mexican Americans had the highest prevalence of vitamin D inadequacy (25-hydroxyvitamin D levels from 30 to less than 50 nmol/L) compared to non-Hispanic Whites (62). However, skin color alone does not explain 25(OH) D levels because behavior in the sun is also an important factor to consider (63).

Sunscreen

Sunscreens are designed to protect the skin from UV radiation. Sunscreen with a sun protection factor (SPF) of 15 was shown to reduce the synthesis of vitamin D_3 in the skin by 99% (11). Studies have shown that regular use of sun screen might impair vitamin D status (13). However, other studies suggest that although sunscreens are effective, many may not actually be blocking UV radiation because they are inadequately applied. Therefore, sunscreen use may not actually diminish vitamin D synthesis from sunlight in real world use (2).

Skin Covering and the Avoidance of Direct Sun Exposure

Experimental studies have shown that clothing inhibits the production of previtamin D and serum vitamin D levels and that the transmission of previtamin D effective radiation depends on fabric type (64). Salih (2004) investigated in vitro study (conducted in laboratory conditions, such as in test tubes) to determine the influence of 15 different fabric samples (woven polyester, spun polyester, a mixture of polyester and cotton, a mixture of polyester and wool with white, light brown, brown and black colors) used in Oman by both men and women on the photo transformation of provitaminD3 (7-dehydrocholesterol) to previtaminD3 (65). Solutions of 7-dehydrocholesterol in methanol were exposed to sunlight in quartz containers for predetermined periods (up to 60 min) either uncovered or covered with the fabric sample. Fabrics under test were graded as the number of threads per square inch (in^2), and their sunlight attenuation was determined. When fabric covered samples of 7-dehydrocholesterol were irradiated, photoproducts were also detected and their concentrations depended on the degree of sunlight attenuation imposed by the fabric. The higher the number of threads per in^2, the more the light attenuation is produced. The investigator concluded that the type of fabrics used by Omanis and by other people may greatly inhibit the photoproduction of vitamin D3 to an extent that would require dietary vitamin D supplementation.

However, some studies have shown that covering of the skin with any type of clothing prevents the cutaneous production of cholecalciferol. For example, a study performed by Matsuoka et al., (1992) in human volunteers wearing fabrics made out of white or black cotton, wool, or polyester exposing to simulated sunlight for up to 40 min showed no elevation in circulating concentrations of vitamin D (66). The religious practice of the veil (niqab or hijab) covering the whole body or all except face and hands of women, have been shown to be an independent factor of vitamin D deficiency in the Middle East and Africa.

In Saudi Arabia, where extensive coverage of clothing is the norm, a study in 321 healthy Saudi Arabian women with a mean 35.4 years of age recruited from the city of Riyadh showed that 52% had a serum 25(OH) D levels ≤ 20 nmol/L (67). In Kuwait, a study in 50 veiled Kuwaiti women aged 14-45 years who had three children or less, and 22 unveiled women with matched age and number of children as a control showed that veiled women had a high prevalence of vitamin D deficiency compared to non-veiled women (68). In United Arab Emirates (UAE), a study on multinational women of childbearing age (from 19-44 years) as conducted between June and September 1994 in Al Ain, UAE, compared serum vitamin D 25(OH)D status of 33 UAE nationals, 25 non-Gulf Arabs (Jordanians, Egyptians, Palestinians, and Lebanese), and 17 Europeans. A clothing score that designed to quantify skin exposure based on mode of dress was calculated for each woman. Using body surface area, head and face, forearm, and leg exposures had exposure scores of 3, 3 and 6, respectively. Hand and feet exposure had scores of 0.5 each. A UV exposure score that takes into account the magnitude of clothing was calculated for each woman by multiplying the clothing score by the total sunlight exposure (total sunlight exposure = average number of minutes per exposure by the number of times exposed in the preceding 7 days and dividing by 60 to determine the exposure in hours per week).The serum 25(OH) D was significantly lower among UAE nationals 21.5 nmol/L and other non-Gulf Arabs 31.5 nmol/L than in Europeans 160.5 nmol/L ($p < 0.0001$). Compared to UAE nationals and other non-Gulf Arabs, Europeans were older, better educated, and less likely cover their head, forearms and legs as part of style of dress while outdoors. Therefore, the clothing score for Europeans was significantly higher than the scores of UAE nationals and non-Gulf Arabs.

The ultraviolet (UV) exposure score, was also significantly higher in European women than in the UAE national and non-gulf Arab groups. The influence of dietary vitamin D intake on vitamin D status was not explored in this study. The researchers concluded that limited skin exposure of Arab women to sunlight due to their traditional, extensive clothing appears to be an important determinant of low vitamin D status in this population (69). Another study assessed the role of clothing on vitamin D levels of 126 healthy adults (40 males, 86 females) aged 18–87 years in Brisbane, Australia, at the end of the 2006 winter (70) and showed that protective clothing in winter was associated with low vitamin D status. However, the study results may have been confounded by obesity, which affected outdoor physical activity. These results draw attention to the need for other sources of vitamin D when photo protective clothing is worn for whatever reason. It also draws attention to the need to use a multivariate model to determine individual, demographic, cultural and geographic factors that may affect vitamin D levels (64).

Aging

Vitamin D deficiency affects all age groups, from the new-born to the elderly, and is dependent on lifestyle factors and environmental conditions. In general, aging is associated with decreased 7-dehydrocholesterol concentrations; the precursor of vitamin D_3 in the skin reduced the capacity of the skin to make vitamin D3 (71). Furthermore, the elderly may be confined indoors for long periods of time, which may compound the problem. Therefore, the elderly have lower 25(OH) D levels compared to the young population from the same regions and with similar skin color (10).

Gender

Vitamin D deficiency affects both men and women in all age groups. However, there is evidence that European women have lower levels of 25 (OH) D concentrations than European men in elderly population (10,72) in a study of healthy aged men and women in New Zealand (73). Moreover, pregnant women and those breast-feeding for prolonged periods of time are at a greater risk of vitamin D deficiency (10). Bailey et al.,(2010) estimated vitamin D intakes from food and supplements for US citizens aged ≥ 1 years using data from the National Health and Nutrition Examination Survey (NHANES), 2005–2006, Average intake levels for men from foods only ranged from 204 to 288 IU/day; for women the range was 144 to 276 IU/day. When a vitamin D supplement was administered, these mean values were increased (37% of the U.S. population used supplements containing vitamin D) (2,74). Gender differences also explain clothing differences where girls and women wearing veils have lower vitamin D levels throughout the year, as compared to their male counterparts (10, 75).

Overweight and Obesity

Vitamin D is fat-soluble and can be stored in the body fat. In 1971, Rosenstreichet et al. first identified adipose tissue as the major site of vitamin D accumulation from experiments in which radiolabeled vitamin D was administrated to vitamin D deficient rats. After measuring tissue levels during vitamin D repletion and during subsequent periods of deprivation, researchers found that adipose tissue acquired the greatest quantity of radioactive compound and had the slowest rate of release (76). Similarly, Mawer et al., (1972) found that adipose tissue is the major site where vitamin D accumulates in human tissues after an injection of radioactive vitamin D_3 (77). Vitamin D obtained from cutaneous synthesis or the diet is promptly taken up by adipose tissue, which stores vitamin D for subsequent release and metabolism when production of vitamin D is reduced, especially during the winter (13, 46, 60).

Overweight and obesity is an individual level factor that affects vitamin D status (25). A lower serum 25(OH)D concentration was observed in obese subjects compared with those of normal body weights in experimental (25), prospective cohort (78), case-control (79), and cross-sectional studies (80, 56). This suggests that the association between serum 25(OH)D and obesity could be explained by an increased storage of 25(OH)D in adipose tissue in obese individuals (79). Sequestering of vitamin D into fat plays a major role in reducing the amount that can be presented to the liver for 25-hydroxylation. As stated previously, vitamin D is absorbed with other lipids as part of chylomicrons and is taken up first by peripheral tissues that express lipoprotein lipase, particularly adipose tissue and skeletal muscle. This pathway predicts that increased adiposity should lead to decrease serum 25(OH) D levels and the weight loss should reduce peripheral sequestration and increase 25(OH)D levels (2). In obese subjects, exposure to the same amount of UV radiation raised plasma 25(OH)D concentrations by only 50% compared with non-obese subjects (25), suggesting that in obese individuals, vitamin D was stored in adipose tissue and not released when needed. Wortsman et al. (2000) studied the ability of the skin to produce vitamin D in whole skin (i.e., the epidermis and the dermis) obtained during surgery from two obese subjects and two non-obese subjects with skin type III (25). The individual skin pieces were measured after being exposed to stimulated sunlight. Investigators found that the content of vitamin D precursor 7 dehydrocholesterol in the skin was not statistically significant between obese and non-obese subjects, and the percentage conversion to previtamin D_3 was similar in the two groups (25). Therefore, the investigators concluded that obesity does not affect the capacity of the skin to produce vitamin D_3, but it may alter the release of vitamin D_3 from the skin into the circulation due to limited mobility and excessive storage of vitamin D in the adipose tissue (25, 81). The implication of these studies is that the bioavailability of vitamin D from dietary and cutaneous sources is decreased in obese subjects because of its deposition in body fat compartments, and that increased amount of subcutaneous fat in obese individuals alters the release of vitamin D and decreased body ability to utilize this nutrient (25). Obese subjects, therefore, may require larger doses of vitamin D supplements to achieve a serum 25(OH)D level comparable to that of normal subjects' counterparts (25,60). Weight reduction studies also support the previous findings and show that serum 25(OH)D increases when obese subjects lose body fat despite no increased intake of vitamin D from diet or sun exposure (2, 82, 83).

Total body fat, as determined by whole body dual energy x-ray has been shown to be strongly inversely associated with serum 25(OH)D. Leg fat was more strongly inversely correlated to 25(OH)D concentration compared with abdominal fat, which might support the idea that endogenously produced vitamin D is stored particularly in the subcutaneous fat depot (13,79).

Vitamin D Deficiency

Vitamin D deficiency is prevalent in many populations. More recently, it has been recognized as a worldwide pandemic (11). The degree of vitamin D deficiency varies from region to region. Serum 25(OH)D levels below 75 nmol/L are common in most populations while levels below 25 nmol/L that constitute severe vitamin D deficiency are frequently seen in populations at higher risk such as the elderly. In some regions of the world, such as South Asia and the Middle East, severe vitamin D deficiency is common in all age groups, from infant to the elderly (10). Vitamin D deficiency decrease calcium and phosphorus absorption, resulting in poor mineralization of the skeleton (13). Severe vitamin D deficiency causes growth retardation and rickets in children (11, 84). In adults, severe vitamin D deficiency presents as osteomalaica, osteopenia, osteoporosis and fracture (1, 11).Vitamin D deficiency has been linked to other health problems beyond bone health. Evidence suggests that low vitamin D status is associated with increased risk of cardiovascular disease, certain types of cancer, tuberculosis, multiple sclerosis and type I diabetes (13). Studies have shown that Vitamin D deficiency increases the risk of developing breast, colon, prostate and other cancers (11). One study found that male and female who were exposed to the most sunlight across their lives were less likely to die of cancer (85). A meta-analysis showed that 1,000 IU of vitamin D intake would be associated with a decreased risk of developing colorectal and breast cancer as much as 50% (11, 86). Vitamin D deficiency increased the risk of type 1 diabetes, multiple sclerosis, and hypertension (11). Hypponen et al. (2001) found that children who received 2,000 IU of vitamin D/day during the first year of their lives and who were followed for thirty one years reduced their risk of developing type 1 diabetes by 78% (87). Munger et al.(2004) found that women who received 400 IU vitamin D per day decreased the risk of developing multiple sclerosis by 40% (88). Krause et al. (1998) studied 18 patients with untreated mild hypertension (8 women, ages 26-66 years), who were exposed to ultraviolet B radiation three times a week for 3 months, and found that 25-hydroxyvitamin D levels increased by approximately 180%, and blood pressure became normal (both systolic and diastolic blood pressure was reduced by 6 mm Hg) (89).

Vitamin D Status in Saudi Arabia

Despite abundant sunshine in Saudi Arabia, a region spanning latitudes between 16˚N to 33˚N and longitudes between 34°E and 55°E (90), studies identified a high prevalence of vitamin D deficiency among Saudi adults. In 1983, Sedrani et al. conducted a study of a small sample of university students n=59 (26 men, 33 women) aged 18-26 and elderly subjects n=24 (13 men, 11 women) from Riyadh, Saudi Arabia and revealed serum 25(OH) D levels ranging between 4 and 12 ng/ml (10-30 nmol/L). In elderly subjects, serum 25(OH) D levels were significantly lower than those of young students of both sexes, with a range of 1.5 to 6.4 ng/ml (3.75 -16 nmol/L). The researchers concluded that low vitamin D levels in Saudis are largely due to avoidance of sunlight exposure, dietary vitamin D deficiency, and the lifestyle of the Saudi population, which involves the complete covering of the skin (except for face and hands) (91). However, although the avoidance of sun exposure is evident in Saudi Arabia, little is known to date about the reasons. More research is needed to clarify the influence of cultural practices and behavior regarding sun exposure in this population.

In 1984, Fonseca et al. reported low serum 25(OH)D levels among 31 Saudi Arabian women aged 22-65 who attended the primary care clinic of the hospital for acute minor illnesses. Serum 25(OH) D was measured using competitive protein-binding assay (CPBA) (92). Although CPBA was valid and performed quite satisfactorily even when compared with modern assays, it was hampered by cumbersome sample extraction and purification (93, 94). The median serum 25 (OH) D levels was 6 ng/ml (15 nmol/L). Only three subjects had serum 25 (OH) D levels within the normal range 10-55 ng/ml (25-138 nmol/L). Vitamin D concentrations were significantly lower in women who lived in apartments than in those living in villas or rural areas, and whose average exposure to sunlight was less than 30 minutes/day. The study findings confirm the importance of inadequate sunlight exposure in the aetiology of vitamin D deficiency.

In the 1990s, studies supported the earlier findings and confirmed that the problem of vitamin D deficiency still remained in the Saudi population. Sedrani et al., (1992) studied the effect of regional and environmental locations on vitamin D status on 4,078 healthy Saudi subjects aged from 6 years up to 90 years. The sample was divided into five groups on the basis of their geographical location and lifestyle. The lowest 25(OH)D plasma concentrations were found in the population living in the Northern Province. The highest levels were observed in the Western province. In the same geographical location, rural adult men and women had significantly higher 25(OH)D than urban adult men and women. As for the house type, subjects who lived in tents have significantly higher 25(OH)D than those living in mud houses, villas or brick houses. Subjects living in tents are usually urbanized Bedouins, therefore, have more exposure to sun. The study has found several interregional, sex, and age differences, and has revealed that even in a sunny country like Saudi Arabia; vitamin D deficiency is frequently seen (95,96). The effects of season on serum 25(OH)D concentrations were also studied, Sedrani et al.(1992) revealed that there is no significant difference between the plasma concentration of 25(OH)D in January and August for adult men and women. This suggests that as the temperature increases during the summer, the exposure to UVB radiation is decreased and, consequently, the concentration of plasma 25(OH) D decreases (97). A study was performed by Ghannam et al. (1999) on 321 healthy premenopausal Saudi women with a mean of age 35.4 years recruited from the city of Riyadh (67). Serum 25OHD concentration was determined by radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA). Nichols Diagnostics used purified human vitamin D-binding protein DBP as the competitive binder, but the validity of this assay has been questioned, with some studies suggesting a significant overestimation of $25(OH)D_3$ and underestimation of $25(OH)D_2$ (94). Severe vitamin D deficiency $\leq$ 20 nmol/L was present in 52% of the subjects (67).

Over the past few years there have been several studies reporting vitamin D deficiency in Saudi Arabian adults. In the study by Alfaraj et al. (2003) the vitamin D status of 360 Saudi men and women (aged 15-52 years) who experienced low back pain in a central province of Saudi Arabia was determined. Serum 25(OH)D measured by radioimmunoassay (RIA) using kits supplied by DiaSorin (Minnesota, USA) (17). The DiaSorin test has been utilized in the vast majority of large-clinical studies worldwide to define ‘normal’ circulating 25(OH) D levels in a variety of disease states (93). Vitamin D status was measured before and after treatments with vitamin D supplementation. Findings showed 83% of the study participants had low vitamin D levels before treatment with vitamin D supplements. Oral therapy with 25-OH cholecalciferol using a dosage of 5000IU/day was given to patients whose weight was less than 50 kg and 10,000 IU/day was given to those with weight exceeding 50 kg. Three months after vitamin D supplements had been provided; clinical improvements were seen in 95% of all subjects (17). Alturki et al., (2008) conducted a study between February and May 2008 in 200 healthy Saudi women between (25-35 years and women of ≥ 50 years) who lived in the eastern region of Saudi Arabia. The prevalence of vitamin D deficiency among young women was 30% and 55% in postmenopausal women. The study indicated that sun exposure and consumption of dairy products were minimal (98). Sadat et al., (2009) studied vitamin D status among healthy Saudi Arabian men (100 males aged 25-35 years and 100 males aged 50 years) living in the eastern region of Saudi Arabia. The prevalence of vitamin D deficiency among men was up to 37%, and the authors suggested that vitamin D deficiency in Saudi men might be related to lack of adequate exposure to sun light, wearing long sleeves and head covers, and inadequate consumption of dairy products (99).

The gender differences in vitamin D deficiency have also been investigated in Saudi Arabia. Elshafie et al.,(2012) conducted a study in the Royal Guard primary health care center in Riyadh, Saudi Arabia, on a consecutive sample of 50 Saudi married couples aged (16-60 years) attending the center without complaints related to vitamin D deficiency. The study compared vitamin D deficiency in men and women through study of married couples, assuming they have similar dietary and general health habits. Quantitative determination of total 25(OH)D was done using the Electro-Chemical Luminescence assay method (Roche Diagnostics, cobas e602/2010/ Japan, Basel, Switzerland) (27). However, the essay is specific for 25(OH) $D_{3,}$ so it will not be a viable product in countries where vitamin D_2 is used clinically (93). Men had higher sun exposure, more use of light clothes at home and more intake of milk. Vitamin D was higher in men with a mean difference of about 9 nmol/L. The prevalence of vitamin D deficiency (< 25 nmol/L) was 70% in women, compared to 40% in men. The researchers concluded that vitamin D deficiency is very high among Saudi married couples, especially wives. Female gender is an independent predictor of lower vitamin D level, in addition to sedentary lifestyle and low milk consumption (27).

Regarding the role of sunlight in vitamin D synthesis, it is unexpected to have high prevalence of vitamin D deficiency in Saudi Arabia (100). Direct exposure to sunlight is not practical for Saudi population. This in large part is explained by excessive heat and cultural practices especially among women who tend to avoid body exposure by wearing *Abaya* (black veils) for cultural and religious reasons (17). In Saudi Arabia, with high summer temperatures, air conditioning is considered a necessity; furthermore, the recent prosperity has led to a rapid urbanization and an increasing section of the Saudi population live in air-conditioned apartments in which direct entry of sunlight is obstructed by walls or glass (92). Skin color is darker in some regions in Saudi Arabia, and obesity is a major public health problem among Saudis (101), and both conditions are known predictors of low vitamin status (16,19). Prolonged breastfeeding without vitamin D supplementation also appears to be an important factor (16). Other factors including inadequate consumption of dairy products (99), inadequacy of the current level of vitamin D fortification of food products, poor dietary vitamin D supplementation and age are also important (19,102).

From a historical perspective the problem of vitamin D deficiency in Saudi Arabia does not seem to be improving. For example, Elshafie et al. (2012) (27) found that the median level of serum 25(OH) D among women about 9 nmol/L is actually lower than that reported more than two decades ago among Saudi adult women 15 nmol/L (92). The investigators explained that this might due to the dietary habit shifts towards a more Westernized pattern. Furthermore, increased literacy and improved health behaviors do not seem to have an impact on some health issues, particularly those related to customs and traditions (27). Christie et al. (2010) found in their study limited to Saudi female student's knowledge regarding vitamin D deficiency and reported limited sun exposure due to intense heat, cultural reasons for covering the body, and an infrastructure that makes sun exposure difficult (39). However, the difference between median levels of serum vitamin D in these two studies may be due to differences in the way the assays measure vitamin D. In the 1984 study, serum

25(OH) D was measured using competitive protein-binding assay (CPBA), whereas in the 2012 study, serum 25(OH) D was measured using the Electro-Chemical Luminescence assay method. Clinical laboratory professionals observed that serum vitamin D assays from different commercial sources produced inconsistent results from the same individual blood sample (103).

Because of the well-documented avoidance of sun exposure among Saudi population, it becomes important for dietary vitamin D intake from both food and supplement compensation to maintain serum 25(OH) D levels when cutaneous synthesis is limited. Studies have shown that serum 25(OH) D levels have effectively improved after vitamin D supplementation (104). Other studies showed that consuming milk fortified with vitamin D over 24 months significantly increased serum 25 (OH) D status of the subjects (105).

Very little food naturally contains vitamin D; therefore, some countries such as United States, Canada, and the European Union have developed strategies for mandatory or optional fortification of certain stable food with vitamin D such as milk, oil and margarine as a preventive measure (7,106). For example, foods that are fortified with vitamin D in the United States are milk, ready-to-eat cereals, grain products and pastas, margarine and juice drinks (7,106).

Recommended Dietary Guidelines for Vitamin D

The guidelines for recommended vitamin D intake differ between countries, although many national recommendations are based on the Dietary Reference Intake in the USA and recommended nutrient intakes (RNIs) published by the World Health Organization (WHO). The recommended nutrient intakes (RNIs) of vitamin D is 200 international units (IU)/day (5 µg/day) from infancy to adulthood, aged 19-50 years including pregnant and lactating women, 400 IU/day (10 µg/day) for 51-65 years, and 600 IU/day (15 µg/day) for 65+ years (107). Several studies have found a strong relationship between serum 25(OH)D and bone status. Therefore, the Institute of Medicine, in conjunction with Health Canada, has selected to use serum 25(OH) D to estimate required intake. The requirement distributions are based on the estimation of the mean group vitamin D intake required to maintain the serum 25(OH) D levels above 27 nmol/L, which is the level necessary to maintain normal bone health. For each population group, the dietary intake of vitamin D was rounded to the nearest 50 IU (1.25mg) and then doubled to cover the needs of all individuals within that group regardless of sunlight exposure. This amount was termed adequate intake (AI), which had been used by the Institute of Medicine in the United States since 1941 (108). The World Health Organization expert consultation decided to use these figures as recommended nutrient intakes (RNIs) because it considered this to be a logical approach to estimating the vitamin D requirements for the global population (107).

In 2010, the Institute of Medicine established new guidelines for vitamin D, representing a daily intake that is sufficient to maintain normal calcium absorption and bone health, where serum 25(OH) D is between 30 and 50 nmol/L, consistent with maximal calcium absorption. Serum 25(OH) D of 30-40 nmol/L might be expected to be consistent with coverage for no more than 50% of the population. The assumption of the other 30% to cover nearly all adults from 19-50 years is appropriate and consistent with a serum 25(OH)D 50 nmol/L. Based on these considerations, the estimated average requirement (EAR) is 400 IU/day (10 µg) and the recommended dietary allowance (RDA) is 600 IU/day (15 µg) for adults 19-50 years. These dietary reference intake (DRI) values assume minimal exposure to sunlight (2). However, RDA is proposed to be a target for the daily intake of an individual. Therefore, RDA is an inappropriate value for assessing nutrient adequacy of groups because it covers the great majority of the nutrient requirements of the population. EAR is used instead of RDA for assessing nutrient adequacy of the population (109).

In the present study, the reference value used to determine the adequacy intake of vitamin D is the U.S Estimated Average Requirements (EAR) of 400 IU/day instead of the recommended nutrient intakes (RNIs) of 200 IU/day published by the World Health Organization (WHO) for adults aged 19-50 years. The rationale behind using the EAR is because the RNI of 200 IU/day adopted by WHO is based on the Adequate Intake (AI) published in 1997 by the USA Food and Nutrition Board (FNB) and the assumption made by the WHO committee that most people aged 19-50 years will also receive vitamin D from exposure of their skin to sunlight (107).However, the EAR is based on more recent data developed by the US Food and Nutrition Board (FNB) and published in 2010. This newer data links vitamin D intake to changes in serum 25(OH) D concentrations assuming minimal exposure to sunlight, therefore reducing the confounding effect of sun exposure on serum 25(OH)D concentrations. This data demonstrates that an intake of vitamin D of 400 IU/day yields a 25(OH) D level of 40 nmol/L, which is consistent with normal calcium absorption for adults aged 19-50 years (2). Therefore, the EAR is considered a suitable and sufficient reference to use in Saudi Arabia, where for cultural and religious reasons Saudi women exposure to sunlight is limited.

Current Intakes of Vitamin D in Saudi Arabia

Very few studies have demonstrated the effects of dietary intake on vitamin D levels among the Saudi population. Alissa et al., (2011) showed that postmenopausal Saudi women had vitamin D intake below the estimated average requirements (7.69–11.54 µg). Other studies have shown a lack of consumption of dairy products and products supplemented with vitamin D (19, 27). In Saudi Arabia, the fortification policy is mandatory fortification of enriched wheat flour and enriched treated flour with vitamin D at a concentration of ≥ 551.15 IU/kg (55.1 IU/100g) (110). Many (but not all) Saudi dairy companies fortify milk and Laban (buttermilk) with vitamin D **(Table 1).**

However, the level of fortification is not the same among different brands, vitamin content in fresh milk and Laban varies from 40 IU/L to 400IU/L. Powdered milk is also fortified with vitamin D and the content varied from 65.6 IU/100g to 350/100g (110). However, other dairy products such as flavored yogurt, ice cream, and cheese are not routinely fortified with vitamin D, which calls attention to a significant public misperception that all dairy products are rich sources of vitamin D (111). Most of the dairy products in Saudi Arabia are either not fortified or contain a lower amount of vitamin D than is recommended in developed countries such as the United States and Canada. Sadat Ali et al. (2013) evaluated the current status of vitamin D fortification in food items, such as dairy products, commonly consumed by the Saudi population and compared them with the similar products used in the American markets. The vitamin D content in food products available in the Saudi marketplace contained a lower amount of vitamin D than the 400 IU/ 250mL recommendation set by the U.S. code of Federal Regulations for the U.S. marketplace (110).

Table 1. Selected vitamin D food sources in the average Saudi diet

Food source	Serving size	Vitamin D (µg)	Vitamin D (IU)**
Milk * (whole, low fat 2%, and skim)	250 mL	2.5	100
Laban drink*(whole, low fat, and skim)	250 mL	2.5	100
Yogurt *(whole, low fat, and skim)	125 g	1.25	50
Milk Powder*	250 mL	1.9-2.7	75-109
Oils *	100 mL	0.5	20
Canned tuna (white)	35 g	2.4	94
Egg	1 large	1.1	44
Meat , liver and poultry	90 g	0.3 – 1.2	10-48
Cereal*	30 g	1.3	52

***Milk, yogurt, some corn oils and cereals (voluntary) are fortified with vitamin D in Saudi Arabia. **IU= International unit**

Vitamin D and Bone Health

The primary function of vitamin D is to maintain serum calcium concentrations within the normal range by increasing intestinal calcium absorption. The absorption of calcium depends on vitamin D status: in a vitamin D-deficient state, the intestine absorbs only 10-15% of dietary calcium, but in a sufficient vitamin D state, 30% is absorbed from the diet. During the periods of growth pregnancy and lactation, calcium can be absorbed by as much as 60-80% if vitamin D is sufficient (1,57). When serum vitamin D levels were low, there was significant decrease in intestinal calcium absorption that was associated with elevated PTH. PTH stimulates the tubular reabsorption of calcium as well as enhances the kidney to produce 1, 25(OH) D. PTH also activates osteoblasts, which enhance the transformation of preosteoclasts, to become mature osteoclasts. Osteoclasts remove the mineralized collagen matrix in bone, causing osteopenia, osteoporosis and increased the risk of bone fracture in adults (1, 57).

Effect of Vitamin D on Bone Health in Adults

Osteomalacia

Vitamin D deficiency reduces the absorption of dietary calcium and phosphorus, which results in poor mineralization of the skeleton that causes rickets in children and osteomalacia in adults (84). Osteomalacia is associated with bone pain and tenderness, muscular weakness, and generalized fatigue (45). One study showed that 93% of patients who were admitted to the hospital in the United States with bone pain and muscle aches and who had a variety of diagnosis such as fibromyalgia and chronic fatigue, had severe vitamin D deficiency (112). In Saudi Arabia, from a total of 360 patients with idiopathic low back pain (324 females, 36 males) aged 15-52 years, 83% of patients n= 299 (275 females, 24 males) were found to have low serum 25(OH) D levels. After treatment with vitamin D supplementation, vitamin D levels returned to normal in all subjects who were initially vitamin D deficient. Clinical improvement in back pain symptoms was seen in all the subjects that had a low level of vitamin D. Measurements of serum 25(OH)D is recommended for presumed osteomalacia in patients with chronic back pain (17).

Osteoporosis

The world health organization (WHO) defined osteoporosis as a "disease characterized by low bone mass and micro-architectural deterioration of bone tissue, enhanced bone fragility and an increase in fracture risk" (113,114). Low bone mass has been found to be the strongest predictor of fracture risk; thus, the WHO definition has defined osteoporosis based on a bone mineral density (BMD) value 2.5 standard deviation or more below the mean for a healthy young adult woman (113). Unlike osteomalacia, osteoporosis is not associated with any bone pain (1). Osteoporosis is most often associated with inadequate calcium intake; however, insufficient vitamin D promotes the development of osteoporosis by reducing calcium absorption (115).

Sufficient storage of vitamin D levels maintain bone strength and might help prevent osteoporosis in postmenopausal women and older adults (48). In many experimental trials supplements of both vitamin D and calcium were beneficial to BMD or reduce fractures in older adults, thus, it is difficult to support the beneficial effect of vitamin D alone (2,48). However, a meta-analysis of vitamin D supplementation [five randomized control trials for hip fracture risk (n=9,294), seven randomized control trials for non-vertebral fracture risk (n=9,820)] supports the beneficial effect of vitamin D supplementation on bone mineral density, and fracture risk in elderly subjects (116). The beneficial effects were obtained when oral vitamin D supplementation is between 700-800 IU/d (17.5-20 μg/d) and when serum 25(OH)

D concentration of 75 nmol/L or more, an oral dose of 400 IU/d is not sufficient for fracture prevention (116).

Bone Mineral Density (BMD)

Clinical measurement of (BMD) using dual energy X-ray absorptiometry (DXA) is considered the “gold standard” for diagnosing osteoporosis and evaluating fracture risk (117). DXA is used to assess bone mineral content (BMC) of the whole body or form specific sites, including those most vulnerable to fracture, such as lumbar spine and femoral neck (118, 119). Bone mineral density (BMD) “is a value obtained from the measurement of bone mineral content (BMC) in grams divided by the projected area in cm^2 of the bone being measured, and therefore , the units of BMD are g/cm^2 ”(120). However, the BMD values (in g/cm^2) are not used for assessing osteoporosis. Instead, the WHO defined osteoporosis on the basis of the T-score “which is the difference between the measured BMD and the mean value of young adults, expressed in standard deviations (SD) for a normal population of the same gender and ethnicity” (118, 120).

Four reference values have been proposed by WHO and modified by the International Osteoporosis Foundation (120). The summary of T scores is presented in **Table 2**. However, if local reference data are available then Z-scores, not T-scores are preferred to define low bone mineral density in premenopausal women as recommended by the International Society for Clinical Densitometry (ISCD). A Z-score of -2.0 or lower is considered "below the expected range for age" or low, and a Z-score above -2.0 is considered to be "within the expected range for age" or normal.

A Z-score below the expected range for age in a patient with chronic corticosteroid treatment and/or recent fractures is consistent with a diagnosis of osteoporosis (121).

Table 2. WHO Standardized reference values in evaluating areal BMD results

Diagnosis	T score
Normal	**> -1.0**
Osteopenia	**< - 1.0 , > - 2.5**
Osteoporosis	**< - 2.5**
Severe osteoporosis	**< - 2.5 plus fragility fractures**

Abbreviation: WHO; world health organization, BMD; bone mineral density .

Vitamin D and Bone Health in Saudi Arabia

Several studies have been published examining bone health of the Saudi Arabian population, in both genders and all age groups, including children, adolescents, adult men, and premenopausal and postmenopausal women. A retrospective study in a pediatric endocrine clinic at King Khalid University Hospital in Riyadh, between January 1990 and December 2009, found that 81 (47 girls, 34 boys) Saudi children and adolescents aged 2-18 years were diagnosed to have rickets or osteomalacia (21). The diagnosis of rickets and osteomalacia was based on clinical, biochemical and radiological data. Fifty eight (71.60%) children with nutritional rickets or osteomalacia have shown clinical symptoms and signs of diseases. Bone pains and aches were the most commonly present symptoms in 39 (67.20%) patients. Short stature was found in 12 (20.69%), while skeletal deformities and pathological fractures were the presenting symptoms in 11 (18.97%) and 4 (6.90%) patients respectively. Muscle weakness was evident in 6 (10.34%) patients; 3 of them had severe weakness. Serum 25(OH) D levels were low, ranging from >10 to 45 nmol/L. In this study, rickets was caused by diversity of disorders; the most common causes were nutritional; either low Vitamin D or calcium, or both.

Chronic low back pain has been well documented as a clinical symptom of osteomalacia. Chronic idiopathic low back pain is commonly caused by vitamin D deficiency in Saudi Arabia, especially among female patients (17). A study of 360 patients (90% women and 10% men) aged 15-52 years, attending spinal and internal medicine clinics over a 6-year period who had experienced low back pain that had no obvious cause for more than six months (17) revealed that 83% of the study patients (n = 299) had an abnormally low level of 25(OH)D, ranging from <10 to 22.4 nmol/L. The investigators concluded that screening and treatment of patients with chronic low back pain for vitamin D deficiency should be mandatory.

In a retrospective study for a six-year period ending in December 2000, 47 female patients aged 13-46 years identified with the diagnosis of osteomalacia and/or vitamin D deficiency associated proximal muscle weakness were included (20). Patients were followed in three main medical centers in western Saudi Arabia. Data were collected on clinical, biochemical, radiological, and electrophysiological findings before and after Vitamin D treatment by chart review. The investigators found that all of the patients exhibited progressive proximal muscle weakness lasting 6-24 months before the evaluation. Six (13 %) of patients had severe muscle weakness, which forced these patients to become wheel chair bound. Sixty-six percent of patients had musculoskeletal pain in the back, hips, or lower limbs. Osteomalacia was the referral diagnosis in only 11 patients, whereas the remaining 36

(77%) patients were misdiagnosed. However, all patients had metabolic and radiological profiles suggestive of osteomalacia. After treatment of oral cholecalciferol (800 IU/day) and calcium supplementation (1200 mg/day), remarkable improvements in symptoms was documented in all patients. Because vitamin D deficiency is a treatable cause of osteomalacic myopathy in Saudi Arabia, the investigators suggested that screening for Vitamin D levels in patients with acquired myopathy is required to identify this disorder.

Adequate levels of vitamin D have an important effect on bone mass in young and old adults. Vitamin D level significantly influences bone mineral density (BMD) reading among Saudi individuals in a number of studies. Most of these studies have shown that low BMD correlates strongly with poor vitamin D status (19, 33, 67, 122, 123, 124). Cross-sectional studies conducted on 400 healthy Saudi men and women in the peak bone mass (PBM) age group and those aged ≥ 50 years recruited from the outpatient department of King Fahd University Hospital, Al Khobar, Saudi Arabia, between February 2008, and May 2008 (124) revealed that subjects with a normal 25(OH)D level (≥75 nmol/L), 50% of women and 7% of men in the PBM age group and 26.4% of women and 49.2% of men aged ≥ 50 years had low bone mass. The majority of subjects with 25(OH)D insufficiency (50 and 75 nmol/L), 84.2% of women and 88.9% of men in the PBM age group and 83.3% of women and 80% of men aged ≥ 50 years had low bone mass, whereas 100% of subjects with 25(OH) D deficiency (<50 nmol/L) had BMD readings in the range of osteopenia or osteoporosis. A prospective study of 96 Saudi female patients aged 20-73 years with clinical and biochemical diagnosis of osteomalacia attended the metabolic bone disease clinic at King Khalid University Hospital (KKUH) in Riyadh (122). Bone mineral density was compared with that of healthy Saudi women matched for age; BMD was significantly lower in women with osteomalacia due to lack of sun exposure and lack of intake of milk and dairy product.

Table 3. Selected studies of vitamin D status and Bone health in Saudi population

Subject groups	Type of study	Findings	Reference
Healthy postmenopausal Saudi women (n=122) , 46-70 years. Jeddah, Saudi Arabia	Cross-sectional study	Most of the sample was found to be vitamin D deficient with a serum vitamin D level < 50 nmol/l. Only BMD of the femoral neck showed significant correlations with serum vitamin D level.	Alissa et al., 2011(33)
Healthy premenopausal n=501, postmenopausal women (n=671), 20-79 years. Jeddah, Saudi Arabia	Cross-sectional study	Women with serum 25(OH) D < 50 nmol/L exhibited decreased BMD values at lumbar spine (L1-L4) or neck femur	Ardawi et al., 2011(19)
Healthy Saudi women n= 321 with a mean of age 35.4 years. Researchers measured BMD in Saudi women and compared to their U.S. counterparts .Riyadh, Saudi Arabia	Cross-sectional study	BMD in healthy Saudi females is significantly lower than their U.S. counterparts. This may due to the increase number of pregnancies and longer duration of lactation together with prevalent vitamin D deficiency.	Ghannam et al., 1999 (67)

Lifestyle Factors and Vitamin D

Physical activity also influences serum 25(OH)D. Higher self-reported activity is linked with higher levels of 25(OH) D in NHANES III, and in other studies (125,126). Whether the association between physical activity and vitamin D metabolism is a direct relationship or is a result of confounding due to the relationship between physical activity and sun exposure or body fat is uncertain. Controlling body fat, however, appears unlikely to fully explain the relationship between physical activity and serum 25(OH)D. Data from NHANES showed that physical activity was associated with lower body fat, and controlling for body fat did not noticeably reduce the relationship between physical activity and serum 25(OH)D (125). Conversely, some studies support the role for confounding from sun exposure. Rock et al., (1999) found no significant relationship between physical activity and serum 25(OH)D after adjusting for hours of sun exposure (127).

Another lifestyle factor related to serum 25(OH)D is smoking. Cigarette smoking has a significant effect on calcium and vitamin D metabolism. Calcium absorption is lower in smokers than in non-smokers; this is attributed to lower PTH and possibly attributed to lower levels of 25(OH)D and 1,25(OH)2D in smokers (128). Low calcium absorption results in accelerated bone loss and decreased usefulness of dietary calcium supplements. Studies have shown that smokers have lower bone mineral density (BMD) compared to former and non-smokers, and smoking is a risk factor for hip fracture and osteoporosis. The lower BMD in smokers can be attributed to the possible suppressant effect of smoking on serum 25(OH) D and 1, 25(OH)2D concentration(128,129),resulting in higher bone turnover. However, the mechanism by which smoking suppresses the levels of PTH, and the levels of 25(OH) D and 1, 25(OH) 2D is still unknown (129).

Knowledge about Vitamin D

The connection between an absence of knowledge regarding vitamin D nutrition and low serum vitamin D levels has been considered in the scientific literature as a possible factor contributing to vitamin D deficiency (37, 38).

Oudshoorn et al. (2011) found that better knowledge of vitamin D and calcium was associated with both higher 25(OH)D levels and a higher daily dietary calcium intake. Only 38% of the older adults were familiar with vitamin D and knowledge of sources. They concluded that improving health knowledge could be a possible intervention to improve vitamin D status and calcium intake in older people (36). On the other hand, Kung and Lee (2006), who conducted a telephone interview survey of 547 middle-aged and elderly Chinese women living in Hong Kong, found that the majority of participants had heard of vitamin D, but knowledge about the role and sources of vitamin D was low. They concluded that improving knowledge and public health education may be an effective first step toward increasing individual responsibility for preventing vitamin D inadequacy and osteoporosis (37). A lack of knowledge regarding the production and intake of vitamin D among Saudis has been noted by Siddique (2007) (24), while Christie et al. (2011) conducted qualitative research on a sample of female Saudi Arabian students to investigate knowledge of, attitudes about and practices to prevent vitamin D deficiency; the researchers found significant limitations in the students' understanding of vitamin D and vitamin D deficiency (39).

Chapter 3: Research Design and Methods

Study Location

This study was conducted in Jeddah city, which is located in the western region of the kingdom of Saudi Arabia (latitude 21.7˚ N) (130).The population of Jeddah is approximately 3.4 million. Jeddah is the second largest city in Saudi Arabia, and represents almost 14% of the total population, estimated at 25.37 million in 2009 (131). This region has a temperate climate, with a mean temperature of 27° - 38° C in summer and a mean temperature of 19° - 29° C in winter (132). Sunlight is abundant throughout the year in Jeddah city; the average duration of sunlight is more than 10 hours per day (133).

Study Participants and Procedure

Sampling

A cross-sectional study of premenopausal Saudi women (aged 20 to ≤ 50) was conducted at King Abdul-Aziz Medical City, in Jeddah, Saudi Arabia. The rationale behind selecting women at age 20 and not younger was that according to the International Society for Clinical Densitometry (ISCD) and World Health Organization, classification for osteopenia in premenopausal women begins at the age of 20. Additionally, the recommended reference database for diagnosing osteopenia among premenopausal women using DXA derived from the young female population starts at age 20 (114). Two hundred fifty-seven participants were selected from attendees of the primary care clinic at King Abdul-Aziz Medical City using systematic sampling. Systematic samples are relatively quick and easy to draw when there is an ordered list of units in the study population (134,135). The sampling frame was derived from daily listing sheets that included only walk-in females who were seeking general outpatient services; women with scheduled appointments proceeded directly to their special clinics in the hospital with no listing required, as was systematically arranged. A systematic sampling method was applied to the list of eligible women who were seen in the primary care clinic during the study period between December 2014 and April 2015.

The daily average number of female visitors to the primary care clinic is 70; approximately 57% (40) of these women are premenopausal. The targeted daily sample number was 6 premenopausal women, and the individual interview time was approximately 30 minutes. However, every third woman was selected from the list, and the total number of potential sample women was 23. The rationale behind selecting 23 women was the mandatory exclusion of postmenopausal women, premenopausal women who were not eligible, and premenopausal women who declined to participate. Therefore, the prediction of 6 women out of a potential sample of 23 was based on the following assumption: 57% of 23 (13 women) were expected to be premenopausal; 50% of those 13 (6 women) were expected to be eligible and would agree to participate in the study. To calculate the sampling interval, we divided the daily average number of women (N=70) who attended the primary care clinic by the number of sample women (23; 70/23=3).

To implement the systematic sampling, a random number between one and three was generated each day. The random number determined which first woman was chosen from the list. Starting with first women chosen, then every third woman on the list was selected for potential inclusion in the sample. The exact process was repeated daily until the targeted sample size (n = 257) was reached. Systematic sampling is an equal-probability method that is functionally similar to simple random sampling when the daily listing sheet order of women is roughly random (134,135). The daily listing sheet registration procedure in this primary care clinic is likely to produce a random order of women for the systematic sample, since it is constructed on the following basis: first come, first serve; registration is made upon check in; medical records and referral to the appropriate physician occurs post registration. Based on this registration procedure, it is unlikely that a women's order in the registration list was related to the variable being measured (serum vitamin D level). As stated by Schwarz (2013), if the population is in a random order relative to the variable being measured, the method is equivalent to a simple random sample (135).

Through the above mentioned systematic sampling method, the nurse in charge of the clinic called every selected woman from the female waiting room, and that woman was directed to a private exam room. Subsequently, the woman was fully briefed about the study objectives and methods by a member of the research team, and the woman was invited to participate in the study. Each woman who agreed to participate was initially questioned with regards to her age- and health-related medical conditions to ensure that she met the inclusion criteria, and eligible women were asked to proceed directly to the same room upon the completion of the medical examination that was the main reason for her visit. In the next step, data collection sessions were conducted under the supervision of the research teams, who interviewed women and reviewed their medical record in case more information was needed, such as vitamin D and calcium supplement dosage and medication. Data collection sessions usually lasted for 25–30 minutes.

Women were eligible for the study if they were citizens of Saudi aged 20 to 50 years. Women were excluded from the study if they were pregnant or lactating or post-menopausal (defined as amenorrhea for at least six months).Women with cancer, diabetes, hyperthyroidism or hypothyroidism, metabolic bone disease, malabsorption, renal diseases, or those taking medications known to effect bone metabolism were also excluded from the study. Preventive measures were used to exclude any relatives or friends who may have accompanied women selected by systematic sampling. These companions may share genetic and/or common diet and nutritional behavior that could result in an association between registration order and serum vitamin D levels. During the initial interview, participants were questioned about anyone who accompanied them to the clinic; their companions' names were recorded and those individuals were excluded from the study. If it is later determined that a relative or friend was mistakenly included in the study, the initial participant remained, and relatives and friends who were added later were excluded. In this way, the exclusion procedure ensured against any possible bias in the systematic selection of subjects and, thus, to the study results. The use of vitamin D supplements and calcium were not exclusionary criteria because we were interested in evaluating how many participants take vitamin D supplements as well as the effects of supplementation on serum 25(OH) D levels and bone mineral density markers.

Participant's Selections for Double Energy X-Ray Absorptiometry (DXA)

To select participants for the DXA scan, each woman chosen through the systematic sampling method described above, was asked to participate in this portion of the study until completion of the required sample for this subgroup (n=102) was achieved. Upon completion of the first visit, each woman was asked to return for a second visit, during which bone mineral density (BMD) measurements was taken via DXA scan. If a participant declined this request, then selection proceeded to the next sample woman. Sociodemographic data (age, education level, marital status, occupation, and economic status) and anthropometric measures of women who refused to participate in the DXA scan was compared to women who accepted the invitation. These data were used for nonresponse analysis to determine the differences between those who chose to participate in the DXA scan and those who did not.

Ethical Approval

The research protocol and the consent documents were approved by the ethics committee of the Institutional Review Board (IRB) at the University of Maryland, College Park and Institutional Review Board (IRB) at King Abdullah International Medical Research Center (KAIMRC), Jeddah, Saudi Arabia. Participants were informed about the aim of the study, of the clinical tests, and of the type of interview. The informed consent process took place in a setting that afforded a sufficient level of privacy for the participants.

Data Collection

Eligible women were invited for face-to-face interviews, during which written informed consent was obtained. Weight, height, and waist circumference were measured. Data were collected using a pre-test questionnaire on (1) demographic and lifestyle; (2) dietary intake of vitamin D and calcium; (3) sun exposure and skin color; (4) vitamin D knowledge and attitude and behavior toward sun exposure. Then, blood samples were drawn by qualified phlebotomists for serum calcium, phosphate, alkaline phosphatase, serum 25-hydroxyvitamin D3 25(OH) D, and parathyroid hormone (PTH).

1. Anthropometric Measurements

The anthropometric measurements were performed by trained staff according to standardized procedures (136). Participants were weighed without their shoes, and were asked to stand in the center of the scale facing the recorder, hands at side, looking straight ahead. After the participant was correctly positioned and the readout on the digital measurement device became stable, weight was reported. Subjects were also asked to stand on the scale with the heels of both feet together in order to measure stature accurately. Weight and height were measured using a SECA 703 High-Capacity Digital Column Scale (SECA, Hanover, MD, USA) and reported to the nearest 0.1 kg and 0.1 cm, respectively. Body Mass Index (BMI) was calculated by dividing the weight (in kilograms) by the square of the height (in meters). Standard BMI cut-points according to World Health Organization criteria (137) were used to categorize the weight status: underweight (> 18.5 kg/m^2, normal (18.5 - 24.9

$kg/m^{2)}$, overweight (25-29.9 kg/m^2), and obese (≥ 30 kg/m^2). Waist circumference (in centimeters) was measured with flexible metal tape (range 0 - 150 cm) placed at mid-point between bottom of the rib cage and above the top of iliac crest. According to the International Diabetes Federation (IDF) abdominal obesity cut-points for people in the Middle East (≥ 80 cm in women), waist circumference was dichotomized into normal and abdominal obesity [normal (< 80 cm); obese (≥ 80 cm)] (138).

2. Questionnaires

The questionnaire used in this research was developed based on a number of sources including the review of associated literature; review of questionnaires obtained from vitamin D research, consultation with experts in the field, and proposed respondents. A pilot study was performed prior to beginning data collection in 30 volunteer women falling into the same age groups, in a similar setting, to screen for potential problems with the questionnaire. The questionnaire was tested both in self-administered and interviewer-administered groups. In the self-administration group, women were instructed to report any problems or difficulties with questions while answering the questionnaire. In the interviewer-administered group, cognitive interviews were carried out; the interviewer read the questions aloud and probed immediately after the respondent answered a particular question. Cognitive interview techniques are widely used in surveys, to ensure the quality and accuracy of survey instruments and to identify and analyze sources of response errors in survey questionnaires (139). Based on the pilot study results, some items were refined and clarified in a later version of the questionnaire.

2.1 Demographic and Lifestyle Variables

Sociodemographic variables included age (years), marital status, e.g., married (living with partner) or unmarried (single, divorced, widowed); Occupation status (employed, unemployed, student, housewife); Monthly household income was divided into categories, according to the Central Department of Statistics and Information (2006/2007) (140) and a previous published study (141), depending on the amount of Saudi Arabian Riyal (1SAR = 0.266USD): (< 4,000; 4,000 to 7,999; 8,000 to 14,999; 15,000 to 24,999; ≥ 25,000); residence (rented apartment , owned apartment , rented house , owned house , others). Participants were asked about the number of years of formal education, and then level of education was categorized as follows: (less than college, college graduate, and post graduate). Participants were asked about the number of pregnancies and number of living children. Breastfeeding variables included number of breastfed children and use of vitamin D supplementation during breastfeeding (yes, no).

Life style factors included physical activity (PA) (inactive, moderately active, active), and smoking status (current, past, never smoked), type of smoking (cigarette, shisha, cigarette and shisha), number of cigarettes or shisha smoked per day. Physical activity (PA) was assessed using the Short Version of the International Physical Activity Questionnaire (142), in which participants are asked to report the number of days and the duration of the walk, moderate-intensity, and vigorous activities they undertook during the past week. The items in the IPAQ form provide separate scores on walking, moderate-intensity and vigorous-intensity activity **(Table1)**. Total PA was expressed as the metabolic equivalent (MET) min/week, by weighing the reported min/week, in each of three activity categories, by the metabolic equivalent specific to each activity **(Table1).** A reliability and validity study of IPAQ was conducted in 12 countries, and demonstrated reasonable test-retest reliability (intra-class correlations range 0.7–0.8) and inter-method validity (median rho = 0.67) (143). The researchers concluded that IPAQ is suitable for any mode of administration, and activity examples can be culturally adapted for local populations.

Table 1. International Physical Activity Questionnaire IPAQ Scoring Protocol

Walking	*Moderate PA	*Vigorous PA	Physical Activity PA Score
MET levels Walking = 3.3 METs	MET levels Moderate intensity = 4.0 METs	MET levels Vigorous intensity = 8.0 METs	______
Walking MET min/week = 3.3 × walking minutes × walking days	Moderate MET min/week = 4.0 × moderate-intensity activity minutes × moderate days	Vigorous MET min/week = 8.0 × vigorous-intensity activity minutes × vigorous-intensity days	MET-min/week = sum of Walking + Moderate + Vigorous MET minutes/week scores.

***Moderate physical activities refer to activities that take hard physical effort and make you breathe much harder than normal (e.g., carrying light loads, bicycling at a regular pace) *Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal (e.g., heavy lifting, digging, aerobics, or fast bicycling).**

2.2 Dietary Vitamin D and Calcium Intake

Dietary intake of vitamin D per day was estimated using the Semi-quantitative Food-Frequency Questionnaire (SFFQ). The SFFQ used was a modified version of a previously validated questionnaire (144), which was also validated in United Arab Emirates and showed positive correlation between the SFFQ and 3-day diet food records were ($r = 0.82$, $p < 0.001$) and ($r = 0.74$, $p < 0.001$) for calcium and vitamin

D, respectively (145). The modified version was designed to take into account the food composition for the Saudi population (146), Arabian Gulf diet (147) and the Middle Eastern diet (148) that are considered to be excellent sources of calcium. Vitamin D values of some food items were obtained from the Arabian Gulf Food Composition Table such as meat, and local fish consumed in this area. However, because vitamin D values for non-local foods such as tuna and salmon are not available in the Arabian Gulf Food Composition Table, the U.S. Department of Agriculture reference data (149) were used. Vitamin D values of supplements and local food items such as vitamin D fortified milk and Laban drink (buttermilk) were obtained from food labels. The items in SFFQ include natural food sources of vitamin D (e.g., tuna, salmon, meat, poultry, liver, and eggs), local, and imported vitamin D fortified food and beverages available in the Saudi marketplace. Supplemental vitamin D and/or calcium and multivitamins were assessed by the SFFQ.

The selection of food items in SFFQ was obtained and reviewed with the investigator using visual estimates (photographs) to help subjects estimate average portion size. The photographs using common items such as household items (plates or bowels) or hands were used to help estimate average portion size for subjects who ate from the communal dish**.** On the SFFQ, respondents were asked to record how often they consumed a single serving of each food listed during the previous month, with possible responses ranging from less than once per month to 2 or more per day. Vitamin D intake and calcium were computed by multiplying the intake frequency by the nutrient content of the portion size of specific items. Intake of vitamin D and calcium were calculated by summing nutrient intake from diet and supplemental sources. Vitamin D and calcium intakes were examined in terms of absolute and energy-adjusted nutrients (intake per 1,000 kcal). Energy-adjusted values, termed nutrients densities, were computed as daily intake from diet and supplements, divided by calories per day, and multiplied by 1,000 (150,151). To determine the adequacy of vitamin D and calcium intake, the values obtained were assessed using Estimated Average Requirements (EAR) of 400 IU/day of vitamin D and 800 mg/day of calcium (2).

2.3 Sun Exposure and Skin Color

The sun exposure recall questionnaire included details of the time of the day of sunlight exposure; length of exposure to sunlight during the previous month on week days and weekends. Cover status when they were outside in the public area was used to determine the area exposed to direct sunlight. Women were classified as either 1) covering the whole body including the face, 2) face covered but hands exposed, 3) face and hands exposed. From the amount of clothing worn outside, the percentage of body surface area (BSA) exposed to sunlight was determined using an adjusted “rule of nines” (9% for face, 1% for each hand, 9% for each arm, and 18% for each leg) used in clinical practice to estimate the burnt area of skin (152). A Sun Exposure Index (SEI) was calculated as the number of hours per week spent in direct sun without sunscreen protection multiplied by the fraction of BSA exposed sunlight to determine the amount of sun exposure for each participant (152). In addition, women were asked the average length time per day they spent in their courtyard. In Saudi culture this is the place where the sunlight exposure occurs because in all other outdoor activities women generally cover their bodies by wearing black abayas (153).

The definition of abaya is a clothing style not only limited to Islamic dressing habits but also to Saudi traditional clothing, which covers the whole body completely from head to toe (39).

Skin color was assessed by the investigator using the Fitzpatrick skin-type scale (154). The system is a numerical classification scheme for determining six different skin types based on a questionnaire related to an individual's genetic constitution, reactions and vulnerability to sunlight or UVB radiation, and tanning habits (154,155). The response to each question was measured on a scale of 0-4. The response for all questions were added together to get the total score corresponding to the Fitzpatrick skin-type scale, which ranges from very fair (skin type 1) to very dark (skin type IV) **Table 2.** Recently, vitamin D researchers suggested that skin type can be best evaluated by using the Fitzpatrick scale (156). Most objective measures of skin color can assess only time period of measurements, while the Fitzpatrick scale takes long-time behaviors into account. One limitation is that there may be inaccuracies in the reports of some questions such as natural skin color, protective behaviors, or perceived skin damage (157). The Fitzpatrick skin color scale has been previously used in Arabian Gulf studies including, United Arab Emirates (158) and Kuwait (38).

Table 2. Fitzpatrick Skin Pigmentation Scale

Skin type	Color	Reaction to Sunlight	Tanning Ability
Type I	**Caucasians; pale white skin, blonde or red hair, blue eyes.**	**Always burns**	**Never tan**
Type II	**Caucasian; fair skin, blonde or red hair, freckles, blue or green eyes.**	**Always burns**	**Sometimes tans**
Type III	**Darker Caucasian, light Asian; fair to medium skin**	**Sometimes burns**	**Always tans gradually**
Type IV	**Mediterranean, Asian, Hispanic; medium skin**	**Rarely burns**	**Always tans well**
Type V	**Middle Eastern, Latin, light skinned black, Indian ; olive or dark skin**	**Very rarely burns**	**Tans very easily**
Type VI	**Dark-skinned black; very dark skin**	**Never burns**	

2.4 Knowledge of Vitamin D and Attitudes and Behavior toward Sun Exposure

The internal consistency reliability of the vitamin D knowledge test was calculated on the dichotomized items (correct / incorrect) using kuder-Richardson Formula 20. The reliability of the test was calculated to be 0.70, indicating that the test demonstrated acceptable reliability (159). The questionnaire consisted of two sections. In the first section, the participants were asked seven multiple-choice questions about their knowledge of the sources and role of vitamin D. The questions were adapted from those used in previous validated surveys (36,37) and were modified for our study population. The first question was used to screen participants. Women were asked if they had heard of vitamin D, and only women who had heard of vitamin D were asked to complete the rest of the survey. Questions 1–7 were used to calculate participants' total knowledge score by adding the points received for each answer. One point was assigned for each correct answer, and 0 points were given for each incorrect answer or “I don’t know” response. If a question had two correct answers, then each correct answer was worth 0.50 points. If a question had three correct answers, then each correct answer was worth 0.33 points. The participants were categorized as more or less knowledgeable about vitamin D based on their number of correct answers. The participants were regarded as more knowledgeable if they correctly answered four or more questions, including a positive response to question 1. The participants who answered question 1 negatively or correctly answered fewer than four questions were considered to be less knowledgeable about vitamin D.

The second section of the questionnaire included nine multiple-choice statements about women’s attitudes and behavior toward sunlight exposure. The statements were adapted from those used in a previous survey (160) and modified for our study population. This section included questions on whether women spend time in the sun or tanning, use sunscreen, wear colored *abaya* during sun exposure, avoided sun exposure, and their reasons for each choice.

2.5 Health Information

A general health questionnaire was also administered to each participant, who provided information on medical history, the consumption of medications, menstrual status and personal fracture history.

3. Biochemical Analysis

Non-fasting venous blood samples were drawn by qualified phlebotomists for serum 25-hydroxyvitamin D 25(OH) D, parathyroid hormone (PTH), adjusted serum calcium (adCa), phosphate (P04), and alkaline phosphatase (ALP) (**Table3).** Serum calcium was measured by Arsenazo III dye (Architect 16000, Abbott, USA). Parathyroid hormone (PTH) was measured by a two-step sandwich immunoassay for the quantitative determination of intact PTH in human serum using CMIA technology (Architect 2000, Abbott Diagnostics, Germany). The PTH assay showed a detection sensitivity of ≤ 3.0 pg/mL, intra-assay coefficient of variation (CV) was 12.9-6.1%, inter-assay (CV) was 3.0-6.4%. The reference range in the study laboratory is 24-114 pg/mL.

Quantitative 25(OH) D in serum was determined by using one step delayed chemiluminescent microparticle immunoassay (CMIA) (Architect, Abbott, Germany).This essay was used to recognize 25(OH) D_2 (ergocalciferol) and 25(OH) D_3 (cholecalciferol) with equal affinity. Quality control samples were run together with each batch of serum samples. The laboratory undertaking the testing is a participant in the proficiency Accuracy Based vitamin D (PABVD) since 2012. The Accuracy Based vitamin D is part of the proficiency testing (PT) kits for the College of American Pathologists (CAP), the vitamin D external quality assurance scheme of vitamin D assays (161). Intra-assay 25(OH) D coefficient of variation (CV) from daily quality control was 1.4- 3.7% and inter-assay (CV) was 2.6 – 4.6%.The fully automated Architect assay is designed to have an imprecision of ≤ 10 %.

Total volume required was 6 mL using two plain vacutainer tubes (BD vacutainer systems, Plymouth, UK). The serum samples were taken in the laboratory in the primary care clinic; after blood collections, samples were left for 10 min for clotting then immediately centrifuged at 3000rpm for 10 min. Then, samples were aliquoted and frozen at -20° C until further analysis. Samples were run simultaneously in a batch. All analyses were performed in the Pathology laboratory at King Abdul-Aziz Medical City.

Baseline serum 25(OH) D levels were classified into three categories using cutoff points reported in the scientific literature (11,162) and according to the Endocrine Society (15). Vitamin D deficiency serum 25(OH) D < 50.0 nmol/L (20ng/mL); insufficiency serum 25(OH) D 50 - < 75 nmol/L (20-30 ng/mL); sufficiency serum 25(OH) D ≥ 75 nmol/L (30ng/mL). For some analyses, the following cut off value was also used to diagnose vitamin D sufficiency: ≥ 50 nmol/L according to World Health Organization and the Institute of Medicine (2,163)

Table 3. Information regarding Sample type, Tube, Instruments, Blood volumes, for all Biochemical Parameters

Test	Sample type	Type of tube	Instrument	Blood Volume
ALP	**serum**	**plain**	**Architect 16000**	**0.5 ml**
Ca	**serum**	**plain**	**Architect 2000**	**0.5 ml**
PO4	**serum**	**plain**	**Architect 2000**	**0.5 ml**
IPTH	**serum**	**plain**	**Architect 2000**	**2.0 ml**
VITD	**serum**	**plain**	**LIAISON**	**2.5 ml**

4. Measurement of Bone Mineral Density (BMD)

Bone mineral density (BMD) was measured using double energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE medical systems, Madison, WI 53718, USA) in a subgroup of 102 women on the lumbar spine (L1 to L4) and the neck of the femur. The scanning precision (coefficient of variance, CV %) was calculated by two repeated measurements in 30 subjects. The precision (CV %) of the measurements were (1.0 -1.2%) and (1.1 – 2.2%) in the lumbar spine and the neck of the femur, respectively. A complete quality control test on DXA equipment was performed each day for BMD and composition calibration using spine phantom as recommended by the manufacturer, to ensure the stability of the system. Additional quality control was also done every six months or when required by the manufacturer's company. DXA scans were performed in the Department of Radiology at King Abdul-Aziz Medical City by licensed Radiological Technologists trained in bone density exams. Prior to scanning, women were asked to remove all jewelry and wear only hospital gowns during scanning procedures. Women were asked to lie on the DXA scanning bed for the duration of testing (approximately 10-15 minutes). The BMD values at the hip were compared with peak bone mass for Caucasian female normative database from the National Health and Nutrition Examination Survey NHANES III data, as recommended by the International Society for clinical Densitometry (164). In addition, the Saudi Osteoporosis Society (SOS) committee released guidelines in 2015 stating that in the absence of local normative reference, the committee suggests to continue using the data from the United States because the (NAHNES III) data are accurate and reliable and it seems to be best option until the local data becomes available (165). BMD values were classified according to WHO criteria, a low bone mass (osteopenia) if BMD T-score is < -1.0, or normal bone mass if BMD T-score is ≥ -1.0 (113).

Sample Size and Power Analyses

An a-priori power analysis was conducted to determine the number of participants required to detect a small effect ($f2 = 0.10$) with power = .80, given the following testing parameters: a multiple forward stepwise regression with 16 predictors and conducted at $\alpha = .05$. A sample that provides adequate power for this purpose would also be large enough to provide adequate power for a bivariate correlation or t-test, or a test of a single regression coefficient of a multiple regression. The analysis indicated that a sample size of 207 would detect a small effect given these parameters. The power analysis was conducted with G*Power 3.1 software package (166). To account for any potential loss of participants, an additional recruit of 20% was considered, and a total sample size was 250 women.

To determine the required number of participants in the DXA subsample, the power analysis was also performed, based on research question (Is there a significant difference in calcium intake, vitamin D intake, and serum vitamin D levels, and serum parathyroid hormone levels between women with normal bone density and women with low bone density in this study population?). Specifically, the sample size needed to detect the effect of a specific predictor ("serum vitamin D level") with a power level of .80, and an alpha level of .05, using a multiple logistic regression with binary response variable, assuming a moderate effect size (odds ratio = 2.50) and the squared multiple correlation coefficient between "serum vitamin D level" and other independent variables in the model = 0.3, was computed. The results of the power analysis indicates that, assuming a medium effect size, a minimum of 76 subjects are required in order to achieve a power of 80% at the 0.05 level of significance.

Statistical and Data Analysis

Data were checked for missing values and outliers, and analyzed using the Statistical Package for Social Science (SPSS) software version 22.0 (SPSS, Inc., Chicago, IL, USA). The characteristics of the study population were described through frequencies and percentages for categorical variables. For continuous variables, medians and range were used to summarize non-normal data, and mean $\pm$ standard deviations (SD) were used to summarize data with normal distribution (only height, bone variables, and knowledge score were normally distributed). Percentages values between groups were compared using the Chi square tests. For skewed variables (e.g., serum vitamin D level, vitamin D intake, length of sun exposure, biochemical values, anthropometric), median values between groups were compared using the Mann-Whitney *U* test when only two groups were considered, and the Kruskal-Wallies test when more than two groups were studied. A nonparametric Wilcoxon signed-rank test was used to determine if vitamin D and calcium intake were statistically significantly different from the U.S. Estimated Average Requirement EARs of 400 IU/day and 800 mg/day, respectively. The relationship between demographic variables and knowledge score (normally distributed, with normality assessed via the QQ plot) was investigated using two-sample *t*-tests when only groups were considered , and a one way analysis of variance (ANOVA) when more than two groups were studied. Spearman rank correlation was used to assess the strength of the relationship of 25 (OH) D concentrations with predictors that were thought to influence 25 (OH) D status such as PTH, age, vitamin D intake, calcium intake and BMI. Additionally, Spearman rank correlations were also used to assess the strength of the relationship of bone mineral density at the lumbar spine and the femur neck with variables such as BMI, parity, and smoking, variables that were thought to influence bone mineral density. Statistical significance was considered at p-value <0.05.

Variables found to be statistically significant associated with serum 25(OH) D concentrations were then included in a forward stepwise multiple linear regression analysis in order to investigate the relationship between possible predictors and vitamin D status. The condition for entry at each iteration was $\alpha = .05$, and the condition for predictor removal at each iteration was $\alpha = .10$. Backward elimination attempts to find a good model by beginning with a model that includes all candidate regressors, it does not perform well in the presence of multicollinearity (a high degree of correlation among two or more independent variables). Issues of multicollinearity include: misleading p-values, large standard errors of the coefficients, small changes in the data producing wide swings in the parameter estimates. Therefore, the forward selection was implemented. The forward selection starts with no predictors in the model. The strongest significant predictor at $\alpha = .05$ was entered in the model's first iteration. Next, the strongest significant predictor at $\alpha = .05$ given model 1 was entered. At each iteration a predictor that was significant in a previous model can be removed if it became non-significant at $\alpha = .10$. In these analyses the dependent variable serum 25 (OH) D concentrations was log transformed since it was not normally distributed. The following independent variables were considered for entry in the stepwise regression: Age, years of formal education, number of pregnancies, number of children, vitamin D intake (diet and supplement), calcium intake (from diet and supplement), sunlight exposure index, BMI, waist circumference, household income, and skin color. Note that household income and skin color were categorical variables, and the remaining variables were continuous variables.

The forward stepwise multiple linear regression was also conducted in order to investigate the relationship between the dependent variable, vitamin D intake (from food and supplement), and the independent variables, the sociodemographic and parity variables. In these analyses the dependent variable vitamin D intake (from food and supplement) was log transformed since it was not normally distributed. The following independent variables were considered for entry in the stepwise regression: age, years of formal education, and number of children, income level, and occupation, type of residence, marital status, and taken vitamin D supplement during breastfeeding period. Note that age, years of formal education, and number of children were continuous variables, and the remaining variables were categorical variables. The assumptions for the two regression models were evaluated using normal quantile-quantile (QQ) plots of the standardized residuals (the assumption of normality) and scatter plots with standardized residuals versus standardized predicted values (the assumption of homogeneous error variance). The assumptions of the regression models were satisfied.

Multiple logistic regressions were implemented to identify and analyze factors that influence BMD markers. Hence, the two dependent variables were BMD at lumbar spine and BMD at femur neck, both binary response variables (low and normal). The independent variables being considered included: Vitamin D intake, calcium intake, serum 25 (OH) D levels, and serum PTH level. The following variables served as possible confounding variables: BMI and energy intake. It should be noted that in multiple logistic regression, the effect of a specific independent variable on the dependent variable is adjusted for the effects of all the other independent variables in the model. The Wald test was used to determine if an effect was statistically significant. Statistical significance was considered at p-value < 0.05. Hosmer-Lemeshow goodness-of-fit test was used to determine the model adequacy p-value > 0.05 indicates good model fit). In this analysis, 88 subjects were used for logistic regression, after excluding subjects with missing values in the study variables.

A multiple linear regression model was conducted to investigate if there was a relationship between knowledge of vitamin D and total vitamin D intake, after controlling for age and years of education (Model 1). Another multiple linear regression model was also conducted to investigate if there was a relationship between knowledge of vitamin D and serum vitamin D level, after controlling for age and years of education (Model 2). A p-value less than 0.05 indicated significance. The assumptions for regression models were evaluated using normal quantile-quantile (QQ) plots of the standardized residuals (the assumption of normality) and scatter plots with standardized residuals versus standardized predicted values (the assumption of homogeneous error variance). The assumptions of the regression models were satisfied.

Fisher's exact tests were conducted to investigate if there was a relationship between attitudes and behaviors toward sun exposure, and serum vitamin D level (a 2-level categorical variable: deficiency vs. sufficiency).

Chapter 4: Results

PAPER I: Vitamin D Status and Predictors of Vitamin D Deficiency among Saudi Premenopausal Women living in Jeddah City

ABSTRACT

Background: Vitamin D deficiency is a global health problem even in sunny countries like Saudi Arabia, where it is prevalent among females. The latest national study reported that its prevalence (serum hydroxyvitamin D 25(OH) D < 50 nmol/L) among young, healthy Saudi women is 72%. Saudi women's traditional clothing limits skin exposure to sunlight, which may be an independent risk factor. However, it is unclear whether traditional clothing determines women's vitamin D status, with or without consideration of contributing individual, demographic, and cultural risk factors. Research studies designed to increase understanding of vitamin D status, particularly in premenopausal women in Saudi Arabia, are needed.

Objective: The study's objective was to identify which potential predictors are associated most with vitamin D deficiency, including dietary vitamin D and calcium intake, sunlight exposure, skin color, anthropometric measures, socioeconomic factors, and lifestyle factors, in premenopausal Saudi women.

Methods: A cross-sectional study was conducted among 257 premenopausal Saudi women aged 20–50 years in Jeddah between December 2014 and April 2015. Anthropometric, dietary, sun exposure, skin color, lifestyle, and data on sociodemographic factors were obtained through pre-tested questionnaires. Serum intact parathyroid hormone, calcium, phosphorus and alkaline phosphatase were measured. Serum 25(OH) D was evaluated using chemiluminescent microparticle immunoassay.

Results: The prevalence of vitamin D deficiency was 77.6% (25(OH) D < 50 nmol/L). Lower serum 25(OH) D levels were associated with increased risk of elevated parathyroid hormone (PTH). Lower income levels, being unemployed, being unmarried, and low dietary and supplement calcium intake, were associated with lower serum 25(OH) D concentrations. In the stepwise regression analysis, major predictors of vitamin D deficiency were low dietary and supplement vitamin D intake, and younger age explaining 41% of the variance in 25(OH) D serum concentrations ($P < 0.001$).

Conclusion: This study confirms that vitamin D deficiency is highly prevalent among premenopausal Saudi women. Public health interventions offering early screenings and increased vitamin D and calcium intake could improve vitamin D status in young Saudi women.

INTRODUCTION

A central issue in public health is the role that vitamins play in preventing disease. Vitamin D is well known for its primary functions in calcium absorption and bone mineral metabolism. Vitamin D deficiency decreases the efficiency of intestinal calcium and phosphorus absorption of dietary calcium and phosphorus, resulting in an increase in parathyroid hormone (PTH) concentrations. Secondary hyperparathyroidism due to low levels of 25 hydroxyvitamin D-25(OH)D may lead to increased bone turnover and bone loss, resulting in osteopenia, osteoporosis, and ultimately an increase in the risk of fractures in adults (2,3 15, 167). Health outcomes of prolonged severe vitamin D deficiency (serum 25(OH)D < 25 nmol/L) which is detrimental to the skeleton, include rickets in children and osteomalacia in adults (2, 3, 84).

In addition to its critical role in musculoskeletal health, vitamin D plays an important role in non-skeletal health. Vitamin D receptors have been found in many other tissues, including the prostate, the heart, blood vessels, muscles, and endocrine glands. Increasing evidence points to possible links between vitamin D insufficiency and a number of non-skeletal disorders, including high blood pressure, heart disease, diabetes, cancer, age-related cognitive decline, multiple sclerosis, and arthritis. Thus, vitamin D deficiency may have broader implications for public health than previously acknowledged (1, 4, 5).

Vitamin D, while universally recognized as one of the four essential fat-soluble vitamins, is obtained through either synthesis in the epidermis with exposure to ultraviolet (UVB) sunlight or the intake of vitamin D-rich foods. It is naturally present in very few foods, including oily fish, egg yolks, veal, beef, liver, and sun-dried mushrooms. Sunlight exposure, rather than diet, has been reported as the main source of vitamin D for the majority of the population (4,7). During exposure to ultraviolet (UVB) radiation from sunlight, 7-dehydrocholesterol in the epidermis is converted to previtamin D3. Vitamin D3, or cholecalciferol, is produced in the skin. Vitamin D2 and D3 ingested in food undergo hydroxylation in the liver to form 25 hydroxyvitamin D, the chief circulating form, and then further hydroxylation in the kidney to produce the biologically-active form, calcitriol or 1, 25-dihydroxyvitamin D (4,10-13, 54).

Serum concentration of 25-hydroxyvitamin D, or simply 25(OH)D, is considered the best indicator of vitamin D nutritional status, as 25(OH)D levels reflect both vitamin D produced in the skin and that acquired from the diet. While the optimal 25(OH) D level is a topic of considerable debate, there is a general consensus that a serum 25(OH)D concentration < 25 nmol/L is considered an indicator for vitamin D deficiency (11,13, 14). According to the US Institute of Medicine's (IOM) 2011 recommendations for vitamin D (12), a serum 25(OH) D concentration < 30 nmol/L qualifies as "deficient" and has a known impact on skeletal health, being associated with calcium malabsorption and secondary hyperparathyroidism and leading to increased bone resorption and osteomalacia in adults and rickets in children. Serum 25(OH) D in the range of 30–50 nmol/L may be "inadequate" in some people, and serum 25(OH)D > 50 nmol/L is "sufficient" for the majority of the population (97.5%) (4, 12). The World Health Organization (WHO) has defined vitamin D "insufficiency" as serum 25(OH)D below 50 nmol/L (163), while serum

25(OH)D below 25 nmol/L is considered to indicate “deficiency.” However, the Endocrine Society released guidelines in 2011 stating that vitamin D deficiency is defined as a blood serum of 25(OH) D < 50 nmol/L, while a blood serum of 25(OH)D levels of 50–75 nmol/L indicate vitamin D insufficiency, and serum 25(OH)D levels ≥ 75 nmol/L indicate sufficiency to maximize the effect of vitamin D on bone and muscle metabolism (15).Using these thresholds, vitamin D deficiency (< 50 nmol/L) is prevalent and has been reported in many populations worldwide.

Most recently, vitamin D deficiency has been recognized as a problem in more than one-third of the population worldwide. A recent systematic review of vitamin D status around the world analyzed almost 200 population-based vitamin D studies from 44 countries in North America, Europe, Asia, and the Middle East (35). Using strict inclusion criteria to filter and compare data, together with consistent threshold values for serum 25(OH)D, the researchers found more than one-third of the studies reported vitamin D status at the deficiency level (< 50 nmol/L). A number of factors associated with vitamin D deficiency worldwide, include primarily limited exposure to sunlight. Common factors known to influence the amount of sunlight reaching the epidermal layers of the skin include: season, latitude, time of day, skin color, clothing, and age. Other factors include dietary factors, particularly the intake of vitamin D-rich food and vitamin D supplements, as well as obesity (168). It has been suggested that the low vitamin D levels associated with obesity are due to sequestration of fat-soluble vitamin D in adipose tissue (25), leading to a reduction in its bioavailability.

Limited exposure to sunlight has consistently been identified as a main contributing factor for low 25(OH)D concentrations in Saudi Arabia, where the prevalence of vitamin D deficiency ranges from 30–93% (100). Despite the abundant sunshine in a country spanning the tropical and subtropical latitudes from 16˚ N to 33˚ N, several factors limit the population's skin exposure to sunlight: cultural practices (e.g., clothing style) that reduce skin exposure, the hot climate that drives people to air-conditioned comfort, and the pigmentation in dark skin that can reduce endogenous vitamin D production (16, 17, 24). People with darker skin have natural skin protection and require longer exposures to sunlight to produce the same amount of vitamin D as light-skinned people do (4,15).

Saudi Arabia reports some of the highest rates of vitamin D deficiency in the world(16,17). Numerous studies have identified a high prevalence of vitamin D deficiency (serum 25(OH)D < 50 nmol/L) in the Saudi population (27, 98, 169-172). This major public health problem has been documented in both genders and all age groups (16). Saudi women are particularly at high risk for vitamin D deficiency due to some of the cultural practices (e.g., clothing style and veils) that limit skin exposure (18). The most recent national study reported that the prevalence of vitamin D deficiency (serum 25(OH)D < 50 nmol/L) among healthy young women is 72%, and 85% among postmenopausal women (19). Although there is a general consensus on the vitamin D concentration of 25(OH)D < 50 nmol/L used in Saudi studies, comparisons of the prevalence of vitamin D deficiency are hampered by the choice of different assay methodologies (4). Saudi studies (17, 20, 21) have highlighted concerns that Saudi adolescents and women of reproductive age are at risk of suboptimal bone health, leading to osteomalacia, chronic back pain, and other bone-related problems, with serum 25(OH)D ranging from less than 10 to 45 nmol/L. Low bone mass has been reported among young (25–35 years), apparently healthy Saudi women with serum 25(OH)D levels less than 50 nmol/L (22).

A comprehensive survey of the leading vitamin D studies (35) noted the need for future research that strengthens our understanding of vitamin D status in key subgroups worldwide. The subgroup of premenopausal women in Saudi Arabia is clearly one in which the understanding of vitamin D status is extremely limited. Furthermore, there has been no systematic investigation of the subgroup-specific factors, including individual, demographic, and cultural factors that are associated with vitamin D deficiency. Thus, the aim of this study was to fill gaps in the understanding of potentially important predictors of vitamin D status in healthy premenopausal women living in Jeddah, the second largest city in Saudi Arabia. Specifically, the study sought to assess the vitamin D status of premenopausal Saudi women living in Jeddah and to identify and comprehensively evaluate potential predictors that are strongly associated with vitamin D deficiency, including socioeconomic factors, sunlight exposure, skin color, anthropometric measurements, diet, and lifestyle-related behaviors.

SUBJECTS AND METHODS

1. Study Location

This study was conducted in Jeddah city, which is located in the western region of the kingdom of Saudi Arabia (latitude 21.7˚N) (130). The population of Jeddah is approximately 3.4 million. Jeddah is the second largest city in Saudi Arabia, and represents almost 14% of the total population, estimated at 25.37 million in 2009 (131). This region has a temperate climate, with a mean temperature of 27° - 38° C in summer and a mean temperature of 19° - 29° C in winter (132). Sunlight is abundant throughout the year in Jeddah city; the average duration of sunlight is more than 10 hours per day (133).

2. Study Design and Participants

A cross-sectional study of premenopausal Saudi women (aged 20 to ≤ 50) was conducted at King Abdul-Aziz Medical City, in Jeddah, Saudi Arabia. Two hundred fifty-seven participants were selected from attendees of the primary care clinic at King Abdul-Aziz Medical City using systematic sampling. The sampling frame was derived from daily listing sheets, which include walk-in females who were seeking general outpatient services. A systematic sampling method was applied to the list of eligible women who were seen in the primary care clinic during the study period between December 2014 and April 2015. To implement the systematic sampling, a random number between 1 and 3 was generated each day. That woman was chosen, and then every third woman on the list was selected for potential inclusion in the sample. The exact process was repeated daily until the targeted sample size (n = 257) was reached. Through the abovementioned systematic sampling method, the nurse in charge of the clinic called every selected woman from the female waiting room, and that woman was directed to a private exam room. Subsequently, the woman was fully briefed about the study objectives and methods by a member of the research team, and the woman was invited to participate in the study. Each woman who agreed to participate was initially questioned with regards to her age- and health-related medical conditions to ensure that she met the inclusion criteria, and eligible women were asked to proceed directly to the same room upon the completion of the medical examination that was the main reason for her visit. In the next step, data collection sessions were conducted under the supervision of the research teams, who interviewed women and reviewed their medical record in case more information was needed, such as vitamin D calcium supplement dosage and medication. Data collection sessions usually lasted for 25–30 minutes.

Women were eligible for the study if they were citizens of Saudi aged 20 to 50 years. Women were excluded from the study if they were pregnant or lactating or post-menopausal (defined as amenorrhea for at least six months). Women with cancer, diabetes, hyperthyroidism or hypothyroidism, metabolic bone disease, malabsorption, renal diseases, or those taking medications known to effect bone metabolism were also excluded from the study. The use of vitamin D supplements was not exclusionary criteria because we were interested in evaluating how many participants take vitamin D supplements as well as the effects of supplementation on serum 25(OH) D levels.

3. Data Collection

Eligible women were invited for face-to-face interviews, during which written informed consent was obtained. Weight, height, and waist circumference were measured. Data were collected using a pre-test questionnaire on (1) demographic and lifestyle; (2) dietary intake of vitamin D; and (3) sun exposure behaviors and skin color. A pilot study was performed prior to beginning data collection in 30 volunteer women falling into the same age groups, in a similar setting, to screen for potential problems with the questionnaire. Each interview was conducted in a private room with only the interviewer and participant present. Then, blood samples were drawn by qualified phlebotomists for serum calcium, phosphate, alkaline phosphates, serum 25-hydroxyvitamin D3 25(OH) D, and parathyroid hormone (PTH)

3.1 Anthropometric Measurements

The anthropometric measurements were performed by trained staff according to standardized procedures (136). Weight and height were measured using a SECA 703 High-Capacity Digital Column Scale (SECA, Hanover, MD, USA) and reported to the nearest 0.1 kg and 0.1 cm, respectively. Body Mass Index (BMI) was calculated by dividing the weight (in kilograms) by the square of the height (in meters). Standard BMI cut-points according to World Health Organization criteria (137) were used to categorize the weight status: underweight (> 18.5 kg/m^2, normal (18.5 - 24.9 kg/$m^{2)}$, overweight (25-29.9 kg/m^2), and obese (≥ 30 kg/m^2). Waist circumference (in centimeters) was measured with flexible metal tape (range 0 - 150 cm) placed at mid-point between bottom of the rib cage and above the top of iliac crest. According to the International Diabetes Federation (IDF) abdominal obesity cut-points for people in the Middle East (≥ 80 cm in women), waist circumference was dichotomized into normal and abdominal obesity [normal (< 80 cm); obese (≥ 80 cm)] (138).

3.2 Demographic Variables

The following variables were considered as potential predictors of serum 25(OH)D level based on previous evidence of their association with serum 25(OH)D level: Sociodemographic variables included age (years), marital status, e.g., married (living with partner) or unmarried (single, divorced/widowed); Occupation status (employed, unemployed, student, housewife); Monthly household income was divided in categories [depending on the amount of Saudi Arabian Riyal (1SAR = 0.266USD)]: (< 4,000; 4,000 to 7,999; 8,000 to 14,999; 15,000 to 24,999; ≥ 25,000); residence (rented apartment , owned apartment , rented house , owned house , others). Participants were asked about the number of years of formal education, and then level of education was categorized as follows: (less than college, college graduate, and post graduate). Participants were asked about the number of pregnancies and number of living children. Breastfeeding variables included number of breastfed children and use of vitamin D supplementation during breastfeeding (yes, no).

3.3 Lifestyle Variables

Life style factors included physical activity (PA) (inactive, moderately active, active), and smoking status (current, past, never smoked), type of smoking (cigarette, shisha, cigarette and shisha), number of cigarettes or shisha smoked per day. Physical activity (PA) was assessed using the Short Version of the International Physical Activity Questionnaire (142), in which participants are asked to report the number of days and the duration of the walk, moderate-intensity, and vigorous activities they undertook during the past week. Total PA was expressed as the metabolic equivalent (MET) min/week, by weighing the reported min/week, in each of three activity categories, by the metabolic equivalent specific to each activity. Active individuals were those who performed a) vigorous activity on at least 3 days/week and a total physical activity at least 1,500 MET-minutes/week or b) any combination of activities and total physical activity at least 3,000 MET-minutes/week. Moderately active individuals were those who practiced a) 3 or more days of vigorous activities at least 20 min/day or b) 5 or more days of moderate activities or walking at least 30 min/day or c) 5 days of any combination of activities and whose total activity levels were lower than highly active individuals. Inactive individuals were those who did not practice or whose activities were not enough to meet the active or moderately active individual's categories. A reliability and validity study of IPAQ was conducted in 12 countries, and demonstrated reasonable test-retest reliability (intra-class correlations range 0.7–0.8) and inter-method validity (median rho = 0.67) (143).

3.4 Sun Exposure

The sun exposure recall questionnaire included details of the time of the day of the sunlight exposure; length of exposure to sunlight during the previous month on week days and weekends. Cover status when they were outside in the public area was used to determine the area exposed to direct sunlight. Women were classified as either 1) covering the whole body including the face, 2) face covered but hands exposed, 3) face and hands exposed. From the amount clothing worn outside, the percentage of body surface area (BSA) exposed to sunlight was determined using an adjusted "rule of nines" (9% for face, 1% for each hand, 9% for each arm, and 18%

for each leg) used in clinical practice to estimate the burnt area of skin (150). A Sun Exposure Index (SEI) was calculated as the number of hours per week spent in direct sun without sunscreen protection multiplied by the fraction of BSA exposed sunlight to determine the amount of sun exposure for each participant (152). In addition, women were asked the average length time per day they spent in their courtyard. In Saudi culture this is the place where the sunlight exposure occurs because in all other outdoor activities women generally cover their bodies by wearing black *abayas* (153). The definition of *abaya* is a clothing style not only limited to Islamic dressing habits but also to Saudi traditional clothing, which covers the whole body completely from head to toe (39).

3.5 Skin Color

Skin color was assessed by the investigator using the Fitzpatrick skin-type scale (154). The system is a numerical classification scheme for determining six different skin types based on a questionnaire related to an individual’s genetic constitution, reactions and vulnerability to sunlight or UVB radiation, and tanning habits (154, 155). The response to each question was measured on a scale of 0-4. The response for all questions were added together to get the total score corresponding to the Fitzpatrick skin-type scale, which ranges from very fair (skin type 1) to very dark (skin type IV). For the analysis, the skin types were grouped into the following: light skinned (skin type I to III), moderately skinned (skin type IV & V), dark skinned (skin type VI). The Fitzpatrick skin color scale has been previously used in Arabian Gulf studies including, United Arab Emirates (158) and Kuwait (38).

3.6 Dietary Calcium and Vitamin D Intake

Dietary intake of vitamin D per day was estimated using the Semi-quantitative Food-Frequency Questionnaire (SFFQ). The SFFQ used was a modified version of a previously validated questionnaire (144), which was also validated in United Arab Emirates and showed positive correlation of daily vitamin D ($r = 0.82$, $p < 0.001$) and calcium intake ($r = 0.74$, $p < 0.001$) derived from SFFQ compared with three-day diet food record (145). The modified version was designed to take into account the food composition for the Saudi population (146), Arabian Gulf diet (147) and the Middle Eastern diet (148) that are considered to be excellent sources of calcium. Vitamin D values of some food items were obtained from the Arabian Gulf Food Composition Table such as meat, and local fish consumed in this area. However, because vitamin D values for non-local foods such as tuna and salmon are not available in the Arabian Gulf Food Composition Table, the U.S. Department of Agriculture reference data (149) was used. Vitamin D values of supplements and local food items such as vitamin D fortified milk and Laban drink (buttermilk) were obtained from food labels. The items in SFFQ include natural food sources of vitamin D (e.g., tuna, salmon, meat, poultry, liver, and eggs), local, and imported vitamin D fortified food and beverages available in the Saudi marketplace. Supplemental vitamin D and/or calcium and multivitamins were assessed by the SFFQ.

The selection of food items in SFFQ was obtained and reviewed with the investigator using visual estimates (photographs) to help subjects estimate average portion size. The photographs using common items such as household items (plates or bowels) or hands were used to help estimate average portion size for subjects who ate from the communal dish**.** On the SFFQ, respondents were asked to record how often they consumed a single serving of each food listed during the previous month, with possible responses ranging from less than once per month to 2 or more per day. Vitamin D intake and calcium were computed by multiplying the intake frequency by the nutrient content of the portion size of specific items. Intake of vitamin D and calcium were calculated by summing nutrient intake from diet and supplemental sources. Vitamin D and calcium intakes were examined in terms of absolute and energy-adjusted nutrients (intake per 1,000 kcal). Energy-adjusted values, termed nutrients densities, were computed as daily intake from diet and supplements, divided by calories per day, and multiplied by 1,000 (150,151).

3.7 Biochemical Analysis

Non-fasting venous blood samples were drawn by qualified phlebotomists for serum 25-hydroxyvitamin D 25(OH) D, parathyroid hormone (PTH), adjusted serum calcium (adCa), phosphate (P04), and alkaline phosphatase (ALP). Serum calcium was measured by Arsenazo III dye (Architect 16000, Abbott, USA). Parathyroid hormone (PTH) was measured by a two-step sandwich immunoassay for the quantitative determination of intact PTH in human serum using CMIA technology (Architect 2000, Abbott Diagnostics, Germany). The PTH assay showed a detection sensitivity of ≤ 3.0 pg/mL, intra-assay coefficient of variation (CV) was 12.9-6.1%, inter-assay (CV) was 3.0-6.4%. The reference range in the study laboratory is 24-114 pg/mL.

Quantitative 25(OH) D in serum was determined by using one step delayed chemiluminescent microparticle immunoassay(CMIA) (Architect, Abbott, Germany).This essay was used to recognize 25(OH) D_2(ergocalciferol) and 25(OH) D_3 (cholecalciferol) with equal affinity. Quality control samples were run together with each batch of serum samples. The laboratory undertaking the testing is a participant in the proficiency Accuracy Based vitamin D (PABVD) since 2012. The Accuracy Based vitamin D is part of the proficiency testing (PT) kits for the College of American Pathologists (CAP), the vitamin D external quality assurance scheme of vitamin D assays (161). Intra-assay 25(OH) D coefficient of variation (CV) from daily quality control was 1.4- 3.7% and inter-assay (CV) was 2.6 – 4.6%.The fully automated Architect assay is designed to have an imprecision of ≤ 10 %.

Total volume required was 6 mL using two plain vacutainer tubes (BD vacutainer systems, Plymouth, UK). The serum samples were taken in the laboratory in the primary care clinic; after blood collections, samples were left for 10 min for clotting then immediately centrifuged at 3000rpm for 10 min. Then, samples were aliquoted and frozen at -20° C until further analysis. Samples were run simultaneously in a batch. All analyses were performed in the Pathology laboratory at King Abdul-Aziz Medical City.

Baseline serum 25(OH) D levels were classified into three categories using cutoff points reported in the scientific literature (11, 162) and according to the Endocrine Society (15). Vitamin D deficiency serum 25(OH) D < 50.0 nmol/L (20ng/mL); insufficiency serum 25(OH) D 50 - < 75 nmol/L (20-30 ng/mL); sufficiency serum 25(OH) D ≥ 75 nmol/L (30ng/mL). For some analyses, the following cut off value was also used to diagnose vitamin D sufficiency: ≥ 50 nmol/L according to World Health Organization and the Institute of Medicine (2, 163).

4. Ethics Statement

The research protocol and the consent documents were approved by the ethics committee of the Institutional Review Board (IRB) at the University of Maryland, College Park and Institutional Review Board (IRB) at King Abdullah International Medical Research Center (KAIMRC), Jeddah, Saudi Arabia. Participants were informed about the aim of the study, of the clinical tests, and of the type of interview. The informed consent process took place in a setting that afforded a sufficient level of privacy for the participants.

5. Sample Size and Power Analyses

An a-priori power analysis was conducted to determine the number of participants required to detect a small effect ($f^2 = 0.10$) with power = .80, given the following testing parameters: a multiple forward stepwise regression with 16 predictors and conducted at $\alpha = .05$. A sample that provides adequate power for this purpose would also be large enough to provide adequate power for a bivariate correlation or t-test, or a test of a single regression coefficient of a multiple regression. The analysis indicated that a sample size of 207 would detect a small effect given these parameters. The power analysis was conducted with G*Power 3.1 software package (166). To account for any potential loss of participants, an additional recruit of 20% was considered, and a total sample size was 250 women.

6. Statistical Analysis

Data were checked for missing values and outliers, and analyzed using the Statistical Package for Social Science (SPSS) software version 22.0 (SPSS, Inc., Chicago, IL, USA). The characteristics of the study population were described through frequencies and percentages for categorical variables. Non-parametric data were presented as median and range, and data with normal distribution were presented as mean $\pm$ standard deviations (SD). Median values between groups were compared using the Mann-Whitney *U* test when only two groups were considered, and the Kruskal-Wallies test when more than two groups were studied. Spearman rank correlation was used to assess the strength of the relationship of 25(OH)D concentrations with predictors that were thought to influence 25(OH)D status such as PTH, age, vitamin D intake, calcium intake and BMI. Statistical significance was considered at p-value <0.05.

Variables found to be statistically significant associated with serum 25(OH)D concentrations were then included in a forward stepwise multiple linear regression analysis in order to investigate the relationship between possible predictors and vitamin D status. The condition for entry at each iteration was $\alpha = .05$, and the condition for predictor removal at each iteration was $\alpha = .10$. The forward selection starts with no predictors in the model. The strongest significant predictor at $\alpha = .05$ was entered in the model's first iteration. Next, the strongest significant predictor at $\alpha = .05$ given model 1 was entered. At each iteration a predictor that was significant in a previous model can be removed if it became non-significant at $\alpha = .10$. In these analyses the dependent variable serum 25(OH)D concentrations was log transformed since it was not normally distributed. The following independent variables were considered for entry in the stepwise regression: Age, years of formal education, number of pregnancies, number of children, vitamin D intake (diet and supplement), calcium intake (from diet and supplement), sunlight exposure index, BMI, waist circumference, household income, and skin color. Note that household income and skin color were categorical variables, and the remaining variables were continuous variables. Additionally, in order to keep the sample size as balanced as possible for each level of the categorical variables, some regrouping was implemented. The new categories for each categorical variable are as follows: Household income level: a 2-level categorical variable (less than 15000 vs. 15000 and above); Skin color: a 2-level categorical variable (skin type II/III vs. skin type IV/V). Although sunlight exposure index, skin color type, and waist circumference were not statistically associated with serum 25(OH)D concentrations in the univariate analysis, we still have considered them because they are known determinants of 25(OH) D concentrations (19, 51, 173).

The assumptions for regression model was evaluated using normal probability plots of the standardized residuals and scatter plots with standardized residuals *v.* standardized predicted values. The assumption of the regression model was satisfied. Variance inflation factors (VIF) and tolerance levels were applied on the final model to ensure model multicollinearity was not impacting the model.

RESULTS

A total of 270 premenopausal Saudi women were approached to participate in this study during their visit to the primary health care clinic at King Abdul-Aziz

Medical City in Jeddah between December 2014 and April 2015. Of these, 8 (3%) women did not meet the inclusion criteria because of varying health reasons, and 5 (2%) women did not complete the questionnaire because they didn't have time. Therefore, 257 (95%) women contributed to the anthropometric data and survey data, and were included in this study. Of these, 250 women completed bloodwork in regards to their serum 25(OH) D levels.

Demographic and Life Style Characteristics

Table 1 presents demographic and lifestyle characteristics. A total of 257 premenopausal Saudi women aged 20-50 years from Jeddah city were enrolled in this study in the winter of 2014. The population was mainly young (59.5%) aged 20-30 years and educated 63.4% (had university or college education and 7.8% had post graduate education). Over half of the participants (51.8%) were employed, while 24.9% of participants were students. Nearly half of participants (49.9%) were in the low middle and middle socio-economic status, while (34.6%) of participants were in the upper middle and high socio-economic status, and over half of the participants were living in owned homes (55.7%). Nearly half of the participants (47.9%) were single (never married), 8.6% were divorced or separated, and 43.6% were married. From those who identified themselves as married, divorced, or separated, approximately 40% had children, and a high proportion had two or more children (32%).

Most of the participants (63.4%) reported "face covered but hands exposed" when they were exposed to sunlight and approximately one third of the study participants (27.6%) used sun-protection. More than half of the participants (51.4 %) reported having courtyards in their homes; among those only a few participants (11.7%) spent time outside in their courtyard in the sun. The majority of participants had skin type III (41.2%) and IV (51.4%) **Figure1.** Most participants (59.1%) reported being moderately active. Only 47 (18.3%) participants reported current smoking; of these 47 women, (10.9%) smoked hookah, while (4.7%) were cigarette smokers and (2.7%) smoked in combination (hookah and cigarettes).

Anthropometric, Dietary and Clinical Characteristics

Anthropometric, dietary, and clinical characteristics are shown in **Table 2**. Among the 257 participants, the mean age was 29.8 $\pm$ 7.4 years. The mean BMI was 26.9 $\pm$ 6.7 kg/m^2 (Table 2), with 41.6% of participants being normal weight ($\geq$ 18.5-24.9), 27.2% being overweight ($\geq$ 25-29.9 kg/m^2) and 27.6% being obese ($\geq$ 30 kg/m^2). The median vitamin D intake from diet and supplements was 236.4 IU (234.6 IU/1000 kcal/day). More than half of the women (65%) fell below the EAR of vitamin D 400 IU/day based on their vitamin D intake from food and supplements. The median calcium intake from diet and supplements was 702.7 mg (804.9 mg/1000 kcal/day). Approximately 61% of the women did not meet their EAR for calcium (800 mg/day). Thirty-seven percent of the women reported using vitamin D supplements, and about 15% reported using calcium supplements.

Comparisons of the anthropometric, dietary and clinical characteristics of participants with 25(OH) D concentrations of lower than 50 nmol/L versus 50 nmol/L or greater are also found in **Table 2**. Participants with low vitamin D concentrations were younger (median 28 years old versus median 31, $p = 0.033$), less likely to consume vitamin D from diet and supplements (median 205.7 IU/1000 kcal/day versus 1352.6 IU/1000 kcal/day, $p < 0.001$), and less likely to consume calcium from diet and supplements (median 789.1 mg/1000 kcal/day versus 895.8 mg/1000 kcal/day, $p = 0.01$). Median BMI was in the overweight range for both groups. Approximately 53% of the women with low serum 25 (OH) D concentrations were overweight or obese compared with 57% of the women with normal serum 25(OH) D concentrations. Among the women with low serum 25(OH) D concentrations, about 83% of the women with normal body weight were below the EAR 400 IU/day for vitamin D compared to 75% of the obese women. The majority of the women with normal serum 25 (OH) D concentrations (86%) met their EAR for vitamin D; only 14% were below the EAR [normal (17%); overweight & obese (13%)]. Median serum calcium (Ca) and phosphate (P04) concentrations were normal and similar between the two groups. There were no significant differences in the median serum alkaline phosphatase (ALP) and parathyroid hormone (PTH) between the two serum 25(OH)D concentration groups. However, we found three women with vitamin D deficiency that had serum ALP levels above the upper normal of the laboratory reference range (39-114). Overall, 14% (26/187) of the participants had serum intact PTH concentrations above the laboratory reference range (24-114 pg/mL), and 11% (22/194) were in the vitamin D deficient group.

Vitamin D Status among Study Population

The distribution of serum 25 (OH) D concentrations among study participants is shown in **Figure2.** Serum 25(OH) D concentrations had a skewed distribution ranged from 7.70 to 180.40 nmol/L (median 34. 2 nmol/L) **(Table 2).** Among the 250 women who had a blood sample, the median concentration of 25(OH) D was 34.2nmol/L. Among those with deficient 25(OH) D levels, the median concentration was 30.0 nmol /L. In contrast, the median concentration was 65.9 nmol /L for those with sufficient levels **(Table 2).** There was a high proportion of participants (77.6%) with vitamin D deficiency (25(OH) D < 50 nmol/L) **(Table 3).** Among those with the deficiency, 52 women were severely deficient (25(OH) D < 25 nmol/L) (52/250, 20.2% of the entire sample). Age differences were observed in 25(OH) D concentrations. The women in their 20s showed the lowest 25(OH) D concentrations and the highest prevalence of vitamin D deficiency: 62.9% of participants aged 30 years and less had vitamin D deficiency (25(OH) D < 50), while just 10.8 % of the participants aged more than 40 years had vitamin D deficiency **(Table3).**

Serum 25(OH) D and PTH Concentrations

Serum PTH concentrations were available for 187 women and are reported as medians due to non-normal distribution. The median serum intact PTH concentration was 76.8 pg/mL (range: 31-298 pg/mL). Regarding vitamin D status, significant inverse relationships were found between serum 25(OH) D and intact PTH concentrations, that is, lower 25(OH) D concentrations were associated with higher levels of PTH (r^2 = - 0.26, p < 0.001). The inverse association between serum 25(OH) D and intact PTH was more evident in women with serum vitamin D < 50 nmol/L (p = 0.002) than women with serum ≥ 50 nmol/L (p = 0.21). As shown in **Figure 3,** when stratified by serum 25(OH) D thresholds, median serum PTH levels decreased from 91 pg/mL (range: 31-281) for 25(OH) D < 25 nmol/L to 69 pg/mL (range: 45-91) for 25(OH) D ≥ 75 nmol/L (p for Kruskal-Wallis test = 0.016). Elevated PTH was detected in 15% (22/148) and 13 % (4/31) of the women with deficiency (25(OH) D < 50 nmol/L) and insufficiency (25(OH) D 50 - > 75 nmol/L), respectively, while no women with vitamin D sufficiency (25(OH) D ≥ 75 nmol/L) had elevated PTH. In addition, PTH was significantly and inversely correlated with serum calcium (r^2 = -0.245, p = 0.001) and serum phosphates (r^2 = -0.14, p = 0.05), while PTH was significantly and directly correlated with serum alkaline phosphates (r^2 = 0.23, p = 0.001).

Circulating 25(OH) D Levels According to Study Variables

Table 4 shows the median serum 25(OH) D levels according to study variables. The results of the Kruskal-Wallis tests showed a significant difference in serum 25(OH) D concentrations among age groups. Follow-up tests were conducted to evaluate pairwise differences among the three age groups. The results of these tests indicated significantly lower serum 25(OH) D concentrations in the young women group 20-30 years with respect to the other two groups, 31-40 and 41-50. Additionally, statistically significant differences between median serum 25(OH) D concentrations of the following were found; married women had higher serum 25(OH) D concentrations than unmarried (single, divorced/widowed) (p= 0.003); women employed had higher serum 25(OH)D concentrations than unemployed women (p = 0.030). Statistically significant differences were also found in the median serum 25(OH) D levels between women with different BMI. Overweight and obese women had significantly higher serum 25(OH) D concentrations compared to non-obese women (p = 0.019). By socioeconomic status, lower 25(OH)D concentrations were observed in participants with a household income <15000 SR per month, median serum 25 (OH)D levels were significantly higher in the higher socioeconomic group (upper middle and high household income) compared to the lower socioeconomic group (low and lower middle household income) ($p = 0.035$). Among dietary factors, dietary vitamin D intake had no influence on vitamin D status. However, vitamin D intake from diet and supplements had a significant influence on the 25(OH)D concentrations. Women with a daily vitamin D intake (diet and supplement) below the median value of the dietary reference intake (400 UL/day) had significantly lower serum 25(OH) concentrations than those with vitamin D intake above this value ($p < 0.001$). Similarly, women who were taking calcium supplements had significantly higher vitamin D levels ($p < 0.001$).

On the other hand, serum 25(OH) D concentrations did not differ significantly between those who were exposed to the sun less than 30 min/day than those who were exposed for a longer period ($p = 0.29$). Similarly, significant differences were not found in serum 25(OH) D concentrations between those who were fully veiled (covered their faces but hands exposed) and those who were partially veiled (exposed their faces and hands) ($p =0.78$). Additionally, there was no significant difference between participants who regularly used sunscreen and participants who did not use sunscreen regularly. Further, no significant difference was found between the median serum 25(OH) D concentrations of women according to their skin color types ($p = 0.19$). Among other variables, the median serum 25(OH) did not differ significantly in relation to educational levels, waist circumference, physical activity hours, and smoking status.

Univariate Analysis for Association with 25 (OH) D Concentrations

Evaluating the correlations between age, total household income, number of pregnancies, number of children, BMI, waist circumference, vitamin D intake (diet and supplement), calcium intake(diet and supplement), sun exposure index, skin color type, sun screen use, physical activity levels and smoking status, we found that 25(OH)D was statistically significant and positively correlated with age (r_2 = 0.33, p < 0.001), household income (r_2 = 0.14, p = 0.035), number of pregnancies (r_2 = 0.19 , p = 0.003), number of children (r_2 = 0.16 , p = 0.012), BMI (r_2 =0.13, p = 0.041), vitamin D intake (diet and supplement) (r_2 = 0.60 , p < 0.001), and calcium intake (diet and supplement) (r_2 = 0.13, p < 0.047), but were not statistically significantly correlated with sun exposure index, skin color type, sunscreen use, physical activity level, and smoking status.

Predictors of Serum 25-hydroxyvitamin D Concentrations

Table 5 shows the final model after the forward stepwise regression, with dependent variable log transformed serum vitamin D concentrations and predictors. Vitamin D intake from diet and supplements and age were positive predictors of serum vitamin D concentrations. There was a statistically significant relationship between log transformed serum vitamin D concentrations and vitamin D intake from diet and supplement (t(247) = 10.757, p < 0.001). The regression coefficient was 0.00031 nmol/L, indicating that for one unit increase of vitamin D intake, the log transformed serum vitamin D concentration would increase by 0.00031nmol/L (i.e., 1.0031 unit of serum vitamin D level). Similarly, there was a statistically significant relationship between log transformed serum vitamin D concentrations and age (t(247) = 3.735, $p < 0.001$). The regression coefficient was 0.012, indicating that for one unit increase of age, the log transformed serum vitamin D concentration would increase by 0.012 nmol/L (i.e., 1.012 unit of serum vitamin D level). The predictive model explained 41% of the variation in the serum 25(OH) D concentration ($R^2 = 0.41$). Dietary calcium intake from diet and supplements, BMI, household income, number of pregnancies, number of children, are statically significant in the bivariate analysis but not in the stepwise regression. There was no statistically significant relationship between log transformed serum vitamin D concentrations and the other independent variables, including years of formal education, sunlight exposure index, sunscreen use, skin color, waist circumference, and total physical activity score.

The residual for the model was checked with most of the data points falling on the 45 degree line, which suggested that the residual was normally distributed. The skewness and kurtosis for the residuals for the model were -0.07and -0.86, respectively. The residual plot for the model suggested that the error variances were homogeneous. The assumptions of the regression model were satisfied and hence we concluded that the fitted model was adequate.

DISCUSSION

Low Vitamin D Status among Study Population

The results of the present cross-sectional study suggest that vitamin D deficiency is highly prevalent among Saudi premenopausal women living in Jeddah City. Approximately 77.6% of women had serum 25(OH) D concentrations below those recommended by the World Health Organization and the Institute of Medicine (< 50 nmol/L). Severe vitamin D deficiency (< 25 nmol/L) was found in 20.2% of the entire sample.

The prevalence of vitamin D deficiency (serum 25(OH)D levels < 50 nmol/L) found in the present study is higher than that reported in previous studies in Jeddah; the prevalence of vitamin D deficiency among premenopausal Saudi women was recorded as about 72.4% by Ardawi et al. (19) and a recent study in Riyadh (174) recorded the prevalence of vitamin D deficiency among premenopausal Saudi as 77.6%. A high prevalence of vitamin D deficiency was also detected in a retrospective observational study among 10,709 patients recruited from King Faisal Specialist Hospital and Research Center in Riyadh over a period of 5 years. Saudi women have a higher prevalence of severe vitamin D deficiency (serum 25(OH)D < 25 nmol/L) compared to men; 44.6% of Saudi women were found to have severe vitamin D deficiency as compared to 28.3% of men (175). A high prevalence of vitamin D deficiency among Saudi women was observed throughout the year, during both summer and winter months (85% and 76% respectively) (169). The variation of assay methodologies between studies complicates comparisons. However, our results of increased vitamin D deficiency in young Saudi women are in accordance with recent national studies (169, 175).

Relationship between 25(OH) D and PTH Concentrations

Although the best indicator of vitamin D status is serum 25 (OH) D concentrations, additional information on the functional role of vitamin D in bone health can be obtained by evaluating PTH concentrations (176). In the present study, we found that PTH concentrations were negatively associated with serum 25(OH) D concentrations ($r = -0.26$, $P < 0.001$). This inverse relationship between serum vitamin D and PTH levels was noted in Saudi women [$r = -0.28$] (67), Emirati women [$r = -0.22$] (177), and Iranian women [$r = -0.25$] (178). A similar negative relationship was also observed in international studies (179, 180, 181). Studies have shown that one important factor that increases PTH is a serum 25(OH) D level below 50 nmol/L (179, 182). In this study, an inverse association between serum 25(OH) D and intact PTH was evident in women with serum vitamin D < 50 nmol/L ($P = 0.002$). We found elevated PTH in 15% of the women with vitamin D deficiency (< 50 nmol/L) and 13% of the women with vitamin D insufficiency (50- < 75 nmol/L), while we did not find any cases among women with vitamin D sufficiency (≥ 75 nmol/L).

An analysis of the biochemical data of the women who had elevated PTH levels in comparison with those who had normal PTH levels showed significant differences in median serum calcium and serum phosphate, which were significantly lower in the elevated PTH group than the group with normal PTH ($p = 0.006$, $p = 0.027$, respectively). A significant difference was also found for serum alkaline phosphate, which was significantly higher in the elevated PTH group than in the normal PTH group ($p = 0.007$). Our data are of particular concern given the observed increase in PTH levels with decreasing 25(OH) D levels in young women (median age 32 years), suggesting that these women are at risk for impaired bone mineralization and bone loss in the short and long term (181). PTH is a major hormone that maintains normal serum calcium and phosphate concentrations and is itself regulated by calcitriol (1, 25-dihydroxyvitamin D3) and serum calcium levels.

A low serum 25(OH) D concentration leads to a small decrease of serum 1,25-dihydroxyvitamin D3 (1,25 (OH) D) and calcium absorption. A lower serum calcium concentration causes an increase of PTH secretion, which stimulates the production of 1, 25(OH)D. This resultant secondary hyperparathyroidism causes an increase of bone loss and fracture (167,183).

Factors Associated with Low Vitamin D Status

Our study showed that multiple factors were associated with low vitamin D status in Saudi premenopausal women. The findings of this study clearly indicated a high prevalence of vitamin D deficiency particularly in young women who were less than 30 years old (62.9.8%). Serum 25(OH) D levels were higher in middle-aged women (median = 42.7 nmol/L) compared to younger women (median = 30.5 nmol/L). Studies from other geographic regions in Saudi Arabia have also shown a high prevalence of vitamin D deficiency in young Saudi women (174, 184). Our findings of higher serum 25(OH) D concentrations in middle-aged women is in line with the results of other studies (185,186). These findings conflict with the current belief that serum 25(OH) D concentrations decrease with age due to a decrease in the skin's 7-dehydrocholesterol concentration (10). However, it is unlikely that the 7-dehydrocholesterol concentration had started to decline for the participants in our study as their ages ranged between 20 to 50 years. The finding of higher serum concentrations in middle-aged women compared to young women may be explained by their higher vitamin D intake from food (median 185.2 IU/1000 kcal/day vs.157.4 IU/1000 kcal/day, p= 0.047) and their higher consumption of calcium rich food, such as milk and dairy products (median 860.5 mg /1000 kcal/day vs. 781.6 mg/1000 kcal/day, p = 0.04), which are among the few food sources fortified with vitamin D in Saudi Arabia. These findings are consistent with another study done in Saudi Arabia; Aljohani et al. (2013) conducted a study on 256 premenopausal women (15-55 years old) who visited shopping centers in Riyadh City (174). The researchers found a lower consumption of milk among young Saudi women as compared to their older counterparts, which may explain why vitamin D deficiency is also more common in the younger age group. Moreover, the eating or dieting behaviors of young women may be another factor contributing to their lower serum 25(OH) D levels, due to a low vitamin D and calcium intake from food. This finding is consistent with another cross sectional study conducted on 77 healthy women (19-66 years old) who were working in nursing homes in Japan. The study clearly demonstrated that women younger than 30 years old had significantly lower serum 25(OH) D (30.0 $\pm$ 11.0 nmol/L) compared to women 30 years old and older (50 $\pm$ 14 nmol/L). The researchers suggested that young women's specific eating behavior, such as dieting, may be a contributing factor to their low intake of vitamin D in food (186). In our study population, taking vitamin D supplements was common among the middle-aged Saudi women (30-50 years old) (median 1500 IU/day vs. 800 IU/day, p = 0.035), because middle-aged women are more likely to consult with their primary care physicians regarding their vitamin D levels, and physicians tend to prescribe vitamin D and calcium supplements as protection against osteoporosis. There was no significant difference in the sun exposure time between young and middle-aged women.

In addition to young women, we found that unmarried women are particularly at risk of vitamin D deficiency; this observation is similar to earlier studies (187, 188). In Korea, a study of 5,847 adults who had participated in the Korean National Health and Nutrition Examination Survey of 2008 (KNHANES) reported that unmarried status was inversely correlated with serum 25(OH) D for both genders. The researchers pointed out that the beneficial impact of marriage on serum 25 (OH) D concentrations may be due to better nutritional status and a healthier lifestyle (187). In the present study, the most obvious explanation for our findings is that married women eat more than unmarried women, particularly in regard to vitamin D intake and calcium rich foods (median calories from calcium rich and vitamin D foods, 981.3 kcal/day vs. 776.1 kcal/day , p = 0.009). Married women eat more vitamin D rich foods (median 185 IU/1000 kcal/day vs. 157 IU/1000 kcal/day), particularly fish consumption (25 IU/1000 kcal/day vs. 20 IU/1000 kcal/day, p = 0.05).

Household income also affected the vitamin D levels in our sample; women from higher socio-economic households had higher serum 25(OH) D concentrations compared to those from lower socio-economic households. Lower intakes of vitamin D and calcium were also observed in lower socio-economic households, along with a lower consumption of vitamin D and calcium rich foods. An epidemiologic data review demonstrated that higher quality diets, such as those that include lean meats, fish, and low-fat dairy products, are consumed in higher socio-economic households (189). In our data, we observed that women with a higher socioeconomic status were more likely to consume calcium rich food and supplements (median 862.5 mg/1000 kcal/day vs. 773.9 mg/1000 kcal/day, p = 0.04) and were more likely to be exposed to sunlight (median 12 min/day vs. 4 min/day, p = 0.02); this may explain the positive influence of higher income on serum 25(OH) D concentrations. Koning et al. (2014) examined age- and sex-specific 25(OH) D testing rates over a one year period among 1,436 census dissemination areas within the city of Calgary in Alberta, Canada, and then they compared the testing rates with sociodemographic variables obtained from the census, such as household income and educational levels (190). A positive association was observed between the serum 25(OH) D testing rate and higher household income. One possible explanation that the researchers suggested was that individuals of higher socioeconomic status are more likely to take dietary supplements and more likely to request serum 25(OH) D testing from their physicians.

Employment status also had an impact on vitamin D levels in our sample, where vitamin D deficiency was more common among housewives and students compared to employed women. Our finding could be explained by the fact that employed women were more likely than housewives and students to use dietary supplements, and more likely to expose to sunlight (median 12 min/day vs. 4 min/day, p= 0.001). In contrast, a study of 465 young adult Saudi women (19-40 years) who were selected from the primary health care centers of King Abdul-Aziz Medical City in Riyadh reported that vitamin D deficiency was more common among working women due to less time for sun exposure compared to housewives who had more time for sun exposure. Additionally, working women eat more fast food, which lacks many important vitamins and minerals (184). Although educational levels are associated with both household income and employment status, we did not find any significant association between serum 25(OH) D concentrations and educational levels.

Vitamin D occurs naturally in a limited number of foods such as fatty fish, egg yolks, meat, and liver, as well as food fortified with vitamin D such as milk products, vegetable oils, and cereals. Dietary vitamin D has been reported as a determinant of vitamin D status in women in a few national studies (19, 27) and international studies (179,191). As with our findings, vitamin D intake (diet and supplement) had a significant influence on the serum 25(OH) D concentrations. Women with a daily vitamin D intake below the median value of the dietary reference intake (400 UL/day) had significantly lower serum 25(OH)D concentrations than those with vitamin D intake above this value. Vitamin D and calcium supplements were positively associated with serum 25(OH)D concentrations; the use of dietary supplements resulted in higher serum 25(OH)D concentrations. Studies have shown that an increased calcium intake reduces the circulation of calcitriol (1, 25 hydroxyvitamin D), which subsequently increases serum 25(OH) D concentrations (192). However, vitamin D intake from food sources had no influence on vitamin D levels in the current study. This may be attributable to the fact that most of the women were young and their milk and fish consumption were low, so the role of supplementation could help in preventing and treating vitamin D deficiency in this age group. To our knowledge, the associations of serum 25(OH) D with daily vitamin D intake in food and supplements in premenopausal women have not been previously reported on in Saudi Arabia.

In this study, we collected label information on the dietary vitamin D supplements used by the study participants. Almost 37% (n = 96) of the women took vitamin D supplements during the last 30 days; of these, 52% (n = 50) took a single or multiple high dose of vitamin D in the range of 15,000-50,000 IU. The form of vitamin D used in a high vitamin D dose can be either D2 (ergocalciferol) or D3 (cholecalciferol); 30% (n = 29) of the women used high-dose vitamin D2 while 22% (n = 21) used high-dose vitamin D3. The remaining 48% (n = 46) of the women used vitamin D3 alone or in combination with calcium or other multivitamins that contained 400-4000 IU vitamin D3 per daily dose. Both forms, vitamin D2 and vitamin D3, effectively raise serum 25(OH) D levels. However, a recent meta-analysis (55) indicated that D3 is more effective at raising serum 25(OH) D concentrations than D2 when taken in large single doses; thus, vitamin D3 could be the preferred choice for vitamin D supplementation, however, this effect was lost with small doses (1000-4000 IU) of daily supplements.

Unlike the studies that have shown a strong relationship between high BMI and vitamin D deficiency, the present study found that obesity was associated with higher serum 25(OH) D concentrations. However, we did not find such a significant association after adjustments was made for vitamin D intake form diet and supplement. It has been suggested that obesity may decrease the bioavailability of vitamin D obtained from food sources or cutaneous synthesis due to the sequestration of vitamin D, which is fat soluble, by adipose tissue (25). The sequestering of vitamin D in fat plays a major role in reducing the amount that can be presented to the liver for 25-hydroxylation (2). In addition, it is also possible that the inverse association between obesity and serum vitamin D levels can be due to other lifestyle factors, such as decreased sun exposure from a sedentary indoor lifestyle among obese individuals (193). In the present study, we did not find any significant difference in sun exposure time (minutes/day) between obese women (BMI > 25kg/m^2) and nonobese women (≤ 25kg/m^2). It is plausible that obesity was associated with higher vitamin D levels in our sample due to the increased consumption of vitamin D rich food (median 174 IU/1000 kcal/day vs. 157 IU/1000 kcal/day) and calcium from food and supplements (median 840 mg/1000 kcal/day vs. 782 mg/1000 kcal/day) among overweight and obese women compared to normal weight women. Waist circumference did not have any association with vitamin D levels. These findings are similar to that of another study on Korean adults; Han et al. (2014) conducted a study using data from 1,697 Korean adults, which was obtained from the second and third years (2008-2009) of the fourth Korean National Health and Nutrition Examination Survey (194). The results showed that BMI and waist circumference were not associated with 25(OH) D in the linear and logistic regression analyses, but that body fat was inversely correlated to serum 25(OH) D levels. This inverse relationship between fat and 25(OH) D was greater in men than in women. Data on vitamin D supplement use were obtained from the subjects, and then they were categorized into three groups: none, < 400 IU/day, or ≥ 400 IU/day. Information on the intake of milk and dairy products containing significant amounts of vitamin D was not available in the KNHANES data. This factor may lead to differences in the serum 25(OH) D levels between subjects with high and low fat mass. In the present study, body fat mass was not measured; the direct measurement of body fat mass may be needed to investigate whether there is any significant correlation with vitamin D status in the Saudi population.

Regular exposure to sunlight is considered an effective approach for preventing vitamin D deficiency. However, studies in the Middle East regions with abundant sunlight have shown a high prevalence of vitamin D deficiency, particularly among women (19, 35,169,195-197). These findings are attributed to the traditional protective clothing and avoidance of the sun. No differences were found in the serum 25(OH) D levels between women who were fully veiled (covered their faces but had hands exposed) and women who were partially veiled (exposed their faces and hands). The variations in sunlight exposure between the two groups are minimal due to the small contribution of the exposed body areas (face and hands) that allow skin synthesis of vitamin D; therefore, there does not seem to be any significant difference in vitamin D levels between fully or partially veiled women. Additionally, we did not find any significant relationship between serum 25(OH) D levels and length of sun exposure. The median duration of sunlight exposure in our study was short (9.4 min/day), reflecting the absence of an important source of vitamin D, because most women tended to spend more time indoors than outdoors. Private courtyards are one of the few places in Saudi culture where women can spend time in sunlight without veils. Unfortunately, most women who happen to have courtyards in their houses tend to avoid direct exposure to sunlight due to the hot climate conditions most of the year or for cosmetic reasons, such as minimizing skin discoloration and adverse effects of laser and chemical peel treatment.

A low percentage of our participants—approximately 27%—were using sunscreen products, and our findings indicate that sunscreen usage has no significant association with serum 25(OH) D concentrations. It seems likely that the two common correlating factors of time spend under the sun and sunscreen use that affect vitamin D concentration in most societies have minimal impact in the Saudi society, due to the fact that Saudi women wear traditional protective clothing and often have limited exposure to the sun or avoid sunlight altogether.

Increased skin pigmentation reduces the capacity of the skin to synthesize vitamin D from sunlight (11). Research shows that a fair skin color produces more serum 25(OH)D when exposed to the sunlight (51, 57, 61). In the present study, women with a darker skin color had slightly lower serum 25(OH) D levels compared to those with a lighter skin color. However, these differences did not reach a significant level. This may simply be because the variations in skin color between our participants (most women having skin type III or VI) were minimal, thus explaining the insignificant difference between the two groups. Moreover, in Saudi Arabia the style of clothing for women may reduce vitamin D synthesis in the skin to the extent that skin color type is no longer a factor influencing vitamin D levels within this specific group.

Smoking has been associated with decreased bone mass and an increased risk for hip fractures (128,129). Studies found that current smokers had significantly reduced serum parathyroid hormone (PTH), and that smoking could possibly be attributed to lower levels of serum 25(OH)D and 1,25(OH) D (128). There was no significant association between smoking and vitamin D deficiency in our sample.

However, serum 25(OH)D levels were slightly higher in smokers compared to nonsmoker women. This may because smoking women report consuming more vitamin D from food and supplement (median 375 IU/1000 kcal/day vs. 222 IU/1000 kcal /day, p=0.043) than nonsmoking women. Other lifestyle factors, such as physical activity and time spent sitting during the weekdays, were not significantly associated with vitamin D status. This is inconsistent with some previous studies (27,191) that indicated a sedentary life and lack of physical activity were associated with lower serum 25(OH)D concentrations.

In the univariate analysis we found that higher concentrations of serum 25(OH)D correlated with increasing age, household income, number of pregnancies, number of children, BMI, vitamin D intake (diet and supplement), and calcium intake (diet and supplement). Other factors such as sun exposure index, skin color, sunscreen use, physical activity, and smoking did not correlate strongly with individual serum 25(OH)D concentrations.

Independent Predictors of Vitamin D Status

Multiple linear regression analysis showed that dietary vitamin D intake from diet and supplements and age are independent predictors of vitamin D status in Saudi premenopausal women. Our model explained 41% of the variance in serum 25(OH) D concentrations. Previous studies often offer percentages comparable to the data observed or somewhat lower proportions of variance (174, 179, 180, 198). Aljohani et al (2013) found that age and milk consumption were significant predictors of vitamin D levels in women living in Riyadh City, and explained 33.9% of the variance in serum 25 (OH)D concentrations (174). Gagnon et al (2010) found that BMI, PTH, travel to a warmer climate, and contraceptive pills use were independently and significantly associated with serum 25(OH)D levels in healthy women of reproductive age living in Canada, and explained 40% of the variance in serum 25(OH)D levels (180). Similarly, a study that analyzed determinates of serum 25(OH)D concentrations in healthy adults living in Johannesburg (179) found that factors such as sun exposure, dietary supplement use, and ethnicity explained between 16% to 17% of the variance in total serum 25(OH)D concentrations. In the present study, of the predictive factors identified, dietary vitamin D intake from diet and supplements accounted for the greatest explainable variations in serum 25(OH)D levels, which is similar to a study involving a US population (191). Other factors that may contribute to variance and were not assessed in the current study are the season (199) and genetic factors (200).

The strengths of the present study include the assessment of a large number of variables that are thought to influence vitamin D status; the use of a detailed questionnaire regarding sun exposure and comprehensive information on vitamin D and calcium intake from both food and supplements; and the procurement of detailed information on milk and dairy products consumed as to whether the product was fortified with vitamin D or not (0 - 100 IU/250 mL). However, this study has a number of limitations. First, a causal relationship between predictors and vitamin D levels cannot be established from this cross-sectional study design. Second, the effect of the season on serum 25(OH) D concentrations was not considered in the analysis due to the fact that data collection lasted for a few months, and this could contribute substantially to the variance in serum 25(OH) D concentrations. Third, the ambient temperature effects on serum vitamin D levels observed in this study may not be similar in other regions in Saudi Arabia, such as northern areas or the mountains of the southwestern regions, where temperature ranges are lower and people may spend more time outside in the sun. Fourth, although participants included in the present study were young and healthy, the fact they were recruited from a primary health clinic at the hospital may mean that they do not represent the entire population of Saudi premenopausal women living in Jeddah city.

CONCLUSION

The present study confirms that vitamin D deficiency was highly prevalent among Saudi premenopausal women living in Jeddah city, particularly young women. More importantly, our data show an increased risk of elevated PTH levels with decreasing 25(OH) D levels in young women. The most important independent predictors of vitamin D deficiency were low daily vitamin D intake from diet and supplements and younger age. Low household income, unmarried status, unemployed status, and a low daily intake of calcium-rich food and supplements were also associated with lower serum 25(OH) D concentrations. Understanding the determinants of serum 25(OH)D concentrations may improve targeted education and public health intervention programs in Saudi Arabia. The findings suggest that an early screening of vitamin D deficiency in young women aged 20 to 30 years could be useful in preventing vitamin D deficiency in this age group. Additionally, young women should be informed about the importance of consuming natural and fortified vitamin D-rich food to improve their vitamin D levels. Further study is required to determine the optimal vitamin D intakes for maintaining sufficient vitamin D levels for Saudi premenopausal women.

APPENDICES1

TABLE 1
Sociodemographic and lifestyle characteristics of Saudi premenopausal women (20-50 years) attending the primary health care center at KAMC*, Jeddah (n=257).

Characteristics	Categories	Frequency (%)
Age (years)	20-30	153 (59.5)
	31-40	73 (28.4)
	41-50	31 (12.1)
Education status	Less than college	74 (28.8)
	College graduate	163(63.4)
	More than College	20 (7.8)
Income level (SR per month)	< 4000	15 (5.8)
	4000 - < 8000	50 (19.5)
	8000 - < 15000	78 (30.4)
	15000 - < 25000	53 (20.6)
	≥ 25000	36 (14.0)
Occupation	Employed	133 (51.8)
	Unemployed	124 (48.2)
Type of residence	Rented (apartment /home)	114 (44.4)
	Owned (apartment /home)	143 (55.6)
Marital status	Single (never married)	123 (47.9)
	Married	112 (43.6)
	Separated/ Divorced	22 (8.6)
Parity [a]	No children	32 (12.5)
	One child	20 (7.8)
	2-3 children	48 (18.7)
	4 or more children	34 (13.2)
Area of skin exposed	Face covered but hands exposed	164 (63.8)
	Face and hands exposed	93 (36.2)
Sunscreen use	No	186 (72.4)
	Yes	71 (27.6)
Skin color type [b]	II	6 (2.3)
	III	106 (41.2)
	IV	132 (51.4)
	V	13 (5.1)
Physical activity	Inactive	47 (18.3)
	Moderate	152 (59.1)
	Active	58 (22.6)
Currently smoking [a]	No	210 (81.7)
	Yes	47 (18.3)

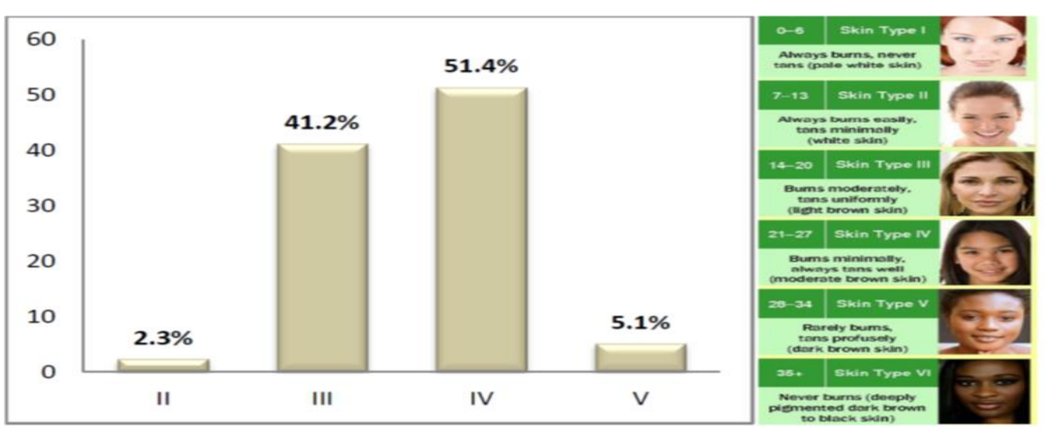
***[a]** Only women who were married/separated/divorced have answered questions regarding number of children(parity) . **[b]** Based on Fitzpatrick skin-type scale classification (Fitzpatrick, 1988), II : fair skin that usually burns and sometimes tan. III: fair to medium skin that moderately burns and always tan gradually. IV: medium skin tone that rarely burns and always tans well. V: olive to dark skin tone that very rarely burns and tans very easily. *Abbreviation; KAMC: King Abdul-Aziz Medical City.*

TABLE 2

Anthropometric, dietary, and clinical characteristics of Saudi premenopausal women attending the primary health care center at KAMC*, Jeddah by serum 25(OH) concentration and in total

Variables	Total (n=257)			Vitamin D < 50 nmol/L (n=194)		Vitamin D ≥ 50 nmol /L (n=56)		
	Mean ± SD	Median	Min-Max	Mean± SD	Median	Mean ± SD	Median	*P-value*
Age(years)	29.8 ± 7.4	28.0	(20.0-50.0)	29.3± 7.3	28.0	31.7 ± 7.9	31.0	0.033*
Weight(kg)	66.7 ± 17.4	63.0	(38.0-160.0)	66.0 ± 17.3	62.6	66.7 ± 17.0	63.0	
Height (cm)	157.2 ± 6.2	157.0	(140.0-174.0)	157.2 ± 6.4	157.0	157.6 ± 5.8	157.5	
BMI (kg/m^2)	26.9 ± 6.7	25.7	(16.5- 56.0)	26.7 ± 6.5	25.4	26.9 ± 6.6	26.5	
Waist circumference (cm)	78.3 ±11.9	77.0	(58.5 – 125.0)	78.1 ±11.7	77.0	77.9 ± 12.2	76.8	
Vitamin D intake from diet (IU/1,000kcal/day)	181.38± 96.1	170.6	(25.1- 624.1)	183.9± 100.1	172.6	172.9± 80.9	164.5	
Vitamin D intake "diet+ supplement" (IU/day)	643.4 ±873.8	236.4	(16.6- 4161.0)	410.4± 602.4	189.2	1510.8 ± 1137.8	1211.2	< 0.001*
Vitamin D intake "diet+ supplement" (IU/1,000kcal/day)	823.2± 1357.6	234.6	(25.1- 99033.9)	528.2 ± 938.9	205.7	1925.9± 1971.3	1352.6	< 0.001*
Calcium from diet (mg/1,000 kcal/day)	788.7± 245.4	762.0	(249.9- 1529.9)	787.1± 244.3	763.9	788.7 ± 241.5	765.6	
Calcium intake "diet+ supplement" (mg/day)	770.9± 421.9	702.7	(56.4-2500.3)	733.9 ± 402.0	680.5	899.3 ± 484.2	783.9	0.024*
Calcium intake "diet+ supplement" (mg/1,000kcal/day)	861.2± 324.3	804.9	(249.9 - 2095.5)	822.7 ± 287.2	789.1	998.3± 403.9	895.8	0.01*
Serum 25(OH)D (nmol/L)	40.4 ±21.9	34.2	(7.7 - 180.4)	31.4 ± 9.3	30.0	71.5 ± 24.4	65.9	
adCa (nmol/L)	2.4± 0.1	2.4	(2.1- 2.6)	2.4± 0.1	2.4	2.4 ± 0.1	2.3	
SerumP04 (mmol/L)	1.2 ± 0.2	1.2	(76.0- 1.6)	1.2 ± 0.2	1.2	1.2± 0.2	1. 2	
Serum ALP (U/L)	65.1 ±18.6	64.0	(27.0-151.0)	66.1±18.9	65.0	61.0 ± 16.7	58.0	0.06
PTH (pg/mL)	85.6 ±40.8	76.8	(31.0-298.0)	88.2 ± 43.0	77.5	75.4 ± 30.0	70.0	0.07

Only 250 women were completed blood work for serum 25(OH)D levels. SD, standard deviation; BMI, Body mass index; adCa, adjusted serum calcium ; P04, serum phosphate ;PTH, serum parathyroid hormone ; ALP, serum alkaline phosphatase 25(OH) D deficiency was defined according to Institute of Medicine as 25(OH) D < 50 nmol/L, insufficiency as 25(OH) D ≥50 - nmol/L. p-value. * indicates significance at the 0.05 level.



Source : www.arpansa.gov.au/

FIGURE1. Distribution of skin color type (154) among 257 Saudi premenopausal women aged 20 to 50 years.

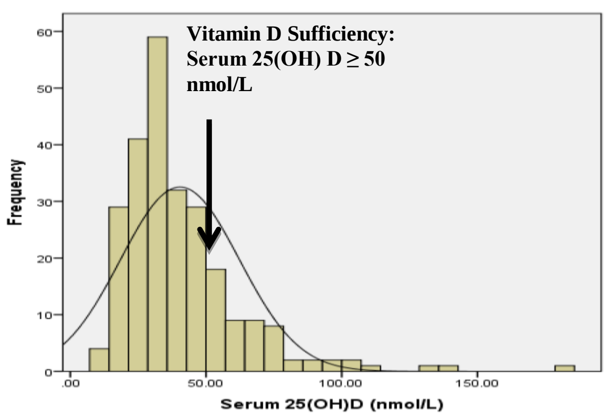


FIGURE 2. Distribution of serum 25-hydroxyvitamin D 25(OH) D among 250 Saudi premenopausal women aged 20 to 50 years

TABLE 3

Distribution of serum 25 hydroxyvitamin D 25(OH) D among 250 Saudi premenopausal women aged 20-50 years in total and according to age group

	Total	20-30	31-40	41-50
25(OH)D nmol/L	**(n = 250)**	**(n = 147)**	**(n = 72)**	**(n= 31)**
Deficiency	**194 (77.6)**	**122(62.9)**	**51(26.3)**	**21(10.8)**
Insufficiency	**43 (17.2)**	**20(46.5)**	**16(37.2)**	**7 (16.3)**
Sufficiency	**13 (5.2)**	**5 (38.5)**	**5 (38.5)**	**3 (23.1)**

25(OH) D dificency was defind as 25(OH) D < 50 nmol/L, insuffecency as 25(OH) D 50 -< 75 nmol/L,and suffeciency as 25(OH) D ≥ 75 nmol/L.

TABLE 4

Circulating 25(OH) D levels according to sociodemographic, anthropometric, dietary , life style factors among Saudi premenopausal women aged 20-50 years attending the primary health care center at KAMC[*] Jeddah (n=250).

Variables Median (range)	Frequency	Serum 25(OH)D nmol/L	*P* value
Age			
20-30	**147**	**30.5 (7.7;103.5)**	**< 0.001***
31-40	**72**	**41.1 (17.6 ;133.5)**	
41-50	**31**	**42.7 (23.0;180.4)**	
Marital status			
Unmarried(single/separated/divorced)	**141**	**33.0 (7.7; 133.5)**	**0.014***
Married	**109**	**37.2 (17.6;180.4)**	
Employment status			
Unemployed	**119**	**32.7 (7.7 ; 180.4)**	**0.030***
Employed	**131**	**37.2 (17.9; 133.5)**	
Income level (SR per month)			
< 15000	**138**	**33.2 (7.7;180.4)**	**0 .035***
≥15000	**88**	**38.1 (10.0; 133.5)**	
BMI Classification			
Nonobese (< 25 kg/m2)	**116**	**31.5 (7.7; 111.2)**	**0.019***
Obese (≥ 25 kg/m2)	**134**	**35.5 (17.6;180.4)**	
Waist circumference			
Normal (< 80 cm)	**147**	**33.2 (7.7; 180.4)**	**0.288**
Abdominal obesity (≥ 80cm)	**103**	**34.8 (17.6;138.7)**	
Daily sun exposure (min/day)			
< 30 min/day	**192**	**34.2 (7.7 ; 180.4)**	**0.289**
≥ 30 min/day	**58**	**34.9 (17.9;138.7)**	
Cover status			
Face covered but hands exposed	**158**	**34.5 (7.7 ; 180.4)**	**0.782**
Face and hands exposed	**91**	**33.2 (10.0 ;133.5)**	
Sunscreen use			
No	**181**	**33.5 (7.7 ;180.4)**	**0.233**
Yes	**69**	**34.8 (15.5;111.2)**	
Skin color type			
II/III	**112**	**36.2 (10.0;138.7)**	**0.197**
IV/V	**138**	**33.1 (7.7; 180.4)**	
Vitamin D intake diet + supplement (IU/day)			
< 400	**160**	**29.5 (7.7; 68.7)**	**< 0.001***
≥ 400	**90**	**51.4 (17.6;180.4)**	
Vitamin D supplement use (IU/day)			
No	**154**	**29.4 (7.70; 68.7)**	**< 0.001***
Yes	**96**	**50.3 (17.6;180.4)**	
Calcium intake D diet +supplement(mg/day)			
<800 DRI	**153**	**33.9 (12.5;111.2)**	**0.211**
≥800 DRI	**97**	**36.4 (7.7; 180.4)**	
Calcium supplement use			
No	**212**	**33.2 (10.0;103.5)**	**< 0.001***
Yes	**38**	**51.6 (7.7 ;180.4)**	

Abbreviation used: 25(OH) D, 25 hydroxyvitamin D; BMI=body mass index; KAMC*: King Abdul-Aziz Medical City.

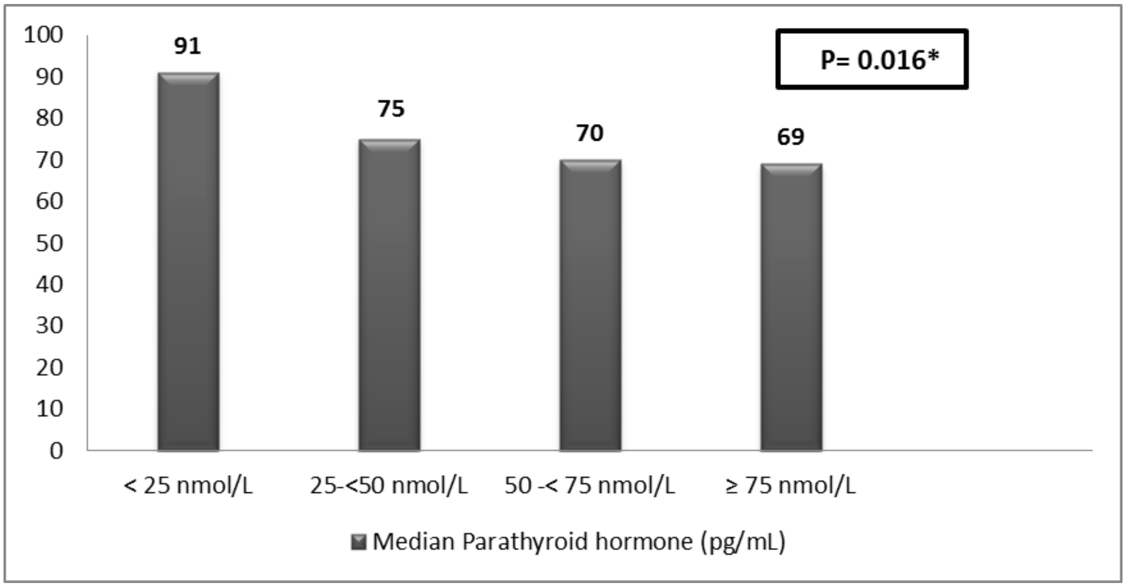


FIGURE3. Median PTH by 25hydroxyvitamin D at thresholds indicated.

TABLE5

Predictors of log-transformed serum 25(OH) D concentrations in forward stepwise regression for Saudi premenopausal women attending the primary health care center at KAMC[*], Jeddah

	Unstandardized Coefficients		Standardized Coefficients			Collinearity Statistics	
	B	**SE**	**Beta**	**t**	**P**	**Tolerance**	**VIF**
Intercept	**3.022**	**0.099**		**30.632**	**0.000***		
Total vitamin D intake	**0.00031**	**0.000029**	**0.567**	**10.757**	**0.000***	**0.959**	**1.043**
Age	**0.012**	**0.003**	**0.197**	**3.735**	**0.000***	**0.959**	**1.043**

Total vitamin D intake; from dietary sources and supplement. Dependent variable = log transformed serum vitamin D level. n = 250. B = Unstandardized regression coefficients. SE = standard error. Beta = Standardized regression coefficients. t = t-statistic. p = p-value. * indicates significance at the 0.05 level. *Abbreviation; KAMC: King Abdul-Aziz Medical City.

PAPER II: Vitamin D Intake among Premenopausal Women Living in Jeddah: Food Sources and Relationship to Demographic Factors and Bone Health

ABSTRACT

Background: Saudi women depend on food sources to maintain their serum 25(OH) D concentrations because covering by traditional clothing and time spent indoors limit their sun exposure. Little is known about vitamin D intake and the main food sources of vitamin D in Saudi Arabia. In addition, the association between bone mineral density (BMD) and vitamin D and calcium intake in premenopausal Saudi women is not well researched.

Objectives: To identify dietary vitamin D sources and to examine potential determinants of vitamin D intake; and to assess bone health and vitamin D and calcium intake in premenopausal women in Jeddah.

Methods: This cross-sectional study was conducted in 257 women aged 20–50 years in Jeddah, Saudi Arabia. Dietary vitamin D and calcium was assessed by semi-quantitative food frequency questionnaire (SFFQ). BMD was measured using double energy X-ray absorptiometry (DXA) in a subset of women (n = 102) at the lumbar spine (L1 to L4) and the neck of the femur.

Results: The median vitamin D intake from diet and supplements was 236.4 IU/day, which is lower than the U.S. Estimated Average Requirement (EAR) of 400 IU/day, and 65% of the participants were below the EAR for vitamin D. Approximately 61% fell below the EAR for calcium. Dairy products, fish, and supplements contributed most to vitamin D intake. Bivariate analysis indicated that being married, multiparty, and of a high household income was positively associated with vitamin D intake.

Multivariate analysis showed that increased age was an independent determinant of sufficient vitamin D intake ($p < 0.001$). The prevalence of osteopenia was 33% and 30% in the lumbar spine and the neck of femur, respectively. There was a significant positive association between calcium intake and BMD at the lumber spine ($p = 0.043$). Serum 25(OH) D, PTH, and vitamin D intake were not significantly different between women with low and normal bone mass.

Conclusion: Premenopausal women in Jeddah have insufficient vitamin D and calcium intakes. Public health strategies to improve nutrition in young women are needed, and expanding fortification programs to include all dairy products would be useful.

INTRODUCTION

Vitamin D is a particularly interesting micronutrient because it can be produced in the body. Vitamins are classically defined as organic chemicals that must be obtained through diet because they are not produced by the body (6). But vitamin D, while universally recognized as one of the four essential fat-soluble vitamins, is obtained through synthesis in the epidermis with exposure to ultraviolet (UVB) sunlight which converts 7-dehydrocholesterol to pre-vitamin D3. It is naturally present in very few foods, mainly vitamin D3 (cholecalciferol) from animal sources including oily fish, egg yolks, veal, beef, and liver (4,7,8). Vitamin D2 (ergocalciferol) comes from plant sources including sun-dried mushrooms (8). Vitamin D, whether obtained from an endogenous or exogenous source, must undergo hepatic hydroxylation to form 25 hydroxyvitamin D 25(OH)D, the chief circulating form, and further renal hydroxylation to convert 25(OH)D to its active form of 1, 25-dihydroxyvitamin D, also known as calcitriol (4, 8-12).

Measuring serum concentrations of 25 hydroxyvitamin D-25(OH) D are considered the best indicator of vitamin D nutritional status, as 25(OH) D levels are directly linked to both vitamin D produced in the skin and acquired from the diet. Several studies have found a strong relationship between serum 25(OH)D and bone status. Therefore, nutritional committees such as the Institute of Medicine, in conjunction with Health Canada, have selected to use serum 25(OH)D to estimate required intake. In 2010, the Institute of Medicine (IOM) established new guidelines for vitamin D. The estimated average requirement (EAR) is 400 IU/day (10 µg) for adults, 19-50 years old. This is in order to achieve 25(OH)D level of 30-40 nmol/L, which meets the requirements of 50% of the healthy population. At the same time, recommended dietary allowance (RDA) is 600 IU/day (15 µg) for adults 19-50 years old in order to achieve 25(OH) D level of 50 nmol/L, which meets the requirements of 97% of the healthy population (2). RDA is an inappropriate value for assessing the nutrient adequacy of groups because it covers the great majority of the nutrient requirements of the population. EAR is used instead of RDA for assessing the nutrient adequacy of the population (109). These dietary reference intake (DRI) values assume minimal exposure to sunlight (2).

Sunlight exposure, rather than diet, has been reported as the main source of vitamin D for the majority of the population (4,7). However, sun exposure has become a less viable source of vitamin D due to widespread use of sunscreen, a more sedentary indoor lifestyle (201), and for certain populations with limited sunlight exposure, such as veiled women (202). Limited exposure to sunlight has consistently been identified as a main contributing factor for low serum 25(OH)D concentrations (< 50 nmol/L) in Saudi Arabia, particularly in women because the traditional clothing covers most of the body. Therefore, dietary intake represents the primary means of attaining vitamin D sufficiency when environmental, cultural, or physiological factors prevent adequate skin exposure to sunlight (7, 26). However, because there are few vitamin D sources in the diet, fortification of staple foods with vitamin D such as milk and margarine have become standard practice worldwide to help the population meet its need.

Fortification measures in Saudi Arabia are limited. Many, but not all, Saudi dairy companies (approximately 11 out of 16) fortify milk and Laban (buttermilk) with vitamin D (400 IU/L). Fortification of other foods, such as cheese, cereal, and vegetable oils, is fairly low and variable in Saudi Arabia. Yet, even a national fortification program may not guarantee adequate vitamin D intake, particularly for at-risk populations with certain dietary patterns, such as low milk consumption (7). A limited number of studies in Saudi Arabia have documented a lack of consumption of dairy products and products supplemented with vitamin D (19,27). No data are available, however, to examine dietary adequacy of vitamin D intake among premenopausal Saudi women. Furthermore, little is known about actual vitamin D intake in Saudi Arabia or the main food sources. No work has been done relating these intake levels to socio-demographic or lifestyle factors, which may help identify sub-populations at risk of inadequate vitamin D intake and potential vitamin D deficiency.

On the other hand, vitamin D and calcium intake are essential nutrients for maximizing peak bone and preventing bone loss, thus decreasing osteoporosis and the risk of fractures over the long term (28, 29), both of which are growing public health and economic issues in Saudi Arabia. The prevalence of low bone mass in Saudi Arabia is higher than in Western countries (67,165). The prevalence of lumbar and femur osteopenia ranges from 7–43.4% and osteoporosis from 2.5–46.7% (30-32). Low bone mass is associated most often with inadequate calcium intake; however, insufficient vitamin D promotes the development of osteoporosis by reducing calcium absorption. Vitamin D deficiency due to limited sun exposure or inadequate amounts of dietary vitamin D decreases the efficiency of intestinal calcium absorption, resulting in increased parathyroid hormone (PTH) concentrations. Secondary hyperparathyroidism due to low levels of 25(OH) D may lead to increased bone loss, resulting in osteopenia, osteoporosis, and an increase in the risk of fractures in adults (2, 3, 15,115, 167). Most Saudi studies investigating the role of nutrition in bone health have utilized only postmenopausal women (30, 33), because these studies focus on osteoporosis prevalence and bone loss rather than bone maintenance. Bone mineral density (BMD) in premenopausal Saudi women has not been studied to a great extent. Young women are ideal candidates for investigating BMD research, as they can optimize their BMD and decrease their risk of fractures through dietary and lifestyle interventions while still young (34).

Thus, the aim of this study is to assess the adequacy of vitamin D intake among premenopausal women in Jeddah as compared to the U.S. Estimated Average Requirement (EAR) of 400 IU/day, to investigate the sources of vitamin D in their diet, and to examine potential determinants of vitamin D intake, including demographic factors. In addition, we evaluated the association between dietary calcium, vitamin D intake, serum vitamin D levels, and serum parathyroid hormone levels with BMD in a subgroup of premenopausal women in Jeddah.

SUBJECTS AND METHODS

1. Study Design and Participants

A cross-sectional study of premenopausal Saudi women (aged 20 to ≤ 50) was conducted at King Abdul-Aziz Medical City in Jeddah, Saudi Arabia. The 257 participants were selected from attendees of the primary care clinic at King Abdul-Aziz Medical City using systematic sampling. The sampling frame was derived from daily listing sheets that included walk-in females seeking general outpatient services. A systematic sampling method was applied to the list of eligible women who were seen in the primary care clinic during the study period between December 2014 and April 2015. To implement the systematic sampling, a random number between one and three was generated each day. The random number determined which first woman was chosen from the list. Starting with first women chosen, then every third woman on the list was selected for potential inclusion in the sample. This process was repeated daily until the targeted sample size (n=257) was reached. Through the above mentioned systematic sampling method, the nurse in charge of the clinic called every selected woman from the female waiting room, and that woman was directed to a private exam room. Subsequently, the woman was fully briefed about the study objectives and methods by a member of the research team, and the woman was invited to participate in the study. Each woman who agreed to participate was initially questioned with regards to her age- and health-related medical conditions to ensure that she met the inclusion criteria, and eligible women were asked to proceed directly to the same room upon the completion of the medical examination that was the main reason for her visit. In the next step, data collection sessions were conducted under the supervision of the research teams, who interviewed women and reviewed their medical record in case more information was needed, such as vitamin D calcium supplement dosage and medication. Data collection sessions usually lasted for 25–30 minutes.

Women were eligible for the study if they were citizens of Saudi aged 20 to 50 years. Women were excluded from the study if they were pregnant or lactating or post-menopausal (defined as amenorrhea for at least six months). Women with cancer, diabetes, hyperthyroidism or hypothyroidism, metabolic bone disease, malabsorption, renal diseases, or those taking medications known to effect bone metabolism were also excluded from the study. The use of vitamin D supplements was not exclusionary criteria because we were interested in evaluating how many participants take vitamin D supplements as well as the effects of supplementation on serum 25(OH) D levels.

To select participants for the DXA scan, each woman, chosen through the systematic sampling method described above, was asked to participate in this portion of the study until completion of the required sample for this subgroup (n=102) was achieved. Upon completion of the first visit, each woman was asked to return for a second visit, during which bone mineral density (BMD) measurements was taken via DXA scan. If a participant declined this request, then selection proceeded to the next sample woman. Sociodemographic data (age, education level, marital status, occupation, and economic status) and anthropometric measures of women who refused to participate in the DXA scan was compared to women who accepted the invitation. These data were used for nonresponse analysis to determine the differences between those who chose to participate in the DXA scan and those who did not.

2. Data Collection

2.1 Anthropometric Measurements

The anthropometric measurements were performed by trained staff according to standardized procedures (136). Weight and height were measured using a SECA 703 High-Capacity Digital Column Scale (SECA, Hanover, MD, USA) and reported to the nearest 0.1 kg and 0.1 cm, respectively. Body Mass Index (BMI) was calculated by dividing the weight (in kilograms) by the square of the height (in meters). Standard BMI cut-points according to World Health Organization criteria (137) were used to categorize the weight status: underweight (> 18.5 kg/m^2, normal (18.5 - 24.9 kg/$m^{2)}$, overweight (25-29.9 kg/m^2), and obese (≥ 30 kg/m^2). Waist circumference (in centimeters) was measured with flexible metal tape (range 0 - 150 cm) placed at mid-point between bottom of the rib cage and above the top of iliac crest. In accordance with the International Diabetes Federation (IDF) guidelines abdominal obesity cut-points for people in the Middle East (≥ 80 cm in women), waist circumference was dichotomized into normal and abdominal obesity [normal (< 80 cm); obese (≥ 80 cm)] (138).

2.2 Demographic Variables

The following variables were considered as potential predictors of serum 25(OH)D level based on previous evidence of their association with serum 25(OH)D level: Sociodemographic variables included age (years), marital status, e.g., married (living with partner) or unmarried (single, divorced/widowed); Occupation status (employed, unemployed, student, housewife); Monthly household income was divided in categories [depending on the amount of Saudi Arabian Riyal (1SAR = 0.266USD)]: (< 4,000; 4,000 to 7,999; 8,000 to 14,999; 15,000 to 24,999; ≥ 25,000) (140,141); residence (rented apartment , owned apartment , rented house , owned house , others). Participants were asked about the number of years of formal education, and then level of education was categorized as follows: (less than college, college graduate, and post graduate). Participants were asked about the number of pregnancies and number of living children. Breastfeeding variables included number of breastfed children and use of vitamin D supplementation during breastfeeding (yes, no).

2.3 Dietary Calcium and Vitamin D Intake

Dietary intake of vitamin D per day was estimated using the Semi-quantitative Food-Frequency Questionnaire (SFFQ). The SFFQ used was a modified version of a previously validated questionnaire (144), which was also validated in United Arab Emirates and showed positive correlation between the SFFQ and 3-day diet food records were ($r = 0.82$, $p < 0.001$) and ($r = 0.74$, $p < 0.001$) for calcium and vitamin D, respectively (145). The modified version was designed to take into account the food composition for the Saudi population (146), Arabian Gulf diet (147) and the Middle Eastern diet (148) that are considered to be excellent sources of calcium. Vitamin D values of some food items were obtained from the Arabian Gulf Food Composition Table such as meat, and local fish consumed in this area. However, because vitamin D values for non-local foods such as tuna and salmon are not available in the Arabian Gulf Food Composition Table, the U.S. Department of Agriculture reference data (149) were used. Vitamin D values of supplements and local food items such as vitamin D fortified milk and Laban drink (buttermilk) were obtained from food labels. The items in SFFQ include natural food sources of vitamin D (e.g., tuna, salmon, meat, poultry, liver, and eggs), local, and imported vitamin D fortified food and beverages available in the Saudi marketplace. Supplemental vitamin D and/or calcium and multivitamins were assessed by the SFFQ.

The selection of food items in SFFQ was obtained and reviewed with the investigator using visual estimates (photographs) to help subjects estimate average portion size. The photographs using common items such as household items (plates or bowels) or hands were used to help estimate average portion size for subjects who ate from the communal dish**.** On the SFFQ, respondents were asked to record how often they consumed a single serving of each food listed during the previous month, with possible responses ranging from less than once per month to 2 or more per day. Vitamin D intake and calcium were computed by multiplying the intake frequency by the nutrient content of the portion size of specific items. Intake of vitamin D and calcium were calculated by summing nutrient intake from diet and supplemental sources. Vitamin D and calcium intakes were examined in terms of absolute and energy-adjusted nutrients (intake per 1,000 kcal). Energy-adjusted values, termed nutrients densities, were computed as daily intake from diet and supplements, divided by calories per day, and multiplied by 1,000 (150,151).

2.4 Lifestyle Variables

Life style factors included physical activity (PA) (inactive, moderately active, active), and smoking status (current, past, never smoked). Physical activity (PA) was assessed using the Short Version of the International Physical Activity Questionnaire (142), in which participants are asked to report the number of days and the duration of the walk, moderate-intensity, and vigorous activities they undertook during the past week. Total PA was expressed as the metabolic equivalent (MET) min/week, by weighing the reported min/week, in each of three activity categories, by the metabolic equivalent specific to each activity. A reliability and validity study of IPAQ was conducted in 12 countries, including Saudi Arabia, and demonstrated reasonable test-retest reliability (intra-class correlations range 0.7–0.8) and inter-method validity (median rho = 0.67) (143).

2.5 Sun Exposure

The sun exposure recall questionnaire included details of the time of the day of the sunlight exposure; length of exposure to sunlight during the previous month on week days and weekends. Cover status when they were outside in the public area was used to determine the area exposed to direct sunlight. Women were classified as either 1) covering the whole body including the face, 2) face covered but hands exposed, 3) face and hands exposed. In addition, women were asked the average length time per day they spent in their courtyard. In Saudi culture this is the place where the sunlight exposure occurs because in all other outdoor activities women generally cover their bodies by wearing black *abayas* (153). The definition of *abaya* is a clothing style not only limited to Islamic dressing habits but also to Saudi traditional clothing, which covers the whole body completely from head to toe (39).

2.6 Biochemical Analysis

Non-fasting venous blood samples were drawn by qualified phlebotomists for serum 25-hydroxyvitamin D 25(OH) D, parathyroid hormone (PTH), adjusted serum calcium (adCa), phosphate (P04), and alkaline phosphatase (ALP). Serum calcium was measured by Arsenazo III dye (Architect 16000, Abbott, USA). Parathyroid hormone (PTH) was measured by a two-step sandwich immunoassay for the quantitative determination of intact PTH in human serum using CMIA technology (Architect 2000, Abbott, Germany). The PTH assay showed a detection sensitivity of ≤ 3.0 pg/mL, intra-assay coefficient of variation (CV) was 12.9-6.1%, inter-assay (CV) was 3.0-6.4%. The reference range for PTH in the study laboratory is (24-114 pg/mL).

Quantitative 25(OH) D in serum was determined by using one step delayed chemiluminescent microparticle immunoassay(CMIA) (Architect, Abbott, Germany).This essay was used to recognize 25(OH) D_2 (ergocalciferol) and 25(OH) D_3 (cholecalciferol) with equal affinity. The laboratory undertaking the testing is a participant in the proficiency Accuracy Based vitamin D (PABVD) since 2012. The Accuracy Based vitamin D is part of the proficiency testing (PT) kits for the College of American Pathologists (CAP), the vitamin D external quality assurance scheme of vitamin D assays (161). Intra-assay 25(OH) D coefficient of variation (CV) from daily quality control was 1.4- 3.7% and inter-assay (CV) was 2.6 – 4.6%.The fully automated Architect assay is designed to have an imprecision of ≤ 10 %.

Total volume required was 6 mL using two plain vacutainer tubes (BD vacutainer systems, Plymouth, UK). The serum samples were taken in the laboratory in the primary care clinic; after blood collections, samples were left for 10 min for clotting then immediately centrifuged at 3000rpm for 10 min. Then, samples were aliquoted and frozen at -20° C until further analysis. Samples were run simultaneously in a batch. All analyses were performed in the Pathology laboratory at King Abdul-Aziz Medical City.

Baseline serum 25(OH) D levels were classified into three categories using cutoff points reported in the scientific literature (11, 162) and according to the Endocrine Society (15). Vitamin D deficiency serum 25(OH) D < 50.0 nmol/L (20ng/mL); insufficiency serum 25(OH) D 50 - $<$ 75 nmol/L (20-30 ng/mL); sufficiency serum 25(OH) D ≥ 75 nmol/L (30ng/mL). For some analyses, the following cut off value was also used to diagnose vitamin D sufficiency: ≥ 50 nmol/L according to World Health Organization and the Institute of Medicine (2,163).

2.7 Measurement of Bone Mineral Density (BMD)

Bone mineral density (BMD) was measured using double energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE medical systems, Madison, WI 53718, USA) in a subgroup of 102 women on the lumbar spine (L1 to L4) and the neck of the femur. The scanning precision (coefficient of variance, CV %) was calculated by two repeated measurements in 30 subjects. The precision (CV %) of the measurements were (1.0 -1.2%) and (1.1 – 2.2%) in the lumbar spine and the neck of the femur, respectively. A complete quality control test on DXA equipment was performed each day for BMD and composition calibration using spine phantom as recommended by the manufacturer, to ensure the stability of the system. Additional quality control was also done every six months or when required by the manufacturer's company. DXA scans were performed in the Department of Radiology at King Abdul-Aziz Medical City by licensed Radiological Technologists trained in bone density exams.

Prior to scanning, women were asked to remove all jewelry and wear only hospital gowns during scanning procedures. Women were asked to lie on the DXA scanning bed for the duration of testing (approximately 10-15 minutes). The BMD values at the hip were compared with peak bone mass for Caucasian female normative database from the National Health and Nutrition Examination Survey NHANES III data, as recommended by the International Society for clinical Densitometry (164). In addition, the Saudi Osteoporosis Society (SOS) committee released guidelines in 2015 stating that in the absence of local normative reference, the committee suggests continuing use data from the United States because the (NAHNES III) data are accurate and reliable and it seems to be best option until local data becomes available (165). BMD values were classified according to WHO criteria, a low bone mass (osteopenia) if BMD T-score is < -1.0, or normal bone mass if BMD T-score is ≥ -1.0 (113).

3. Ethics Statement

The research protocol and the consent documents were approved by the ethics committee of the Institutional Review Board (IRB) at the University of Maryland, College Park and Institutional Review Board (IRB) at King Abdullah International Medical Research Center (KAIMRC), Jeddah, Saudi Arabia. Participants were informed about the aim of the study, of the clinical tests, and of the type of interview. The informed consent process took place in a setting that afforded a sufficient level of privacy for the participants.

4. Sample Size and Power Analyses

An a-priori power analysis was conducted to determine the number of participants required to detect a small effect (f2 = 0.10) with power = .80, given the following testing parameters: a multiple forward stepwise regression with 16 predictors and conducted at $\alpha = .05$. A sample that provides adequate power for this purpose would also be large enough to provide adequate power for a bivariate correlation or t-test, or a test of a single regression coefficient of a multiple regression. The analysis indicated that a sample size of 207 would detect a small effect given these parameters. The power analysis was conducted with G*Power 3.1 software package (166). To account for any potential loss of participants, an additional recruit of 20% was considered, and a total sample size was 250 women.

To determine the required number of participants in the DXA subsample, the power analysis was also performed, based on research question (Is there a significant difference in calcium intake, vitamin D intake, and serum vitamin D levels, and serum parathyroid hormone levels between women with normal bone density and women with low bone density in this study population?). Specifically, the sample size needed to detect the effect of a specific predictor ("serum vitamin D level") with a power level of .80, and an alpha level of .05, using a multiple logistic regression with binary response variable, assuming a moderate effect size (odds ratio = 2.50) and the squared multiple correlation coefficient between "serum vitamin D level" and other independent variables in the model = 0.3, was computed. The results of the power analysis indicates that, assuming a medium effect size, a minimum of 76 subjects are required in order to achieve a power of 80% at the 0.05 level of significance.

5. Statistical Analysis

Data were checked for missing values and outliers, and analyzed using the Statistical Package for Social Science (SPSS) software version 22.0 (SPSS, Inc., Chicago, IL, USA). The characteristics of the study population were described through frequencies and percentages for categorical variables. For continuous variables, medians and inter quartile ranges (IQR) were used to summarize non-normal data, and mean ± standard deviations (SD) were used to summarize data with normal distribution (only height and bone variables were normally distributed). The 257 women were divided into two groups: 167 women with insufficient vitamin D intake from food and supplement ISVDI (< 400 IU/day) and 90 women with sufficient vitamin D intake from food and supplement SVDI (≥ 400 IU/day)]. Median anthropometric, dietary and biochemical values between groups were compared using nonparametric Mann-Whitney *U* tests. A nonparametric Wilcoxon signed-rank test was used to determine if vitamin D and calcium intake were statistically significantly different from the U.S. Estimated Average Requirement EARs of 400 IU/day and 800 mg/day, respectively. Percentages values between groups were compared using the Chi square tests. Kruskal-Wallis tests were performed to test for differences in intakes of vitamin D and calcium among age groups. Spearman rank correlations were used to assess the strength of the relationship of bone mineral density at the lumbar spine and the femur neck with variables such as BMI, parity, and smoking, variables that were thought to influence bone mineral density. Statistical significance was considered at p-value <0.05.

The forward stepwise multiple linear regression was conducted in order to investigate the relationship between the dependent variable, vitamin D intake (from food and supplement), and the independent variables, the sociodemographic and parity variables. The condition for entry at each iteration was α = .05, and the condition for predictor removal at each iteration was α= .10. In these analyses the dependent variable vitamin D intake (from food and supplement) was log transformed since it was not normally distributed. The following independent variables were considered for entry in the stepwise regression: age, years of formal education, and number of children, income level, and occupation, type of residence, marital status, and taken vitamin D supplement during breastfeeding period. Note that age, years of formal education, and number of children were continuous variables, and the remaining variables were categorical variables. Additionally, in order to keep the sample size as balanced as possible for each level of the categorical variables, some regrouping was implemented. The new categories for each categorical variable are as follows: Household income level: a two-level categorical variable (less than 15000 versus 15000 and above); Occupation: a two-level categorical variable (employed versus not employed); Type of residence: a two-level categorical variable (owned versus not owned); Marital status: a two-level categorical variable (married) versus unmarried (single/separated/divorced); Taken vitamin D supplement during breastfeeding period: a two-level categorical variable (yes versus no). For single women (never married) who did not answer questions regarding number of children, and vitamin D supplement during breastfeeding, their responses for these questions were assumed to be 0 (number of children) or no (vitamin D supplement during breastfeeding). The assumptions for regression model were evaluated using normal quantile-quantile (QQ) plots of the standardized residuals (the assumption of normality) and scatter plots with standardized residuals versus standardized predicted values (the assumption of homogeneous error variance). The assumptions of the regression model were satisfied.

Multiple logistic regressions were implemented to identify and analyze factors that influence BMD markers. Hence, the two dependent variables were BMD at lumbar spine and BMD at femur neck, both binary response variables (low and normal). The independent variables being considered included: Vitamin D intake, calcium intake, serum 25 (OH) D levels, and serum PTH level. The following variables served as possible confounding variables: BMI and energy intake. It should be noted that in multiple logistic regression, the effect of a specific independent variable on the dependent variable is adjusted for the effects of all the other independent variables in the model. The Wald test was used to determine if an effect was statistically significant. Statistical significance was considered at p-value < 0.05. Hosmer-Lemeshow goodness-of-fit test was used to determine the model adequacy p-value > 0.05 indicates good model fit). In this analysis, 88 subjects were used for logistic regression, after excluding subjects with missing values in the study variables.

RESULTS

Characteristics of the Study Population

Demographic, anthropometric, dietary intake and biochemical characteristics of the 257 premenopausal Saudi women aged 20-50 years, who were divided into two groups— insufficient vitamin D intake (ISVDI) (< 400 IU/day) and sufficient vitamin D intake (SVDI) (≥ 400 IU/day)—are presented in **Table 1**. Participants with ISVDI were younger (median 27 years versus median 31 years, $p < 0.001$), less likely to consume vitamin D from dietary sources (median 132.1 IU/day versus 157.6 IU/day, $p < 0.001$), less likely to consume calcium from diet and supplements (median 665.2 mg/day versus 811.3 mg/day, $p = 0.02$), than participants with SVDI. Approximately 38.3% of the women were overweight or obese (≥ 25 kg/m^2) in SVDI compared to 61.7; 0% in ISVDI, while 31% of women were nonobese (< 25 kg/m^2) in SVDI compared to 69. 0% in ISVDI, but there was no statistically significant association between BMI category and SVDI/ISVDI. A significantly higher percentage (95%) of vitamin D deficiency (serum 25 OH D < 50 nmol/L) was observed in premenopausal women in ISVDI group compared with 47% in SVDI group ($p < 0.001$). Socioeconomic status did not vary significantly between ISVDI and SVDI. In both groups, more women completed college education (ISVDI=61.7%; SVDI= 66.7%) when compared to women who had less than college education (ISVDI=30 %; SVDI= 26.7%), but there was no statistically significant association between education level and SVDI/ISVDI.

Vitamin D and Calcium Intake

The median daily intakes of vitamin D and calcium from all sources (food and supplement) are presented in **Table2**, for the whole sample and according to age groups. Overall, median daily vitamin D intake from diet and supplement were 236.4 IU/day, which is lower than Estimated Average Requirements (EAR); however, this difference was not statically significant (p=0.52) (**Table2).** Approximately 97% of women fell below the EAR of vitamin D 400 IU/day based on their vitamin D intake from food alone (data not shown). When including supplements, more than half of women (65%) were below the EAR for vitamin D (**Table2)**. Thirty seven percent of women reported using vitamin D supplements or multivitamins containing vitamin D in the past 30 days. The proportion of women reporting use of vitamin D supplement ≥ 400 IU/day was 31%**.** Median vitamin D intake from food and supplement was significantly lower in young women 20-30 years compared with women age 31-40 (p =0.029) or 41-50 years ($p < 0.001$). Additionally, a higher proportion of young women 20-30 years ate less than the EAR for vitamin D (20-30= 74%; 31-40=57.5%; 41-50=35.5, $p < 0.001$). For calcium intake, median daily intake from diet and supplement was significantly lower (p=0.005) than the EAR **(Table2).** Approximately 61% of women did not meet their EAR for calcium. About 15% of women reported using calcium supplements in the past 30 days. The daily intake of calcium from food and supplement showed a significant difference among age groups ($p < 0.015$). The median intake of calcium was significantly higher in women aged (41-50) when compared to the other two younger age groups. A higher proportion of young women 20-30 and 31-40 years ate less than the EAR for calcium (20-

30=66.7%; 31-40=58.9%; 41-50=35.5%, p=0.005). The intakes of vitamin D and calcium were found to be strongly correlated (r_2=0.73, $p < 0.001$) in this study population. The sources of vitamin D (% of total vitamin D intake) in 20-50 years old women are presented in **Table3.** Overall, the estimated sources of vitamin D were as follows: 36.5 % dairy products, 31.0% vitamin D supplements, 12.2% fish, 8.4 % meats, 6.9 % eggs, food made with milk 3% and 1.1 % fortified cereals. For women between 20 to 30 years old, the top three vitamin D sources were: dairy products (38.4%), supplement (25.2%), and fish (12.1%); for women between 31 to 40 years old, the top three vitamin D sources were: supplement (37.9%), dairy products (32.7%), and fish (12.5%); for women between 41 to 50 years old, the top three vitamin D sources were: supplement (43.8%), dairy products (35.9%), and fish (7.9%).

Association of Vitamin D Intake with Sociodemographic Factors

Evaluating the correlations between vitamin D intake and sociodemographic factors, we found that vitamin D intake was statistically significant and positively correlated with age (r_2 = 0.27, $p < 0.001$), marital status (r_2 = 0.14, p = 0.03), gravidity (r_2 = 0.18 , p = 0.004), parity (r_2 = 0.14 , p = 0.02), BMI (r_2 =0.13, p = 0.041).

The results of the forward stepwise regression suggest that there was a statistically significant relationship between log transformed vitamin D intake (diet and supplement) and age (t (230) = 3.754, $p < 0.001$) **(Table4).** The regression coefficient was 0.039, indicating that for one unit increase of age, the log transformed vitamin D intake (diet and supplement) would increase by 0.039 units (vitamin D intake would increase by 1.04 (= exp (0.039) units), i.e., there was a positive relationship between age and (log transformed) vitamin D intake. The R^2 = 0.10, indicates that 10% of the variation in the dependent variable can be accounted for by the model. There was no statistically significant relationship between log transformed vitamin D intake and the other independent variables, including income level, years of formal education, occupation, type of residence, marital status, number of children, and taken vitamin D supplement during breastfeeding period. The residual for the model was checked with most of the data points falling on the 45 degree line of the QQ plot, which suggested that the residuals were normally distributed. The skewness and kurtosis for the residuals were 0.23 and -0.42, respectively. The residual plot for the model suggested that the error variance was homogeneous. The assumptions of the regression model were satisfied and hence we conclude that the fitted model was adequate.

Bone Mineral Density (BMD) Measurements

Out of 257 premenopausal women, 102 women agreed to undergo DXA scan. We compared sociodemographic, anthropometric, dietary between those who did DXA scan and who did not using a Mann-Whitney U test. We found significant difference in women’s age: women who got a DXA scan done were older than women who did not get a DXA scan (median 30.5 years versus 27 years; p = < 0.001). Additionally, a high proportion of women who got a DXA scan done had completed college education 71.6% compared to 58% of women who had not done a

DXA scan. We found no significant difference (p > 0.05) among other variables (data not shown).

Anthropometric and dietary factors for the DXA subsample (n=102) are presented in **Table 5.** Of the women in this analysis, half of the women 50% were 20-30 years-old. Almost 30% of women were obese (BMI ≥ 30 kg/m^2). Approximately 60% of women had lower calcium intake than the EAR for calcium (800 mg/day), whereas 54% of women had lower vitamin D intake than the EAR for vitamin D (400IU/day).

Biochemical parameters used to assess bone health of study participants are presented in **Table 5.** All women had normal serum calcium levels (mean 2.3 ± 0.1 mmol/L). The majority of women had normal serum phosphates (1.2 ± 0.02 mmol/L) and normal alkaline phosphatase levels (63.7 ± 20.2 U/L). However, five women had high serum phosphates levels (mean 1.50 ± 0.04 mmol/L, laboratory reference range 0.80-1.44) while two women had high serum alkaline phosphates levels (mean 173.5 ±19.1U/L, laboratory reference range 39-114). Median serum 25 (OH) D levels were low (mean 42.9 ± 22.8, median 40 nmol/L). Of the women in this analysis, there was a high proportion, 77.5% of women, with vitamin D deficiency (25 (OH) D < 50 nmol/L) and, of these, approximately 14% had severe vitamin D deficiency (25 (OH) D < 25 nmol/L). Elevated PTH was seen in 16% (14/88) of the women (PTH mean 160.7 ± 41 pg/mL, laboratory reference range 24-114 pg/mL), among those with elevated PTH 78.6% (11/14) women had vitamin D deficiency (25 (OH) D < 50 nmol/L). BMD results are also presented in **Table 5**. The BMD data were normally distributed at the femoral neck the p-value for Shapiro-Wilk =0.17, whereas p-values at the lumbar spine indicting the data may not be from normal distribution ($p = 0.04$). However, according to QQ plot, points would fall very closely to the 45 degree line. Hence, the conclusion is that the data were normally distributed. The mean BMD at the lumbar spine was 1.1 $\pm$ 0.1 (g/cm^2), while the mean BMD at the neck of the femur was 0.93 $\pm$ 0.1 (g/cm^2). The prevalence of osteopenia was 33 % and 30% in the lumbar spine and the neck of the femur, respectively. The results of the chi-square test of independence suggested that the prevalence of osteopenia between the lumbar spine and the neck of the femur was statistically significantly different ($\chi2(1, N = 102) = 12.26$, $p < 0.001$). Of the 102 women, 55 (54%) women did not suffer from osteopenia in both sites, whereas 18 (17.6%) women had osteopenia in both sites. The remaining 29 women (28.4%) had osteopenia in one of the sites. Approximately 45.5% (5/11) of our participants who experienced vitamin D deficiency and elevated PTH had lower BMD at either site.

Association of Vitamin D Intake, Calcium Intake, Serum 25(OH) D, Serum PTH with Bone Mineral Density (BMD)

In univariate analysis (Spearman correlation), the measures of BMD at the lumbar spine and the femoral neck showed significant positive relationship with anthropometric measurements. There was a significant positive correlation between height and BMD ($r_2 =0.27$, $p = 0.006$ at the lumbar spine; and $r_2 = 0 .34$; $p < 0.001$ at the femoral neck). Similarly, there was a significant positive correlation between BMD at the lumbar spine and at the femoral neck and weight, and BMI, ($r_2 = 0 .44$, $p < 0.001$; and $r_2 = 0 .37$, $p = < 0.001$, respectively). No significant correlation was found between serum 25(OH) D and BMD ($r_2= -0.10$, $p=0.31$ at the lumbar spine; and

r_2= - 0.11, p=0.28 at the femoral neck). No significant association was found between BMD and age, sun exposure, physical activity, parity, and smoking.

Multiple logistic regression for binary responses was used to investigate the relationship between vitamin D and calcium intake, serum vitamin D and serum PTH (independent variables) and the probability that a case is a member of one of the categories of the dependent variable, BMD classification (low and normal) at the lumbar spine or at the neck of femur, after controlling for BMI and energy intake.

Table 6-a shows the results of the logistic regression with dependent variable = BMD at the lumbar spine (Model 1). The Hosmer-Lemeshow goodness-of-fit tests suggest that the fitted model was adequate (p = 0.73). The analysis results suggested that there was a statistically significant relationship between BMD at the lumbar spine and calcium intake ($\chi2$ (1, N = 88) = 4.100, p = 0.043). The odds ratio was 1.002 with 95% confidence = (1.000, 1.005), suggesting that for one unit increase of calcium intake, the odds of having BMD classification at the lumbar spine = “Normal bone mass” would increase by 0.2%.There was a statistically significant relationship between BMD at the lumbar spine and BMI ($\chi2$ (1, N = 88) = 6.06 , p = 0.014). The odds ratio was 1.117 with 95% confidence = (1.023, 1.219), suggesting that for one unit increase of BMI, the odds of having BMD classification at the lumbar spine = “Normal bone mass” would increase by 11.7%. There was a statistically significant relationship between BMD classification at the lumbar spine and energy intake ($\chi2$ (1, N = 88) = 7.362, p = 0.007). The odds ratio was 0.997 with 95% confidence = (0.995, 0.999), suggesting that for one unit increase of energy intake, the odds of having BMD classification at the lumbar spine = “Normal bone mass” would decrease by

0.3%. There was no statistically significant relationship between BMD classification at the lumbar spine and serum 25(OH) D, serum PTH, and vitamin D intake.

Table 6-b shows the results of the logistic regression (Model 2). The Hosmer-Lemeshow goodness-of-fit tests suggest that the fitted model was adequate ($p = 0.96$). The analysis results suggested that there was a statistically significant relationship between BMD classification at the femur neck and BMI ($\chi2$ (1, N = 88) = 8.251, $p = 0.004$). The odds ratio was 1.122 with 95% confidence = (1.037, 1.214), suggesting that for one unit increase of BMI, the odds of having BMD classification at the femur neck spine = "Normal bone mass" would increase by 12.2%. There was no statistically significant relationship between BMD classification at the femur neck and calcium intake, serum 25(OH) D, serum PTH, and vitamin D intake.

DISCUSSION

Dietary Adequacy of Vitamin D and Calcium

The results of the current study showed that this sample of premenopausal women (aged 20–50 years) in Jeddah did not consume sufficient amounts of dietary vitamin D and calcium to meet the estimated average requirement (EAR) for this age group. The low vitamin D intake observed in the present study is consistent with that which has been previously reported in the Middle East. In a study of Bahraini women (n=325, mean age=28 years), Alhadad et al. (2014) reported a mean dietary intake of 187 IU/day vitamin D, which was insufficient to meet bodily requirements (9). Production companies in Bahrain voluntarily fortify dairy products, while milk and dairy products imported from Saudi Arabia are also fortified with 400 IU/L vitamin

D. It appears that the current fortification measures used in Saudi Arabia and Bahrain are not sufficient to meet the EAR for vitamin D intake in women in both countries. However, the overall estimates of this vitamin D intake still appear to be much higher than those previously reported in other Middle Eastern countries, in which there is an absence of vitamin D fortification in milk-derived food products. Gannage-Yared et al. (2005) reported that the mean daily vitamin D intake of Lebanese women (n=214, aged 20–50 years) was 89.0 IU/day from food sources, whereas the vitamin D intake from food sources (excluding supplement use) by the women in the current study was 141 IU/day (203). The absence of vitamin D fortification in milk-derived products, such as Laban (buttermilk) and yogurt, which are highly consumed in Lebanon and Saudi Arabia, may explain these differences.

It has also been shown that daily vitamin D intake is low in Europe, including the United Kingdom, where no fortification of milk is required; the average dietary vitamin D intake reported among young and middle-aged (35–49 years) British women was 148 IU/day (7). In the United Sates, where fortification of staple foods, such as milk and margarine, is mandatory to help the population meet its needs, the average vitamin D intake from food and supplements among young and-middle aged (20–50 years) Caucasian American women was 293.2 IU/day, with 124 IU/day of this amount being contributed by fortified food (7).

Vitamin D Sources and Serum 25 (OH) D Concentrations

In analyzing the various vitamin D sources, we found that women have a high consumption rate of fortified dairy products and vitamin D-containing supplements, which explains the importance of dairy products, particularly Laban and yogurt, as major sources of vitamin D. In addition, vitamin D supplements and canned fish, such as tuna, are considered to be good sources of this vitamin among women in Jeddah. An international comparison of vitamin D intake, and its major sources of published information with regard to Australia, Japan, Norway, Lebanon, Taiwan, the US, and the UK, along with the results of the current study, is summarized in **Table 7** (7,8, 202, 203). Differences in food-fortification policies or food composition between countries may have led to differences in vitamin D intake and in the main contributory food sources (204). Saudi Arabian dietary patterns are comparable to those of the Lebanese population **(Table7);** Saudi and Lebanese individuals have a high consumption rate of dairy products (36.5, and 30.2%, respectively). However, the consumption of canned fish, such as tuna, meat, and organ meat, such as liver, is higher in Lebanese individuals. The observed percentage differences between the studies may be explained by the proportion (37%) of women who took vitamin D supplements in the present study, which contributed 31% of their vitamin D intake; these supplements were an exclusion criterion in the Lebanese study (203). In the current study, almost 37% (n=96) of the women took vitamin D supplements during the last 30 days; of these, 52% (n=50) took single or multiple high doses, ranging from 15,000–50,000 IU. Approximately 30% (n=29) of the women took high-dose vitamin D2 (ergocalciferol), 22% (n=21) took high-dose vitamin D3 (cholecalciferol), while the remaining 48% (n=46) of the women took vitamin D3 alone or in combination with calcium or other multivitamins that contained 400–4000 IU vitamin D3 per daily dose.

Vitamin D intake from food and supplements was associated with improved vitamin D status among women in the present study. Women whose vitamin D intake was below the EAR had a significantly higher percentage (95%) of vitamin D deficiency (serum 25(OH)D < 50 nmol/L) than those whose intake was above the EAR (47%; $p < 0.001$). Some studies have reported that participants' vitamin D intake is highly correlated with their serum 25(OH) D concentrations, and that this relationship may become stronger as the influence of solar ultraviolet B radiation weakens, particularly among people with limited sun exposure (205). The median duration of sunlight exposure for women in the current study was short (9.4 min/day), which reflects the absence of an important source of vitamin D. Our data support the fact that sufficient vitamin D intake from food and supplements is important, and we found a statistically significant and positive association between such intake and serum 25(OH)D concentrations ($r_2 = 0.60$, $p < 0.001$). Furthermore, Saudi women are dependent on food sources, rather than sun exposure, to maintain their serum 25(OH) D concentrations. This is partly because their fully-covering traditional clothing limits exposure to the sun, and partly due to the fact that they tend to spend more time indoors than outdoors because of the hot climatic conditions that prevail for most of the year.

Sufficient vitamin D intake among premenopausal women in Jeddah is unlikely to be achieved through dietary sources alone. Although vitamin D-fortified food may help to maintain vitamin D status in the general population, it may not be effective in correcting vitamin D deficiency in at-risk groups (202). In Saudi Arabia, manufacturing companies fortify dairy products with vitamin D, particularly fresh milk and Laban (400 IU/L), and yogurt (40 IU/100g), and fortification information is declared on product labels. Powdered milk is also fortified with vitamin D, at a content ranging from 65.6 IU/100g–350 IU/100g (110). Fortification of other foods, such as cheese, cereal, and vegetable oils, is fairly low and variable in Saudi Arabia. However, a recent study evaluating the current status of vitamin D fortification in food items, such as dairy products, commonly consumed by the Saudi population found that those products contained a lower amount of vitamin D than the 400 IU/ 250mL that is recommended by guidelines set for the U.S. marketplace (110). It would be useful to expand food fortification programs to include other type of dairy products, particularly cheese and yogurt, in our population with a high level of vitamin D deficiency, as we found that dairy products were the highest contributor to vitamin D intake, and are highly consumed in Saudi Arabia. Vitamin D supplements significantly improved dietary intake in the current study; 86.5% of women who took a vitamin D supplement met the EAR of vitamin D, compared to 4.3% of women who did not take a supplement of this kind ($p<0.001$). In addition, 51% of the women who took a vitamin D supplement achieved a sufficient serum vitamin D level (25(OH)D $\geq$ 50 nmol/L), compared to 4.5% of women who did not take a supplement ($p < 0.001$). Therefore, it is important to consider supplementation as an effective approach in helping to ensure sufficient vitamin D intake and to address vitamin D deficiency among women in Jeddah.

Vitamin D Intake and Sociodemographic Factors

Multiple linear regression analysis showed that only age was an independent predictor of vitamin D intake in premenopausal women in Jeddah. Previous studies on this topic are limited; none have investigated the demographic determinants of vitamin D intake, particularly among non-pregnant women. A recent study conducted in 68,447 pregnant Danish women found a lower level of sufficient vitamin D intake from diet and supplements, suggesting that young women may be less conscious of health matters compared to older women (206). In the present study, we observed a lower level of sufficient vitamin D intake (≥ 400 IU/day) among young women (20–30 years) compared to middle-aged women (31–50 years). These differences may be explained by the higher consumption of vitamin D-rich food in middle-aged women, compared to young women (median 185.2 IU/1000 kcal/day vs. 157.4 IU/1000 kcal/day, p= 0.04), and a higher consumption of vitamin D-containing supplements (47.1% vs. 30.7%, p=0.01). A high prevalence of vitamin D deficiency was also observed in young women, compared to middle-aged women (83% vs. 70%, p=0.02). The results of the present study suggest that young women at greatest risk of vitamin D deficiency were consuming the lowest amount of vitamin D from dietary and supplementary sources, which confirms the results of previous reports. In a study conducted in healthy young Canadian women (n=107, age 18–30 years), Gozdzik et al. (2008) underlined the fact that vitamin D intake was low among those who were at greatest risk of vitamin D insufficiency (25(OH) D < 75nmol/L) (207). In the present study, the importance of dietary supplements as a source of vitamin D increased from 25% at the age of 20–30 years to 44% at the age of 41–50 years. It appears clear that dietary vitamin D supplementation is required; however, it is important to consider whether this is an acceptable and effective intervention, as vitamin D intake from supplements is quite low among young women (208).

In the bivariate analyses, being married and multiparty was positively associated with vitamin D intake. Our finding is inconsistent with the results of a previous study conducted in a Danish birth cohort (206), which showed that the use of vitamin D supplementation was inversely associated with parity, and that multiparous women tended to have a lower overall vitamin D intake. An increased adherence to vitamin D and calcium supplements in the present study was more likely in women who more often attended antenatal care, where they received supplements.

Some previous studies found that high household income and education was strongly associated with increased vitamin intake from food (209, 210). Although this difference was not observed in the present study, we found that women with a high household income (≥ 15000 SR) were more likely to consume vitamin D supplements than women with a low household income (< 15000 SR) (47.2% vs. 32.2%, p=0.02), which may explain the higher serum 25(OH) D concentrations that were found in women with high household income compared to those with low household income (median 25(OH)D; 38.1nmol/l vs. 33.2 nmol/L, p=0.035). This relationship has also previously been found. McCormack et al. (2015) investigated the prevalence of vitamin D supplement use in Canadians aged 45 years and over who participated in the Healthy Aging module of the Canadian Community Health Survey from 2008–2009 (211). They found that vitamin D supplement use increased with household income, and asserted that this may have been because individuals with low household income consider the taking of supplements to be less important, particularly when faced with a shortage of food and money. Body mass index (BMI) was positively associated with vitamin D intake from diet and supplements in the bivariate analysis (r = 0.14, p=0.030), and this association remained significant after adjusting for energy intake. Our analysis may help to inform public health interventions with regard to improving dietary vitamin D intake behaviors among populations at greatest risk of vitamin D deficiency.

Dietary Vitamin D and Calcium Intake, Serum 25(OH) D, PTH and Bone Health

The prevalence of osteopenia found in the present study is in accordance with the findings of other studies that were conducted in Saudi Arabia. For example, Youssef (2015) found that the prevalence of osteopenia was 39.7% in female students (aged 17–25 years, n=267) in Makkah (212). Another study indicated that the prevalence of osteopenia among young Saudi women (aged 20–25 years, n=101) in Riyadh was 37% and 34% in the spine and in the neck of the femur, respectively (213). The studies conducted in Saudi Arabia have shown that the prevalence of osteoporosis among postmenopausal women is higher than that observed in Western countries (67, 165); this prevalence ranged from 23% to 44.5% across different regions of the country (214, 215, 216). The number of hip fractures was estimated in 2004 at King Fahd University Hospital in Khobar, Saudi Arabia (217). The direct cost of treating the hip fractures for 23 male and 20 female patients with an average age of 72 years with hospital stays for 760 days was SR2.09 million (US$ 557,333.00) at the rate of SR48,712 (US$ 12,9890.90) per patient.

In the present study, we evaluated the relationship between dietary intake of calcium and vitamin D and serum 25(OH) D and serum parathyroid hormone PTH in women with low and normal BMD. The findings are in accordance with the generally accepted risk factors for low bone mass, namely dietary calcium intake (28) and BMI (218, 219), whereas vitamin D intake, serum 25(OH) D and PTH are not significant predictors of BMD in premenopausal women in this study. Calcium and vitamin D are essential nutrients for maximization of peak bone mass. This increase in bone density can prevent bone loss, and thus reduce the risk of osteoporosis and fracture later in life (28). The observed shortfall in calcium intake of below the EAR for more than half of the participants is in accordance with the results of other studies conducted in Saudi Arabia. In a study of postmenopausal women (n=122, age 46–70 years) in Jeddah, Alissa et al. (2011) found that approximately 60% of the participants had lower calcium intake than the EAR (33), while Youssef (2015) found that the mean calcium intake of a group of female students (n=267, age 17–25 years) in Makkah was 559.3 ± 32.2, with approximately 86.1% of the sample consuming less than 800 mg/day calcium (212). Different types of dietary assessment methods may explain the observed differences between our study and the one conducted in Makkah (212). The calcium intake from diet and supplements in our study was assessed using a semi-quantitative food frequency questionnaire, while calcium intake was assessed by 3-day recall in the investigation conducted in Makkah. It has been found that when a calcium-rich food frequency questionnaire is compared with 3-day recall, participants may identify foods in the questionnaire but may not happen to have consumed those foods on the specific day of recall (220, 221). In the present study, calcium intake insufficiency was more pronounced in women with low lumbar spine bone mass than in women with normal bone mass, and we observed a statistically significant relationship between lumbar spine BMD and calcium intake.

This was similar to the results of the study conducted in Makkah, whereby Youssef (2015) found a significant difference in spinal BMD according to calcium intake ($p=0.04$) (212).

Low vitamin D intake was also observed in the present study; over half of the women had a lower intake than the EAR. Vitamin D insufficiency was similar in women with low and normal bone mass, and thus this may have had no major effect on the outcome of this research. This finding is consistent with those of previous studies (33, 222), which reported no significant association between vitamin D intake and BMD measurements at the lumbar spine or hip in women. However, we found a high prevalence of vitamin D supplement use in women with low bone mass (55%) compared to women with normal bone mass (41%). A possible explanation for this could be that women with low bone mass had already consulted their primary care physicians regarding their low serum vitamin D levels, and had started taking vitamin D supplements prior to DXA screening as a precaution against osteoporosis. This confirms the need for more intense strategies to inform young women of the importance of sufficient dietary intake of vitamin D and calcium in optimizing bone health. Nevertheless, there is a need for a longitudinal study of the effects of prolonged dietary and supplementary vitamin D and calcium intake on BMD, targeting at-risk groups. In one prospective population-based study, 9,382 Canadian men and women aged ≥ 25 years were followed for 10 years to assess changes in calcium and vitamin D intake over time and their associations with BMD. The results showed that a cumulatively higher intake of vitamin D and calcium over time contributed to improved bone health maintenance at the lumbar spine and hip in adult women (28).

A low serum 25(OH) D concentration is considered to be an important risk factor for low BMD, and previous studies have shown positive correlations between serum 25(OH) D and BMD at the lumbar spine and at the neck of femur in premenopausal women (218, 223). However, we did not observe a significant association between serum 25(OH) D and BMD at the lumbar spine or at the neck of femur in the present study. Overall, 22.5% of the women had vitamin D sufficiency (25OH D ≥ 50 nmol/L), whereas the remaining 77.5% had vitamin D deficiency (25OH D < 50 nmol/L). The lack of association between serum 25(OH) D and BMD may be attributable to the high prevalence of vitamin D deficiency in our sample. Similar findings have also been reported in previous studies in premenopausal (224) and postmenopausal (30, 33) Saudi women. These studies concluded that serum vitamin D concentrations may not be a reliable indicator of low BMD among these women, due to the fact that the majority of women suffer from vitamin D deficiency (30).

In contrast, an inverse association between serum 25(OH) D and intact PTH was evident in women with serum vitamin D < 50 nmol/L ($r = -0.27$, $p=0.03$). The association between PTH and BMD has not been consistently observed. Some studies have shown that elevated serum PTH levels are significantly associated with lower BMD (223, 225), while others have failed to demonstrate any significant relationship (33). In the present study, the inverse association between PTH and BMD at the lumbar spine and at the neck of femur did not reach statistical significance. However, 7% of our participants who experienced vitamin D deficiency and elevated PTH had lower BMD at one of the two sites. These findings reveal the need for intervention and educational campaigns to improve low bone mass in young women, as, when left untreated, it may lead to osteoporosis and fractures in the long term. PTH and 25(OH) D are the major hormonal regulators of calcium homeostasis. It has been shown that when serum 25(OH) D is 50 nmol/L or lower, there is a significant decrease in intestinal calcium absorption, and that this decrease is associated with elevated PTH (1). PTH maintains calcium homeostasis by enhancing tubular reabsorption of calcium, and stimulates the kidney to activate 25(OH) D to 1,25OHD. Elevated PTH also stimulates the process of dissolving the bone matrix to liberate minerals, leading to osteopenia and osteoporosis and increasing the risk of fracture (1, 226).

Body weight is an important risk factor for osteoporosis. In the present study, we found a strong positive relationship between BMI and BMD at both the lumbar spine and the neck of femur. Women with higher BMI tended to have higher BMD, and this positive association has previously been well documented (196, 218, 219, 224).

The present study provides new insights regarding daily vitamin D intake and examined the food sources that make the biggest contribution among premenopausal women in Jeddah and the subgroup most vulnerable to insufficient vitamin D intake. In addition, this study is the first assessment of dietary intake of vitamin D and calcium among premenopausal women in Jeddah and its association with the bone health parameter, BMD. However, we must consider its limitations. First, SFFQ may not be the most accurate tool for use in the assessment of dietary sufficiency, thus dietary vitamin D and calcium intake may be subject to measurement error. However, we attempted to minimize this error by using an interviewer to administer the questionnaire to help participants to accurately estimate portion sizes, rather than using a self-reported questionnaire. In addition, the SFFQ used in this study had previously been validated in Arabian Gulf regions and was found to accurately reflect the intake of these populations (145); the correlation coefficients between the SFFQ and 3-day diet food records were 0.82 and 0.74 for vitamin D and calcium, respectively. Second, the use of Caucasian normative data for peak adult bone mass may have led to over diagnosis of the prevalence of osteopenia. Standardization of BMD by DXA to a database that is culturally specific for Saudi Arabia is necessary and recommended to ensure reliable interpretation of the individual DXA data and to capture those women at risk. Third, the comparison of the characteristics of those tested and not tested with DXA revealed no significant differences, with the exception of age and education. Women who had undergone a DXA scan may have chosen to take part because they are more interested in their personal health and well-being than the average Saudi woman. Conversely, women may have chosen to take part because they thought they were at higher risk of osteopenia due to certain symptoms, such as bone pain. Finally, although the participants included in the present study were young and healthy, our results are limited to premenopausal women attending the primary health center at King Abdul-Aziz Medical City in Jeddah and not all women residing in Jeddah or in other regions of Saudi Arabia.

CONCLUSION

Insufficient intake of vitamin D and calcium is a growing concern for premenopausal women in Jeddah. Our data indicated that vitamin D intake was particularly low among young women who were at greatest risk of vitamin D deficiency. Calcium intake was positively associated with BMD in this age group and could potentially be manipulated to optimize bone mass. This research supports the need for intense public health strategies to improve nutrition among young women. Strategies should start in adolescence, before peak bone mass is reached to provide a diet rich in calcium and vitamin D and promote physical activity. Dairy products provide most calcium and vitamin D in the diet; therefore, it is important for girls to meet the recommended intake of dairy products during peak height velocity (227). Our results suggest the need to educate young women to select products fortified with vitamin D and to educate lactose intolerant women to make appropriate substitutions, such as well-tolerated dairy products like Laban and yogurt. Current levels of vitamin D fortification in Saudi Arabia are not sufficient to meet the EAR for vitamin D intake. Therefore, it is recommended that the Saudi Food and Drug Authority implement an appropriate monitoring program to control the fortification process. Also beneficial would be introducing new vitamin D–fortified products to the Saudi market, such as cheese and flavored yogurt, which are consumed in substantial quantity by young women in our study population. Further study is required to investigate whether nutrition interventions are effective in increasing BMD in young women in Jeddah.

APPENDICES 2

TABLE 1

Demographic, anthropometric, and clinical characteristics of Jeddah premenopausal women (20-50 years) with sufficient vitamin D intake *vs.* insufficient vitamin D intake (n=257).

Characteristics	Insufficient vitamin D intake (n = 167) Median (P25-P75)	Sufficient vitamin D intake (n = 90) Median (P25-P75)	*P-value*
Age (y)	27.0 (23.0-32.0)	31.0(26.5-39.0)	< 0.001*[b]
BMI (kg/m2)	25.3 (22.1-29.3)	27.2 (22.6-32.1)	0.09 [b]
Vitamin D intake (IU/d) (diet and supplement)	159.1(90.3-228.7)	1630.2(649.5-1842.4)	< 0.001*
Dietary vitamin D intake (IU/d)	132.1(76.4 – 210.2)	157.6 (91.5- 255.6)	0. 05*[b]
Vitamin D intake from fish (IU/d)	15.6 (4.9-31.5)	21.8 (9.4-43.8)	0.04*[b]
Calcium intake (mg/d) (diet and supplement)	665.2 (413.7-912.5)	811.3 (572.8-1089.0)	0.02* [b]
Serum 25(OH) D (nmol/L)	29.5 (23.2-35.2)	51.4 (41.1 -68.9)	< 0.001* [b]
BMI (kg/m2)	n (%)	n (%)	
Nonobese (< 25 kg/m^2)	80 (69)	36 (31.0)	0.22[c]
Obese (≥ 25 kg/m^2)	87 (61.7)	54 (38.3)	
Serum 25(OH) D (nmol/L)			
Deficiency (< 50 nmol/L)	152 (78.4)	42 (21.6)	<0.001*[c]
Sufficiency (≥ 50 nmol/L)	8 (14.3)	48 (85.7)	
Education status			
Less than college	50 (67.6)	24 (32.4)	0.72 [c]
College graduate	103(63.2)	60 (36.8)	
More than college	14 (70.0)	6 (30.0)	
Income level (SR per month)			
< 15000	97 (67.8)	46 (32.2)	0.15 [c]
≥15000	52 (58.4)	37 (41.6)	

BMI, body mass index; WC, waist circumference; P25-P75, 25 percentile and 75 percentile.
a Sufficient vitamin D intake consumed ≥ 400 IU/day and insufficient vitamin D intake consumed < 400 IU/day b Non-parametric Mann Whitney U test was performed. c A Chi-square test was performed * indicates significance at the 0.05 level.

TABLE 2
Vitamin D and calcium intake and percent below the EAR[a] among Jeddah premenopausal women residents (n=257) stratified by age.

	Total (n=257)	P- value[b]	20-30 years (n = 153)	31-40 years (n = 73)	41-50 years (n = 31)	P -value [c]
Vitamin D intake from diet and supplement (IU/day)						
Mean ± SD	643.4 ±873.1		488.24 ± 718.74	854.54± 1060.29	912.31± 946.85	
Median (IQR -P25-P 75)	236.4(114.4-748.4)	0.52	206.8 (99.5 - 403.9)	276.9 (124.6 - 1632.1)	556.1 (228.9-1693.8)	< 0.001
n (%) below EAR[a] (400 IU/day)[d]	167 (65)		114 (74)	42 (57.5)	11 (35.5)	< 0.001*
Calcium intake from diet and supplement intake (mg/day)						
Mean ± SD	770.9 ± 421.9		725.2± 378.1	788. 1± 475.9	955.76 ± 448.21	
Median (IQR-P 25-P 75)	702.7 (469.6-981.2)	0.005*	681.3 (440.1 - 925.6)	713.7 (416. 4-989.1)	962.8 (626.3-1100.0)	0.015*
n (%) below EAR[a] (800 mg/day)[d]	156 (61)		102 (66.7)	43 (58.9)	11 (35.5)	0.005*

a. EAR, Estimated Average requirement; P25-P75, 25 percentile and 75 percentile; SD, standard deviation. b. Wilcoxon Signed Rank Test was performed to compare differences between median nutrient intake and EAR . c. Non-parametric Kruskal-Wallis test was performed to compare differences in intake among age categories. d. Adequacy was determined using the Estimated Average Requirements levels of 400IU for vitamin D and 800 mg/day for calcium for women aged 19-50 years.

TABLE 3
Sources of vitamin D intake in 20-50 years Jeddah premenopausal women, % of total vitamin D intake

Vitamin D sources (IU/day)	Total N=257	Age category 20-30 years (N = 153)	31-40 years (N = 73)	41-50 years (N = 31)
Supplement	31.0	25.2	37.9	43.8
Milk, Laban, yogurt	36.5	38.4	32.7	35.9
Cereal	1.1	1.6	0.5	0.3
Food made with milk	3.1	3.8	2.3	1.3
Fish	12.2	12.1	12.5	7.9
Meat	8.4	9.4	7.8	5.0
Egg	6.9	7.7	5.9	5.4

Only food groups that contributed 1% or more of total vitamin D intake are presented.

TABLE 4
Sociodemographic factors associated with log-transformed vitamin D intake in forward stepwise regression for Jeddah premenopausal women (20-50 years) residents.

	Unstandardized Coefficients		Standardized Coefficients		
	B	SE	Beta	t	P
Intercept	4.551	0.323		14.074	0.000*
Age	0.039	0.010	0.240	3.754	0.000*

Note: Dependent variable = log transformed vitamin D intake from food and supplement. N = 257. B = Unstandardized regression coefficients. SE = standard error. Beta = Standardized regression coefficients. t = t-statistic. p = p-value. * indicates significance at the 0.05 level.

TABLE 5
Descriptive, dietary characteristics, biochemical parameters and bone measurements of subsample (n=102) Jeddah premenopausal women (20-50 years) underwent a DXA scan

Variables	Mean ±SD	Median	P25	P75
Age(years)	32.2 ± 7.8	30.5	26.0	36.3
Height (cm)	157.3 ± 6.6	157.0	153.0	162.0
Weight (kg)	68.4 ± 19.9	64.0	55.4	77.3
Body Mass index (kg/m^2)	27.6 ± 7.7	25.7	22.4	31.2
Waist circumference (cm)	80.0 ± 13.5	78.3	69	86.6
Vitamin D intake from food only (IU/day)	164.8 ± 115.9	133.8	74.4	216.5
Vitamin D intake from food and supplements (IU/day)	697.7 ± 798.6	331.8	126.5	1098.4
Calcium intake from food only (mg/day)	707.6 ± 402.8	628.8	387.8	906.6
Calcium intake from food and supplements (mg/day)	764.8 ± 421.5	702.1	418.5	968.6
Adjusted calcium adCa (mmol/L)	2.3 ± 0.1	2.3	2.3	2.4
Serum phosphate (mmol/L)	1.2 ± 0.02	1.1	1.1	1.2
Serum alkaline phosphates (U/L)	63.7 ± 20.2	60.0	49.0	74.3
Serum vitamin D 25(OH)D (nmol/L)	42.9 ± 22.8	40.2	29.4	48.2
Parathyroid hormone PTH (pg/mL)	84.6 ±40.2	74.5	60	90.8
BMD (gm/cm2), lumber spine	1.1 ± 0.1	1.1	1.0	1.2
BMD (gm/cm2), femoral neck	0.93 ± 0.1	0.9	0.8	1.0

BMI classification	Frequency	Percentages
Underweight	4	3.9
Normal	40	39.2
Overweight	27	26.5
Obese	31	30.4
Current smoker		
No	77	75.5
Yes	25	24.5
Fracture history		
No	95	93.1
Yes	7	6.9
Serum 25(OH) D		
Deficiency < 50 nmol/L	79	77.5
Sufficiency ≥ 50 nmol/L	23	22.5

Note: N = 102. For Serum calcium and serum phosphate, N = 99. For parathyroid hormone, N = 88. P25-P75, 25 percentile and 75 percentile, SD = standard deviation, BMD=bone mineral density, BMI=body mass index , 25(OH) D =serum 25hydroxyvitamin D

TABLE 6-a
Logistic regression analysis for association of vitamin D and calcium intake, serum 25(OH) D and serum PTH levels with bone mineral density at lumber spine (Model 1)

				95% CI	
Variables	**Wald**	**p**	**OR**	**Lower**	**Upper**
Vitamin D intake	**0.504**	**0.478**	**1.000**	**0.999**	**1.000**
Calcium intake	**4.100**	**0.043***	**1.002**	**1.000**	**1.005**
Serum 25(OH)D	**0.154**	**0.695**	**0.995**	**0.971**	**1.019**
PTH	**0.145**	**0.703**	**0.997**	**0.984**	**1.011**
BMI	**6.069**	**0.014***	**1.117**	**1.023**	**1.219**
Energy intake	**7.362**	**0.007***	**0.997**	**0.994**	**0.999**
Constant	**0.019**	**0.889**	**0.775**		

Note: Wald = Wald chi-square statistic, p = p-value, OR= odds ratio, CI = confidence interval.
**** indicates significance at the 0.05 level.***

TABLE 6-b
Logistic regression analysis for association of vitamin D and calcium intake, serum 25(OH)D and serum PTH with bone mineral density at femur neck (Model 2)

				95% CI	
Variables	**Wald**	**p**	**OR**	**Lower**	**Upper**
Vitamin D intake	**1.067**	**0.302**	**1.000**	**0.999**	**1.000**
Calcium intake	**1.066**	**0.302**	**1.001**	**0.999**	**1.003**
Serum 25(OH)D	**0.004**	**0.947**	**0.999**	**0.978**	**1.021**
Serum PTH	**2.449**	**0.118**	**0.989**	**0.975**	**1.003**
BMI	**8.251**	**0.004***	**1.122**	**1.037**	**1.214**
Energy intake	**0.636**	**0.425**	**0.999**	**0.998**	**1.001**
Constant	**2.759**	**0.097**	**0.053**		

Note: Wald = Wald chi-square statistic, p = p-value, OR = odds ratio, CI = confidence interval.
**** indicates significance at the 0.05 level.***

TABLE 7
The average dietary intake of vitamin D and major food sources of selected countries including current study.

Country	Women age (Years)	Vitamin D intake from food (IU/day)	Vitamin D intake from food and supplement (IU/day)	Major sources (Top three)
Current study	**20-50**	**141**	**236.4**	**Dairy products (36.5 %), Supplements (31%), Fish (12.2%)**
Australia (202)	**adult women**	**80-96**		**Margarine (48%), Canned fish (16%), eggs (10%).**
Japan (7)	**≥ 40**	**284**		**Fish (91%), eggs (3%), meat (2%)**
Lebanon (203)	**30-50**	**89**		**Milk (30.2%), Meat (28%), Fish (25.5%)**
Norway (7)	**25-69**		**236**	**Supplements (49%), Fish (26%), Margarine (23%)**
Taiwan (8)	**19-44**	**154**	**176**	**Fish (39%) , Dairy products (14%), Supplement (12%)**
United Kingdom (7)	**35-49**		**148**	**Supplements (34%), Fish (19%), Cereal (16%)**
United States (7)	**20-49**		**293**	**Supplements (40%), Milk (39%), Cereal (3%)**

Note: IU = International Unit.

PAPER III: Knowledge and Attitudes about vitamin D and Sunlight Exposure in Premenopausal Women Living in Jeddah, and their Relationship with Serum Vitamin D Levels

ABSTRACT

Background: Saudi women are at risk of vitamin D deficiency because they are fully covered by traditional clothing and because of their indoor lifestyle. The latest national study reported that vitamin D deficiency (serum 25(OH) D < 50 nmol/L) affects 72% of young Saudi women. Because little information is available regarding knowledge on vitamin D, attitudes toward sun exposure, and the vitamin D status of premenopausal women in Jeddah, more research is necessary in order to develop effective intervention programs.

Objectives: The purpose of this study is to explore how the relationship between knowledge of vitamin D and attitudes about sun exposure affect the serum vitamin D levels in premenopausal women attending the primary care clinic in Jeddah.

Methods: This cross-sectional study included 257 women aged 20–50 years in Jeddah, Saudi Arabia. Participants completed questionnaires about dietary vitamin D intake and sun exposure, and were tested on their knowledge of vitamin D. Serum 25(OH) D was evaluated in participants, and their bone mineral density (BMD) was measured using double energy X-ray absorptiometry in a subset of women (n = 102).

Results: Vitamin D deficiency (25(OH) D < 50 nmol/L) was present in 77.6% of the participants. The prevalence of osteopenia was 33% in the lumbar spine and 30% in the femur neck. Although 99% of participants had heard of vitamin D, and 91% knew that sunlight exposure is a primary source of vitamin D, participants expressed insufficient knowledge of vitamin D sources and had negative attitudes toward sun exposure. High fish consumption, high household income, and employment were associated with a higher vitamin D knowledge score. Education was a significant predictor of vitamin D knowledge score ($p < 0.001$). Wearing colored *abaya* was significantly associated with increased vitamin D levels.

Conclusion: Suboptimal vitamin D status and insufficient knowledge of vitamin D intake sources are common in premenopausal women in Jeddah. Based on this data, health professionals could provide medical intervention to the most vulnerable female patients, as well as offer clear guidelines and information to the general public.

INTRODUCTION

Though vitamins play a pivotal role in preventing diseases, their ability to aid in maintaining good health was unknown for centuries and is still not fully comprehended in many parts of the world. Indeed, it was not until 1920 that a protracted search for a cure for rickets–a bone disease that is most common in children and infants and can lead to permanent deformities–yielded the discovery of vitamin D. The vitamin was found to be essential for calcium absorption, while a severe deficit of it decreases the efficiency and absorption of intestinal calcium and phosphorous, resulting in increased parathyroid hormone (PTH) concentrations. Secondary hyperparathyroidism due to low levels of 25 hydroxyvitamin D-25(OH) D may lead to increased bone turnover and loss. Thus, a severe vitamin D deficiency harms not only children, but it can also cause osteomalacia, osteopenia, osteoporosis, and an increased risk of fracture in adults (1-3).

Vitamin D, which is an essential fat-soluble nutrient, is obtained through either synthesis in the epidermis upon exposure to ultraviolet (UVB) sunlight or by the intake of vitamin D-rich foods such as oily fish, egg yolks, veal, beef, liver, and sun-dried mushrooms. Serum concentration of 25-hydroxyvitamin D [25(OH)D] is considered the best indicator of vitamin D nutritional status, as it reflects both vitamin D produced in the skin and that acquired from the diet. While the optimal 25(OH) D level is a topic of considerable debate, the general consensus is that a serum 25(OH)D concentration < 25 nmol/L may indicate vitamin D deficiency (11,13, 14). As per the US Institute of Medicine's (IOM) 2011 conclusions, a serum 25(OH)D concentration < 30 nmol/L qualifies as "deficient" and has a known impact on skeletal health, calcium malabsorption, and secondary hyperparathyroidism, and leads to rickets in children and increased bone resorption and osteomalacia in adults. Additionally, serum 25(OH)D in the range of 30–50 nmol/L may be “inadequate” in some people, while serum 25(OH)D > 50 nmol/L is “sufficient” for a majority (97.5%) of the population (2,4). Given those thresholds, vitamin D deficiency (< 50 nmol/L) is prevalent in many populations worldwide.

In fact, Saudi Arabia reports some of the world’s highest rates of vitamin D deficiency (16,17), with Saudi women having a particularly high risk for the condition due to the traditional clothing limiting their exposure to sunlight (18). The most recent national study reported that the prevalence of vitamin D deficiency among both young and postmenopausal women is 72% and 85%, respectively (19). Saudi studies (17, 20, 21) have highlighted concerns that Saudi women of reproductive age who have serum 25(OH) D of 10-45 nmol/L are at risk for suboptimal bone health—which can lead to osteomalacia, chronic back pain, and other bone-related problems. Twenty-three Saudi adolescents with vitamin D deficiency (25(OH)D < 50 nmol/L) were diagnosed with osteomalacia in a retrospective hospital-based study in Riyadh from January 1990 to December 2014 (22). Low back pain was prevalent among Saudi patients, ranging from 53.2% to 79.17% in Saudi patients (23), and vitamin D deficiency was the major risk factor for chronic low back pain (17, 23). Low bone mass has been reported among young (25–35 years), apparently healthy Saudi women with serum 25(OH)D levels below 50 nmol/L (124). The prevalence of low bone mass in Saudi Arabia is higher than in

Western countries (67, 165). The prevalence of lumbar and femur osteopenia ranges from 7–43.4% and osteoporosis from 2.5–46.7% (30-32).

Limited exposure to sunlight is a main contributor to the low 25(OH)D concentrations in Saudi Arabia and is a direct result of cultural practices (e.g., clothing that fully covers the skin), the hot climate that drives people to seek air-conditioned comfort, and the pigmentation in dark skin that can reduce endogenous vitamin D production (16,17, 24). Moreover, when compared to individuals with lighter skin, those with dark skin require longer periods of exposure to sunlight in order to produce the same amount of vitamin D (4,15).

In addition to behaviors related to sun exposure, Oudshoorn et al. (2011), Kung & Lee (2006) and Almutairi et al. (2012) (36-38) all consider a lack of knowledge regarding nutrition and the importance of the micronutrient to overall health to be another contributing factor to vitamin D deficiency. A lack of knowledge regarding the production and intake of vitamin D among Saudis has been noted by Siddique (2007) (24), while Christie et al. (2011) conducted qualitative research on female Saudi Arabian students and found significant limitations in their understanding of vitamin D nutrition and how to prevent a deficiency of the micronutrient (39).

A better understanding of vitamin D, the prevailing attitudes regarding sun exposure, and the effect that these may have on vitamin D status is necessary before developing effective intervention programs. In fact, the scarcity of data relevant to these issues from the general public in Saudi Arabia–and young women in particular–makes further studies imperative. Surveys in Saudi Arabia suggest that a high proportion of the population is unaware of vitamin D nutrition and vitamin D deficiency; however, given that only a small group of students representing an educated sector of society was studied, the findings were limited (39). The general lack of knowledge points to the need for quantitative research into the level of awareness in Saudi women as to the importance of vitamin D intake, sun exposure, and how such things have impacted their serum 25(OH)D levels (39,40). Thus, the present study focuses on premenopausal women attending the primary care clinic at King Abdul-Aziz Medical City in Jeddah in an effort to examine the women overall knowledge of vitamin D, their attitudes toward sun exposure, and to explore the associations between knowledge and attitudes and their serum vitamin D levels.

SUBJECTS AND METHODS

1. Study Design and Participants

A cross-sectional study of premenopausal Saudi women (aged 20 to ≤ 50) was conducted at King Abdul-Aziz Medical City in Jeddah, Saudi Arabia. The 257 participants were selected from attendees of the primary care clinic at King Abdul-Aziz Medical City using systematic sampling. The sampling frame was derived from daily listing sheets that included walk-in females seeking general outpatient services. A systematic sampling method was applied to the list of eligible women who were seen in the primary care clinic during the study period between December 2014 and April 2015. To implement the systematic sampling, a random number between one and three was generated each day. The random number determined which first woman was chosen from the list. Starting with first women chosen, then every third woman on the list was selected for potential inclusion in the sample. This process was repeated daily until the targeted sample size (n=257) was reached. Through the above mentioned systematic sampling method, the nurse in charge of the clinic called every selected woman from the female waiting room, and that woman was directed to a private exam room. Subsequently, the woman was fully briefed about the study objectives and methods by a member of the research team, and the woman was invited to participate in the study. Each woman who agreed to participate was initially questioned with regards to her age- and health-related medical conditions to ensure that she met the inclusion criteria, and eligible women were asked to proceed directly to the same room upon the completion of the medical examination that was the main reason for her visit. In the next step, written informed consent was obtained at the very start of the interview. Then data collection sessions were conducted under the supervision of the research teams, who interviewed women and reviewed their medical record in case more information was needed, such as vitamin D calcium supplement dosage and medication. Data collection sessions usually lasted for 25–30 minutes.

To select participants for the DXA scan, each woman, chosen through the systematic sampling method described above, was asked to participate in this portion of the study until completion of the required sample for this subgroup (n=102) was achieved. Upon completion of the first visit, each woman was asked to return for a second visit, during which bone mineral density (BMD) measurements was taken via DXA scan. If a participant declined this request, then selection proceeded to the next sample woman.

Women were eligible for the study if they were citizens of Saudi aged 20 to 50 years. Women were excluded from the study if they were pregnant or lactating or post-menopausal (defined as amenorrhea for at least six months). Women with cancer, diabetes, hyperthyroidism or hypothyroidism, metabolic bone disease, malabsorption, renal diseases, or those taking medications known to effect bone metabolism were also excluded from the study. The use of vitamin D supplements was not exclusionary criteria because we were interested in evaluating how many participants take vitamin D supplements as well as the effects of supplementation on serum 25(OH) D levels.

2. Data Collection

2.1 Demographic Variables

Sociodemographic variables included age (years), marital status, e.g., married (living with partner) or unmarried (single, divorced/widowed); Occupation status (employed, unemployed, student, housewife); Monthly household income was divided into categories, according to the Central Department of Statistics and Information (2006/2007) (140)and a previous published study (141), depending on the amount of Saudi Arabian Riyal (1SAR = 0.266USD): (< 4,000; 4,000 to 7,999; 8,000 to 14,999; 15,000 to 24,999; ≥ 25,000); residence (rented apartment , owned apartment , rented house , owned house, others) Participants were asked about the number of years of formal education, and then level of education was categorized as follows: (less than college, college graduate, and post graduate).

2.2 Dietary Vitamin D Intake

Dietary intake of vitamin D per day was estimated using the Semi-quantitative Food-Frequency Questionnaire (SFFQ). The SFFQ used was a modified version of a previously validated questionnaire (144), which was also validated in United Arab Emirates and showed positive correlation between the SFFQ and 3-day diet food records was ($r = 0.74$, $p < 0.001$) for vitamin D (145). Vitamin D values of some food items were obtained from the Arabian Gulf Food Composition Table (147) such as meat, and local fish consumed in this area. However, because vitamin D values for non-local foods such as tuna and salmon are not available in the Arabian Gulf Food Composition Table, the U.S. Department of Agriculture reference data (149) were used. Vitamin D values of supplements and local food items such as vitamin D fortified milk and Laban drink (buttermilk) were obtained from food labels. The items in SFFQ include natural food sources of vitamin D (e.g., tuna, salmon, meat, poultry, liver, and eggs), local, and imported vitamin D fortified food and beverages available in the Saudi marketplace. Supplemental vitamin D and multivitamins were assessed by the SFFQ.

The selection of food items in SFFQ was obtained and reviewed with the investigator using visual estimates (photographs) to help subjects estimate average portion size. The photographs using common items such as household items (plates or bowels) or hands were used to help estimate average portion size for subjects who ate from the communal dish**.** On the SFFQ, respondents were asked to record how often they consumed a single serving of each food listed during the previous month, with possible responses ranging from less than once per month to 2 or more per day.

Vitamin D intake was computed by multiplying the intake frequency by the nutrient content of the portion size of specific items. Intake of vitamin D was calculated by summing nutrient intake from diet and supplemental sources. To determine the adequacy of vitamin D, the value obtained was assessed using Estimated Average Requirements (EAR) of 400 IU/day (2).

2.3 Sun Exposure and Skin Type

The sun exposure recall questionnaire included details of the time of the day of the sunlight exposure; length of exposure to sunlight during the previous month on week days and weekends. Cover status when they were outside in the public area was used to determine the area exposed to direct sunlight. Women were classified as either 1) face covered but hands exposed, 2) face and hands exposed. In addition, women were asked the average length time per day they spent in their courtyard. In Saudi culture this is the place where the sunlight exposure occurs because in all other outdoor activities women generally cover their bodies by wearing black abayas (153).

Skin color was assessed by the investigator using the validated Fitzpatrick skin-type scale (154). The system is a numerical classification scheme for determining six different skin types based on a questionnaire related to an individual's genetic constitution, reactions and vulnerability to sunlight or UVB radiation, and tanning habits (154,155). The response to each question was measured on a scale of 0-4. The response for all questions will be added together to get the total score corresponding to the Fitzpatrick skin-type scale, which ranges from very fair (skin type 1) to very dark (skin type IV). The Fitzpatrick skin color scale has been previously used in Arabian Gulf studies including, United Arab Emirates (158) and Kuwait (38).

2.4 Knowledge of Vitamin D and Attitudes and Behavior toward Sun Exposure

The content and face validity of the questions were ensured by reviewing relevant literature and questionnaires on vitamin D research and consulting with experts in the field. In addition, the questionnaire was pre-tested in pilot interviews in a similar setting with 30 volunteer women of the same age groups. Based on the results, some items were refined and clarified in a later version of the questionnaire. The internal consistency reliability of the vitamin D knowledge test was calculated on the dichotomized items (correct / incorrect) using Kuder-Richardson Formula 20. The reliability of the test was calculated to be 0.70, indicating that the test demonstrated acceptable reliability (159).

The questionnaire consisted of two sections. In the first section, the participants were asked seven multiple-choice questions about their knowledge of the sources and role of vitamin D. The questions were adapted from those used in previous validated surveys (36, 37) and were modified for our study population. The first question was used to screen participants. Women were asked if they had heard of vitamin D, and only women who had heard of vitamin D were asked to complete the rest of the survey. Questions 1–7 were used to calculate participants' total knowledge score by adding the points received for each answer. One point was assigned for each correct answer, and 0 points were given for each incorrect answer or "I don't know" response. If a question had two correct answers, then each correct answer was worth 0.50 points. If a question had three correct answers, then each correct answer was worth 0.33 points. The participants were categorized as more or less knowledgeable about vitamin D based on their number of correct answers. The participants were regarded as more knowledgeable if they correctly answered four or more questions, including a positive response to question 1. The participants who answered question 1 negatively or correctly answered fewer than four questions were considered to be less knowledgeable about vitamin D.

The second section of the questionnaire included eight multiple-choice statements about women's attitudes and behavior toward sunlight exposure. The statements were adapted from those used in a previous survey (160) and modified for our study population. This section included questions on whether women spend time in the sun or tanning, use sunscreen, wear a colored *abayas* during sun exposure, avoided sun exposure, and their reasons why.

2.5 Biochemical Analysis

Non-fasting venous blood samples were drawn by qualified phlebotomists for serum 25-hydroxyvitamin D 25(OH) D, parathyroid hormone (PTH), adjusted serum calcium (adCa), phosphate (P04), and alkaline phosphatase (ALP). Serum calcium was measured by Arsenazo III dye (Architect 16000, Abbott, USA). Parathyroid hormone (PTH) was measured by a two-step sandwich immunoassay for the quantitative determination of intact PTH in human serum using CMIA technology (Architect 2000, Abbott, Germany). The PTH assay showed a detection sensitivity of $\leq$ 3.0 pg/mL, intra-assay coefficient of variation (CV) was 12.9-6.1%, inter-assay (CV) was 3.0-6.4%. The reference range for PTH in the study laboratory is (24-114 pg/mL).

Quantitative 25(OH) D in serum was determined by using one step delayed chemiluminescent microparticle immunoassay (CMIA) (Architect, Abbott, Germany). This essay was used to recognize 25(OH) D_2 (ergocalciferol) and 25(OH) D_3 (cholecalciferol) with equal affinity. The laboratory undertaking the testing is a participant in the proficiency Accuracy Based vitamin D (PABVD) since 2012. The Accuracy Based vitamin D is part of the proficiency testing (PT) kits for the College of American Pathologists (CAP), the vitamin D external quality assurance scheme of vitamin D assays (161). Intra-assay 25(OH) D coefficient of variation (CV) from daily quality control was 1.4- 3.7% and inter-assay (CV) was 2.6– 4.6%. The fully automated Architect assay is designed to have an imprecision of ≤ 10 %.

Total volume required was 6 mL using two plain vacutainer tubes (BD vacutainer systems, Plymouth, UK). The serum samples were taken in the laboratory in the primary care clinic; after blood collections, samples were left for 10 min for clotting then immediately centrifuged at 3000rpm for 10 min. Then, samples were aliquoted and frozen at -20° C until further analysis. Samples were run simultaneously in a batch. All analyses were performed in the Pathology laboratory at King Abdul-Aziz Medical City.

Baseline serum 25(OH) D levels were classified into three categories using cutoff points reported in the scientific literature (11,162) and according to the Endocrine Society (15). Vitamin D deficiency serum 25(OH) D < 50.0 nmol/L (20ng/mL); insufficiency serum 25(OH) D 50 - < 75 nmol/L (20-30 ng/mL); sufficiency serum 25(OH) D ≥ 75 nmol/L (30ng/mL). For some analyses, the following cut off value was also used to diagnose vitamin D sufficiency: ≥ 50 nmol/L according to World Health Organization and the Institute of Medicine (2,163).

2.6 Measurement of Bone Mineral Density (BMD)

Bone mineral density (BMD) was measured using double energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE medical systems, Madison, WI 53718, USA) in a subgroup of 102 women on the lumbar spine (L1 to L4) and the neck of the femur. The scanning precision (coefficient of variance, CV %) was calculated by two repeated measurements in 30 subjects. The precision (CV %) of the measurements were (1.0 -1.2%) and (1.1 – 2.2%) in the lumbar spine and the neck of the femur, respectively. A complete quality control test on DXA equipment was performed each day for BMD and composition calibration using spine phantom as recommended by the manufacturer, to ensure the stability of the system. Additional quality control was also done every six months or when required by the manufacturer's company. DXA scans were performed in the Department of Radiology at King Abdul-Aziz Medical City by licensed Radiological Technologists trained in bone density exams. Prior to scanning, women were asked to remove all jewelry and wear only hospital gowns during scanning procedures. Women were asked to lie on the DXA scanning bed for the duration of testing (approximately 10-15 minutes). The BMD values at the hip were compared with peak bone mass for Caucasian female normative database from the National Health and Nutrition Examination Survey NHANES III data, as recommended by the International Society for clinical Densitometry (164). In addition, the Saudi Osteoporosis Society (SOS) committee released guidelines in 2015 stating that in the absence of local normative reference, the committee suggests to continue using the data from the United States because the (NAHNES III) data are accurate and reliable and it seems to be best option until the local data becomes available (165). BMD values were classified according to WHO criteria, a low bone mass (osteopenia) if BMD T-score is < -1.0, or normal bone mass if BMD T-score is ≥ -1.0 (113).

3. Ethics Statement

The research protocol and the consent documents were approved by the ethics committee of the Institutional Review Board (IRB) at the University of Maryland, College Park and Institutional Review Board (IRB) at King Abdullah International Medical Research Center (KAIMRC), Jeddah, Saudi Arabia. Participants were informed about the aim of the study, of the clinical tests, and of the type of interview. The written informed consent process took place in a setting that afforded a sufficient level of privacy for the participants.

4. Sample Size and Power Analyses

An a-priori power analysis was conducted to determine the number of participants required to detect a small effect ($f2 = 0.10$) with power = 0.80, and at $\alpha = 0.05$. The analysis indicated that a sample size of 207 would be sufficient. The power analysis was conducted with G*Power 3.1 software package (166). To account for any potential loss of participants, an additional recruit of 20% was considered, and a total sample size was 250 women.

5. Statistical Analysis

Data were checked for missing values and outliers, and analyzed using the Statistical Package for Social Science (SPSS) software version 22.0 (SPSS, Inc., Chicago, IL, USA). The characteristics of the study population were described through frequencies and percentages for categorical variables. Non-parametric data were presented as median and range, and data with normal distribution were presented as mean $\pm$ standard deviation (SD). Percentage values between groups were compared using the Chi square tests. The relationship between demographic variables and knowledge score (normally distributed, with normality assessed via the QQ plot) was investigated using the following methods: 1) two-sample *t*-tests were conducted between knowledge score and marital status, employment status and income level, and 2) a one way analysis of variance (ANOVA) was conducted between knowledge score and age and education groups. For skewed variables (serum vitamin D, vitamin D intake, fish intake, length of sun exposure), differences between the two groups (vitamin knowledge groups) were tested using Mann-Whitney *U* test and Kruskal-Wallies test when more than two groups (attitude toward sun exposure group) were studied. Spearman rank correlation was used to assess the strength of the relationship of serum 25 (OH) D with intact PTH concentrations.

A multiple linear regression model was conducted to investigate if there was a relationship between knowledge of vitamin D and total vitamin D intake, after controlling for age and years of education (Model 1). Another multiple linear regression model was also conducted to investigate if there was a relationship between knowledge of vitamin D and serum vitamin D level, after controlling for age and years of education (Model 2). A p-value less than 0.05 indicated significance. The assumptions for regression models were evaluated using normal quantile-quantile (QQ) plots of the standardized residuals (the assumption of normality) and scatter plots with standardized residuals versus standardized predicted values (the assumption of homogeneous error variance). The assumptions of the regression models were satisfied.

Fisher's exact tests were conducted to investigate if there was a relationship between attitudes and behaviors toward sun exposure, and serum vitamin D level (a 2-level categorical variable: deficiency vs. sufficiency).

RESULTS

Characteristics of the Study Population

A total of 257 premenopausal Saudi women aged 20-50 years from Jeddah city were enrolled in this study between December 2014 and April 2015. The mean age was 29.8 $\pm$ 7.4 years, and 59.5% of the participants were within the young age group (20-30 years). The median vitamin D intake from diet and supplement was 236.4 IU/day. More than half of the women 65% were below the EAR for vitamin D 400 IU/day. Thirty seven percent of women reported using vitamin D supplements or multivitamins containing vitamin D in the past 30 days. The median duration of sunlight exposure in our study was 9.4 min/day, and 22.6% women reported daily exposure to sun more than 30 min/day between 10 am to 4 pm, and 77.7% women reported exposure to sun less than 30 min/day. Further population characteristics are shown in **Table 1**.

Serum 25(OH) D, PTH Concentrations and Bone Health

Among the 250 women who had a blood sample, the median concentration of 25(OH) D was 34.2 nmol/L. There was a high proportion of participants (77.6%) with vitamin D deficiency (25(OH) D < 50 nmol/L) (**Table1)**. Among those with the deficiency, 52 women were severely deficient (25(OH) D < 25 nmol/L) (52/250, 20.2% of the entire sample). Serum PTH concentrations were available for 187 women and are reported as medians due to non-normal distribution. The median serum intact PTH concentration was 76.8 pg/mL (range: 31-298 pg/mL). Regarding vitamin D status, significant inverse relationships were found between serum 25(OH) D and intact PTH concentrations, that is, lower 25(OH) D concentrations were associated with higher levels of PTH ($r^2 = -0.26$, $p < 0.001$). The inverse association between serum 25(OH) D and intact PTH was more evident in women with serum vitamin D < 50 nmol/L ($p = 0.002$) than women with serum ≥ 50 nmol/L ($p = 0.21$). Elevated PTH was detected in 15% (22/148) and 13 % (4/31) of the women with deficiency (25(OH) D < 50 nmol/L) and insufficiency (25(OH) D 50 - > 75 nmol/L), respectively, while no women with vitamin D sufficiency (25(OH) D ≥ 75 nmol/L) had elevated PTH. In addition, PTH was significantly and inversely correlated with serum calcium ($r^2 = -0.245$, $p = 0.001$) and serum phosphates ($r^2 = -0.14$, $p = 0.05$), while PTH was significantly and directly correlated with serum alkaline phosphates ($r^2 = 0.23$, $p = 0.001$). For the DXA subsample (n=102), the prevalence of osteopenia was 33 % and 30% in the lumbar spine and the neck of the femur, respectively **(Table1).** The results of the chi-square test of independence suggested that the prevalence of osteopenia between the lumbar spine and the neck of the femur was statistically significantly different ($\chi 2$ (1, N = 102) = 12.26, $p < 0.001$). Of the 102 women, 55 (54%) women did not suffer from osteopenia in both sites, whereas 18 (17.6%) women had osteopenia in both sites. The remaining 29 women (28.4%) had osteopenia in one of the sites. Approximately 45.5% (5/11) of our participants who experienced vitamin D deficiency and elevated PTH had lower BMD at either site.

Knowledge of Vitamin D

Knowledge of vitamin D for the participants is summarized in **Table 2.** Of the 257 participants, 99 % have heard of vitamin D, and 97% of participants thought vitamin D was important for their health. Sunlight was correctly identified as the single most important source of vitamin D by 91% of the participants. Only 37% of the participants have heard about vitamin D fortification in food. The top two dietary sources for vitamin D correctly identified by the participants were oily fish (48%) and fortified dairy products (36%). Only 5% thought meat was source of vitamin D, while 33% of participants indicated incorrect vitamin D sources such as leafy vegetables and fruits. According to the participants, the most important effects of vitamin D were osteoporosis prevention (75.7%) and bone health (72.9%). Of the 102 women underwent a DXA scan, approximately 32% of women who identified osteoporosis prevention and 30% of those who identified bone health as the two most important effects of vitamin D deficiency had osteopenia. Less than half 48% of the participants identified incorrect health effects of vitamin D such as vision health, and skin softness. Nearly 80% of participants believed that avoiding sun exposure would decrease the amount of vitamin D an individual can get followed by sun screen use

(25.9%) and older age (22.7%), and approximately 9% indicated an incorrect response such as fatty diets.

Association between Knowledge of Vitamin D and Sociodemographic Factors

The average score of knowledge of vitamin was 4.40 $\pm$ 1.1 with a minimum score of 0 and a maximum score of 6.75. Approximately 63% participants were more knowledgeable on vitamin D compared to 37% who were less knowledgeable on vitamin D "(refer to page 10 for the categorization of more/less knowledgeable on vitamin D)". **Table3** shows the analysis results of the relationship between knowledge of vitamin D and sociodemographic factors. The results of one way analysis of variance (ANOVA) showed a significant difference in vitamin D knowledge score among participants with different education levels ($F(2, 254) = 11.38$ $p < 0.001$). Levane's test for homogeneity of variances was non-significant therefore the assumption of homogeneity of equal variance was met. Follow-up tests (Tukeys's HSD) were conducted to evaluate pairwise differences among the three education levels. The results of the pairwise comparisons indicated participants did not attend colleges had significantly lower vitamin D knowledge score than participants attended colleges or participants attended graduate schools. There was no significant difference regarding vitamin D knowledge score among different age groups ($p = 0.686$, **Table3)**. However, some items in vitamin D knowledge questionnaire differed significantly among age groups. Younger participants were more aware of sunlight as source of vitamin D than older participants ($\chi 2$ (2, 255) = 10.097, $p = 0.007$, **Figure 1**). Conversely, older participants were more aware of oily fish as source of vitamin D ($p = 0.02$, **Figure2**).

The results of t tests demonstrated significant differences in mean vitamin D knowledge scores based on marital status, employment status, and income level (**Table3).** In particular, employed women had better vitamin D knowledge than unemployed women ($t(255) = 2.051$, $p = 0.041$). Women with a household income ≥ 15000 SR per month (upper middle and high household income) had better vitamin D knowledge than women with a household income < 15000 SR per month (low and lower middle household income) ($t(230)= -2.692$, $p = 0.008$). Unmarried women (single, divorced/widowed) had better vitamin D knowledge than married women, but this difference did not reach the significance level ($t(255) = 1.95$, $p = 0.052$).

Association between Knowledge of Vitamin D and Vitamin D Intake

In the bivariate analysis, the association between vitamin D knowledge and fish intake is shown in **Figure3.** Median fish intake in the vitamin D knowledgeable group (20.1 IU/day) was significantly higher compared to vitamin D intake in the less knowledgeable group (16.4 IU/day) ($p=0.046$). The association remained significant after adjustment for energy intake or after exclusion of women that used vitamin D supplementation. No significant difference was observed between vitamin D knowledge groups and other vitamin D intake sources.

The results of the multiple linear regression model with knowledge of vitamin D score as the dependent variable and total vitamin D intake, age, and education as the independent variables **(Table4-a)** suggested that there was no statistically significant relationship between knowledge of vitamin D and total vitamin D intake, after controlling for age and years of education ($t(253) = 0.380$, $p = 0.705$). There was no statistically significant relationship between knowledge of vitamin D and age (t(253) = 1.443, p = 0.150), but there was a statistically significant relationship between knowledge of vitamin D and years of education (t(253) = 5.899, $p < 0.001$). The R^2 = 0.124, indicates that 12% of the variation in the dependent variable can be accounted for by the model. The QQ plot of the residuals indicated most of the data points fell on the 45 degree line, which suggested that the residuals were normally distributed. The skewness and kurtosis for the residuals were -0.30 and -0.77, respectively. The residual plot suggested that the error variance was homogeneous. The assumptions of the regression model were satisfied and hence we conclude that the fitted model was adequate.

Association between Knowledge of Vitamin D and Serum 25 (OH) D Level

The results of multiple linear regression model with knowledge of vitamin D score as the dependent variable and serum vitamin D level , age, and education as the independent variables **(Table 4-b)** suggested that there was no statistically significant relationship between knowledge of vitamin D and serum vitamin D level, after controlling for age and years of education (t(246) = 0.820, p = 0.413). There was no statistically significant relationship between knowledge of vitamin D and age (t(246) = 0.928, p = 0.354), but there was a statistically significant relationship between knowledge of vitamin D and years of education (t(246) = 5.596, $p < 0.001$). The R^2 = 0.120, indicates that 12% of the variation in the dependent variable can be accounted for by the model. The QQ plot of the residuals indicated that most of the data points fall on the 45 degree line, which suggested that the residuals were normally distributed. The skewness and kurtosis for the residuals were -0.17 and 0.33, respectively. The residual plot suggested that the error variance was homogeneous. The assumptions of the regression model were satisfied and hence we conclude that the fitted model was adequate.

Attitudes and Behavior toward Sunlight Exposure and Serum Vitamin D Level

Responses from 257 women who answered questions regarding sun exposure are summarized in **Table 5.** 57.6% of women believed that sunlight exposure is good and healthy. Half of the participants 50% rarely like going in the sun, and about 45% did not use sun screen products. The majority of women 70% never wore colored *abaya* (wore the traditional black *abaya*) when they went outside in the sun. Natural and artificial tanning was not common practice among our participants; about 63% reported never exposed to the sun for this intension and 93% reported never seek a suntan from artificial tan such as tanning bed. The main reasons for avoiding sun were prevent skin pigmentation and specific health reasons such as headache and hot weather.

Table 6 shows the two-way table of attitudes and behavior toward sunlight exposure and serum vitamin D level. There was no statistically significant association between the responses of questions regarding sunlight exposure and vitamin D status, except of question 5 (I wear colored *abaya* when I go outside in the sun). In particular, participants never wearing colored *abaya* when going outside in the sun were more likely to have deficient serum vitamin D (25 (OH)D < 50 nmol/L) ($p = 0.016$). For further analysis, we dichotomized the response for “wear colored *abaya*” to yes (always, sometimes) versus no (never) to investigate if there was a relationship between women who wear colored *abaya* and women who wear black *abaya* and serum vitamin D level. The results of the Mann-Whitney test showed higher serum 25(OH) D levels in women who wear colored *abaya* (always, sometimes) compared to women who wear black *abaya* ($p = 0.004$, **Figure 4)**. Furthermore, we found that women who wear colored *abaya* spent more time in the sun in the past week compared to women who wear black *abaya* ($p < 0.001$, **Figure 5**). We compared sociodemographic characteristics between women who wear colored *abaya* (always, sometimes) and women who never wear colored *abaya* (wear black *abaya* only). In our data, we observed that women who wear colored *abaya* were more likely to be unmarried (Chi-square test 64% vs. 46%, $p < 0.01$), had attained higher levels of education (Mann-Whitney *U* test, $p = 0.003$), were more likely to be employed (Chi-square tests 64% vs. 46%, $p < 0.01$), and had higher household income ≥ 15,000 SAR per month (Chi-square tests 56% vs. 31%, $p < 0.001$) compared to women who never wear colored *abaya*. There was no significant difference ($p > 0.05$) in the mean age of women between the two groups (data not shown). Although there was no association between the response to the question "I like going outside in the sun" and serum vitamin D levels (Fisher exact test, p=0.072) **(Table 6),** the results of the Kruskal-Wallis tests showed a significant difference in serum 25(OH) D levels among the different responses to this question. The median serum vitamin D level were significantly higher (p=0.002) in women who said they often like going outside in the sun (40 nmol/L), compared to women who said that rarely (34 nmol/L) or never (29.6 nmol/L) like going outside in the sun (**Figure 6)**. Additionally, we found that women who said they often like going outside in the sun spent more time in the sun in the past week (median 12.2 min/day) compared to women who said that rarely (9.2 min/day) or never (2.1 min/day) like going outside in the sun (p < 0.005, **Figure** 7).

DISCUSSION

Knowledge of Vitamin D

This study provides insight regarding the current knowledge of the importance of vitamin D and attitudes toward sun exposure among premenopausal women attending the primary care clinic in Jeddah. Understanding the attitudes, behaviors, and knowledge of the community may help in developing an effective and appropriate intervention program (228). Overall, it seems that women in Jeddah are more aware of vitamin D than those reported in the published literature (37, 228, 230); for example, almost 99% of the women in the current study had heard of vitamin D, compared to 90% of parents of children (n= 1752) in Jeddah (231) and 63% of the men and women (n = 503, age 20-40 years old) in Sharjah, United Arab Emirates (229). In comparison, one internationally published report stated that 73% of Chinese middle aged and elderly men and women (n = 547) had heard about vitamin D (37), while 69% had heard about vitamin D in Australia (men and women, n = 1971) (230). Moreover, a higher proportion of our participants identified the sun as the most important source of vitamin D, when compared to previous studies conducted in Sharjah (229), Hong Kong (37), Australia (230), and India (232) (91% vs. 43%, 23%, 83%, and 53.3%, respectively). Gaps in the basic knowledge about vitamin D have been observed in previous studies among Saudi school girls (233), female Saudi university students (39), and Saudi women (40). The differences observed between the present study and previous studies may reflect the medical information about vitamin D provided to our participants while attending the primary health center, which may not be available to the general population.

In contrast, we observed a lack of knowledge when the women in this study were asked about vitamin D fortification or vitamin D intake sources, and less than half of the women were able to identify oily fish and fortified dairy products as sources of vitamin D. A minority of the women could name meat as a source of vitamin D, while about one-third of the women identified incorrect food sources of vitamin D, such as vegetables and fruits. These findings confirm the results of previous reports (234, 235). In addition, a lack of knowledge regarding the intake of vitamin D among Saudis has been noted by Christie et al. (2011), who conducted qualitative research on a sample of female Saudi university students (n = 17, age 20-25 years old), and found significant limitations in the students' understanding of vitamin D fortification (39). In another study conducted in Canadian university students (n = 1088), Boland et al. (2014) underscored the fact that university students have poor knowledge of vitamin D, which is particularly of concern because the food sources of vitamin D that were least identified are the sources that Canadians must rely during their limited sun exposure for half of the year because of the high latitude of Canada (234). In the present study, the lack of knowledge about vitamin D food sources is also troubling among our participants who rely more on vitamin D intake sources than sun exposure. This is largely due to the covering of the body by traditional clothing (black *abaya*) and time spent indoors, which limits their sun exposure. Overall, these findings highlight the need for increased awareness and improved knowledge of vitamin D among women, particularly vitamin D intake sources, in future intervention programs.

Although more than two-thirds of the women in the current study were aware of the major benefits of vitamin D on bone health and the prevention of osteoporosis, their actual vitamin D dietary intake was only 236.4 IU/day, which is lower than the U.S. Estimated Average Requirement (EAR) of 400 IU/day. Limited knowledge regarding vitamin D sources could be one of the factors associated with the insufficient intake of vitamin D, and this relationship has been identified previously. For example, Boland et al. (2014) found that the lack of vitamin D knowledge was associated with vitamin D insufficiency among Canadian university students. Researchers suggest that any efforts to address the lack of vitamin D knowledge among students could lead to changes in their knowledge and behavior toward healthier vitamin D intake choices (234).

The majority of the women in the current study were knowledgeable about the fact that avoiding sun exposure decreases the amount of vitamin D an individual takes in. However, they were less knowledgeable about the effects of other factors, such as sunscreen usage, older age, and skin color on the amount of vitamin D obtained from the sun, and potentially, on their serum vitamin D levels. These factors must be addressed in future public health programs aimed to decrease vitamin D deficiency.

Association between Demographic Factors and Vitamin D Knowledge

Previous studies have indicated significant associations between vitamin D knowledge and age, with some finding that the knowledge of vitamin D increased with age (236). Other researchers have found that younger individuals had more knowledge of vitamin D when compared to the older members of the population (37). In the present study, we did not find a significant association between the knowledge score and age, which is similar to the results of a study conducted by Salmanpour et al. (2016), who found that the age of the study participants had no influence on the mean vitamin D knowledge score (229). However, in the current study, we found that women 20-30 years old and 31-40 years old were more aware of sunlight as a main source of vitamin D than older women (41-50 years old), but they were not exposing themselves to the sun more often than the older women. This is consistent with research done in Hong Kong, in which Kung et al. (2006) found that older women were less aware of vitamin D than women of a younger age, but they were more likely to seek sun exposure when compared to younger women (37). Conversely, with regard to food sources of vitamin D, we found that middle aged women (31-50 years old) were more aware of oily fish as a source of vitamin D when compared to younger women (20-30 years old). This awareness has been associated with certain behaviors among middle aged women (31-50 years old), who tended to eat more fish when compared to younger women (20-30 years old). In addition, the results of the present study indicated that women aged 20-30 years old were the least knowledgeable about the health benefits of vitamin D for building bone strength. This may be the result of the many public health education programs in Saudi Arabia that have targeted the prevention of osteoporosis through the education of middle-aged women about the health benefits of vitamin D and calcium on bones and the prevention of osteoporosis. Our results highlight the need to further educate young women while they can still optimize their bone mass through dietary and lifestyle interventions.

The direct association between the educational level and vitamin D knowledge in the current study is not surprising, since previous studies have also found positive associations between knowledge and educational levels (228, 229). In addition, employed women and women with household incomes ≥ 15,000 Saudi Arabian Riyals (SAR) per month had better vitamin D knowledge than unemployed women and women with household incomes < 15,000 SR per month. These findings suggest that vitamin D educational programs should target specific population groups (i.e., low education, low household income, and unemployed) with the greatest need for information about vitamin D to implement any lifestyle changes (228).

Associations between Vitamin D Intake, Serum 25 (OH) D Level, and Vitamin D Knowledge

In this study, we observed insufficient vitamin D intake in women with more and women with less knowledge about vitamin D. The median dietary vitamin D intake for both groups was below the EAR of 400 IU/day. Due to the cross-sectional nature of this study, however, we cannot conclude whether increasing an individual's knowledge of vitamin D would result in behavior changes. However, in a previous intervention study of American young women (n = 80, 19–30 years old), Bohaty et al. (2008) found that although the participants gained knowledge from educational intervention about the importance of calcium and vitamin D in preventing osteoporosis, they showed no changes in their dietary intake of vitamin D or dairy products (237). In addition, the study showed that even after the educational intervention, the young women's intake of vitamin D and dairy products failed to meet the recommended daily allowance (RDA). Therefore, the researchers suggest that, in addition to providing information about foods high in calcium and vitamin D to young female patients at risk for osteoporosis, health practitioners could ask these young women to set specific behavioral goals for increasing their intake of vitamin D and calcium. Follow up visits could be scheduled to ensure that they are meeting these goals (237).

Being aware of fish as a rich source of vitamin D resulted in an increase in its consumption among our sample, with the median fish intake in the vitamin D knowledgeable group being significantly higher than in the less knowledgeable group. Undoubtedly, improving the knowledge and public health education with regard to vitamin D in Saudi Arabia can motivate young women to adopt healthy eating behaviors, and may be an effective step toward increasing women's responsibility for preventing vitamin D deficiency and osteoporosis over the long term (37).

Overall, low serum vitamin D was observed in the present study, with 75.5% of the women having vitamin D deficiency [25(OH)D < 50 nmol/l]. The vitamin D deficiency was similar between the women with both more and less knowledge about vitamin D, which is inconsistent with a previous study conducted in the Netherlands by Oudshoorn et al. (2011), finding that the participants with the most knowledge about vitamin D had a mean serum 25(OH) D level of 58.5 nmol/l (36). This level was approximately twice that of those participants with the least knowledge about vitamin D.

In the present study, the multivariate analysis of the determinants of vitamin D knowledge showed that the educational level of the women was the only predictor of vitamin D knowledge. These findings agree with the results obtained in a study of Vietnamese men and women (n = 1,536, age 14-85 years old), which reported that an awareness of vitamin D, particularly its benefits in bone health, was significantly related to a higher educational level (228). Our results were of special concern, because so many of the participants were highly educated women (college graduate), which suggests that the general population in Jeddah may be even less aware of vitamin D than the participants in the current study. Our findings emphasize the importance of effective intervention strategies targeting young women with low educational levels, to educate them about vitamin D sources and the prevention of vitamin D deficiency.

Attitudes and Behaviors toward Sunlight Exposure

Based on previous recommendations, 5-30 minutes of sun exposure between 10 am and 3 pm twice or three times each week during the summer season is sufficient to maintain optimum levels of vitamin D (1). In the present study, the duration of sunlight exposure was low, and more than half of the women were either never exposed to the sun or exposed less than 10 min per day. These results are consistent with the findings of other studies conducted in Saudi Arabia (40) and Kuwait (238). For example, Habib et al. (2014) reported that 46% of Saudi women in Riyadh (n = 310, age ≥ 20 years old) did not expose themselves to the sun daily, and among those who did expose themselves to the sun daily, 16.6% exposed themselves less than 10 min per day (40). Moreover, a lack of consistency between the attitudes toward sun exposure and knowledge of vitamin D was found in this study. While the majority of the women believed that sun exposure was good for their health, and that the human body can obtain vitamin D through exposure to sunlight, more than half of the women disliked being exposed to the sun.

Concerns about the strength of the sun in Jeddah, symptoms such as fatigue and headache, a desire to prevent darkening of the skin, cultural norms requiring women to cover their bodies, and hot climatic conditions for most of the year were the most common reasons for avoiding the sun. Our results were in accordance with the findings of another study conducted in Saudi Arabia, in which Christie et al. (2011) reported that the hot climate and cultural norms requiring women to cover their bodies were major barriers to sun exposure among Saudi university students (39). Despite being allowed only limited sun exposure in public areas for cultural reasons, many of the participants reported that they had private courtyards in their houses where they could spend some time in the sun without veils. Unfortunately, most of the women who did have courtyards in their houses tended to avoid direct exposure to sunlight because of the heat or for cosmetic reasons (e.g., minimizing skin discoloration and laser peel treatments).

However, a concern about the risk of skin cancer was not the main reason for avoiding the sun in the current study, which was dissimilar to the results of a previous study in New Zealand ($n = 235$, age ≥ 20 years old); Von Hurst et al. (2009) reported that skin cancer was the most prevalent reason for a lack of sun exposure, and that half of the participants would spend more time in the sun if they were not worried about skin cancer (160). These findings reflect the public health messages about sun safety, and the awareness of the dangers of skin cancer in New Zealand (160, 239). Moreover, lifestyles and personal choices were also identified as reasons to avoid the sun. More than half of our participants were employed, and they reported their long working hours indoors as the reason for not getting enough sun. These women would spend more time in the sun if they had more time. Studies of employed women in New Zealand (160) and Hong Kong (37) also reported long working hours, working in office buildings, and driving to work as reasons they lacked exposure to the sun.

In the current study, there was a lack of information and clear advice regarding the amount of time that the women should be exposed to ultraviolet B radiation (UVB), and about what time of day an individual could obtain adequate sun exposure in order to improve their serum 25(OH) D levels. Therefore, the optimum sun exposure times during the summer in four different cities [northern (Arar), southern (Abha), western (Makkah), and eastern (Dammam) regions] in Saudi Arabia were determined based on previtamin D3 conversion (240). Ampoules containing 7-dehydocholestoral in ethanol were exposed to the sun every hour from sunrise to sunset, and the investigators found that the geographical location, as well as the time of the day, influenced the vitamin D production. The study suggested that the optimum sun exposure times for vitamin D3 production in the northern, southern, and western regions during the summer months were from 8:30 am to 10:30 am and 2:00 pm to 4:00 pm, while the maximum UVB hours were between 10:30 am and 2:00 pm. These hours are believed to be most responsible for sun burns and skin cancer. In the eastern region (Dammam city), a significant reduction (around 50%) was found in the conversion of previtamin D3 when compared to the other cities. Dammam is an industrial city, and air pollution could explain this reduction (240). The optimal sun exposure times in Dammam were from 8:30 am to 10:00 am and 1:00 pm to 2:30 pm. In another study in Riyadh city, Alshahrani et al. (2013) found that the optimum time for sun exposure for the production of vitamin D3 in Riyadh was from 10:00 am until 2:00 pm during the winter (241).

Healthcare professionals should be informed and updated about the guidelines for sun exposure in order to educate the general community, which may effectively help the general public increase their vitamin D levels and prevent vitamin D deficiency throughout the country. A recent study in Saudi Arabia (199) suggested increasing the dietary intake of vitamin D (food and supplements) during the summer, because of the reduced amount of outdoor activity and extreme heat. However, Algamdi et al. (2015) reported that 66% of Saudis spend extra time outdoors during the winter, when compared to the summer (242); therefore, sunlight exposure is a cost free and relatively risk free option for restoring serum vitamin D levels (241). More studies need to be done to determine the optimum sun exposure times during the winter in other regions in Saudi Arabia.

Sunscreen with a sun protection factor of 15 should absorb 99% of all solar UVB radiation, resulting in a 99% decrease in the production of previtamin D3 (11). A high proportion of our sample was uncertain about whether or not sunscreen affected serum vitamin D levels, and only 26% of the women believed that the use of sunscreen actually affected those levels. However, this proportion still appeared to be much higher than that previously reported (11%) among participants in Australia who believed that sunscreen might lead to a lack of vitamin D. In Australia and New

Zealand, public health campaigns recommend sunscreen use whenever the skin is exposed to the sun to prevent skin cancer; therefore, the participants were more concerned about preventing skin cancer than about getting enough vitamin D from the sun (230, 239).

Darker skin pigmentation is also an effective sunscreen, and reduces the capacity of the skin to synthesize vitamin D from sunlight (11). For example, individuals with skin color types 5 and 6 (never burn and always tan) require 5-10 times longer exposure to the sun when compared to Caucasians in order to make the same amount of vitamin D (11). It has been suggested that 2-6 minutes of sun exposure per day in the summer, and 4-17 minutes per day in the winter are sufficient for vitamin D production in fair skinned individuals (230). However, a high proportion of our sample was unaware of the effects of skin color on vitamin D production. Further emphasis is needed in public education regarding this issue, since the majority of our participants have skin types 3 and 4, and require longer sun exposure times than fair skinned individuals (skin types 1 and 2).

Protective traditional clothing, rather than sunscreen, was identified as the most commonly used sun protection measure in Saudi Arabia (242). Saudi women usually wear traditional black veils (called *abaya*) over their clothing, covering the whole body, with the exception of the hands and face (in some women), which are exposed to sunlight. In the present study, no differences were found in the serum 25(OH) D levels between those women who were fully veiled (covered their faces, but their hands were exposed) and partially veiled (exposed their faces and hands). Recently, some young Saudi women have begun wearing colored *abayas* (e.g., off-white, beige, gray, blue) particularly in Jeddah. In the current study, about 7% of the women usually wore colored *abayas*, and 23% of the women wore colored *abayas* only occasionally. The women who wore colored *abayas* in the sun had significantly higher serum vitamin D levels when compared to those women who never wore colored *abayas*. However, it is not possible to identify whether these difference are because of the color or fabric of the *abaya*.

Previous studies on the association between the color and fabric of clothing and serum vitamin D levels are limited; however, experimental biology studies have shown that clothing inhibits the production of previtamin D and reduces serum vitamin D levels, and that the transmission of previtamin D effective radiation depends on the fabric type (64). In one study in Oman, Salih (2004) investigated the influence of 15 different fabric samples (woven polyester, spun polyester, a mixture of polyester and cotton, a mixture of polyester and wool, and white, light brown, brown, and black colors) used in Oman by both men and women on the photo transformation of 7-dehydrocholesterol to previtamin D3 (65). The investigator found that the type of fabric used may greatly inhibit the photoproduction of vitamin D3 to the extent that an individual would require dietary vitamin D supplementation. With regard to the color of the fabric, the investigator found that white fabric attenuates more light than other colors. However, Parisi et al. (2005) found that the effect of color was more related to the fabric type, with higher levels of synthesized of previtamin D under white than black eyelet fabric, while the reverse was observed for jersey fabric (243). Moreover, some studies have shown that covering the skin with any type of clothing prevents the cutaneous production of cholecalciferol. For example, a study performed by Matsuoka et al. (1992) in human volunteers wearing fabrics made out of white or black cotton, wool, or polyester exposed to simulated sunlight for up to 40 min showed no elevation in the circulating concentrations of vitamin D (66). Overall, our results highlight the need for further investigation, considering the magnitude of sunlight attenuation by the different colors and fabric types of the *abayas* of Saudi women, and their effects on the photoproduction of vitamin D.

To the best of our knowledge, this is the first study to examine the relationships between vitamin D knowledge, attitudes, and practices toward sun exposure, and serum vitamin D levels among premenopausal women living in Jeddah city. Our data provides important information for building appropriate intervention strategies for the prevention of vitamin D deficiency among young women in Jeddah. However, a few limitations have been identified; for example, the sample was derived from the primary health center at King Abdul-Aziz Medical City in Jeddah, and therefore, may not be generalizable to all women residing in Jeddah or in the other regions of Saudi Arabia. Furthermore, response bias may influence the results due to the high educational levels of our participants, which may account for the greater knowledge of vitamin D than the general population of women in Jeddah. Sun exposure practices could also be more common in this group than in the general population, since educational level has been linked to sun exposure (16, 39).

CONCLUSION

Recently, it has been recommended that the people of Saudi Arabia should increase their vitamin D intake from food and supplements during the summer because of the reduced amount of outdoor activity and extreme heat (199). However, the lack of knowledge of vitamin D sources, and the factors affecting serum vitamin D levels remains high in this sample of premenopausal women in Jeddah. Moreover, there is conflicting knowledge regarding the benefits of sunlight as a main source of vitamin D and a negative attitude towards sun exposure among many young women who were at the greatest risk of vitamin D deficiency. Given the importance of vitamin D in maintaining bone health, this negative attitude may have an adverse effect on women's bones in the long term. This research supports the need for clear advice and accessible reliable information concerning vitamin D sources and the effects of exposure to the sun, with the aim of specifically targeting the most vulnerable sector of the population: young women with a low level of education, and low household income. Educational programs should place more emphasis on the importance of sunlight and provide information concerning the best time of day for obtaining adequate sun exposure, particularly during winter, since exposure to the sun is a cost free and relatively risk free option for restoring serum vitamin D levels (241). It would also be beneficial to encourage women who have private courtyards in their houses to spend some time in the sun without veils and to create more private areas for those who don't have their own space. Further studies considering the magnitude of sunlight attenuation by *abayas* of different colors and fabric may be needed to determine whether specific colors and/or fabrics will directly affect the photoproduction of vitamin D.

APPENDICES 3

TABLE 1

Sociodemographic, lifestyle characteristics of Saudi premenopausal women (20-50 years) attending the primary health care center at KAMC*, Jeddah (n=257).

Characteristics	Categories	Frequency (%)
Age (years)	20-30	153 (59.5)
	31-40	73 (28.4)
	41-50	31 (12.1)
Education status	Less than college	74 (28.8)
	College graduate	163(63.4)
	More than College	20 (7.8)
Income level (SR per month)	< 4000	15 (5.8)
	4000 - < 8000	50 (19.5)
	8000 - < 15000	78 (30.4)
	15000 - < 25000	53 (20.6)
	≥ 25000	36 (14.0)
Occupation	Employed	133 (51.8)
	Unemployed	124 (48.2)
Type of residence	Rented (apartment /home)	114 (44.4)
	Owned (apartment /home)	143 (55.6)
Marital status	Unmarried(single/separated/divorced)	145 (56.4)
	Married	112 (43.6)
Daily sun exposure (min/day)	< 30 min/day	199 (77.4)
	≥ 30 min/day	58 (22.6)
Area of skin exposed	Face covered but hands exposed	164 (63.8)
	Face and hands exposed	93 (36.2)
Skin color type [a]	II	6 (2.3)
	III	106 (41.2)
	IV	132 (51.4)
	V	13 (5.1)
Vitamin D intake (IU/day) diet + supplement	< 400	167 (65.0)
	≥ 400	90 (35.0)
Vitamin D supplement use (IU/day)	No	161 (62.6)
	Yes	96 (37.4)
Serum 25(OH)D level (nmol/L)	Deficiency < 50 nmol/L	194 (77.6)
	insufficiency 50 - < 75 nmol/L	43 (17.2)
	Sufficiency ≥ 75 nmol/L	13 (5.2)
BMD at lumber spine[b]	Low bone mass	34 (33.3)
	Normal bone mass	68 (66.7)
BMD at femur neck[b]	Low bone mass	31 (30.4)
	Normal bone mass	71 (69.6)

****Abbreviation; KAMC: King Abdul-Aziz Medical City; 25(OH) D : serum 25hydroxyvitamin D; IU: international unit. SR; Saudi Riyals; BMD: bone mineral density. [a] Based on Fitzpatrick skin-type scale classification (Fitzpatrick, 1988), II : fair skin that usually burns and sometimes tan. III: fair to medium skin that moderately burns and always tan gradually. IV: medium skin tone that rarely burns and always tans we V: olive to dark skin tone that very rarely burns and tans very easily .[b] Bone mineral density of subsample (n=102) underwent a DXA scan.***

TABLE 2
Knowledge of vitamin D among Saudi premenopausal women aged 20-50 years attending the primary health care center at KAMC Jeddah (n=257).

Knowledge of vitamin D	Frequency (%)
Having heard/learnt about vitamin D	
Yes	255 (99.2)
No	2 (0.8)
Vitamin D is important for health	
Yes	248 (96.5)
Don't know	7 (2.7)
Most important source of vitamin D	
Natural food sources	6 (2.3)
Sunlight**	233 (90.7)
Supplements	4 (1.6)
Do not know	12 (4.7)
Heard about vitamin D fortification in food	
Yes	95 (37.0)
No	160 (62.3)
Dietary sources for vitamin D[a]	
Oily fish**	122 (47.5)
Fortified dairy products **	92 (35.8)
Leafy vegetables	65 (25.5)
Eggs **	64 (25.1)
Citrus fruits	19 (7.5)
Meat**	12 (4.7)
Most important effect of vitamin D[a]	
Bone health**	186 (72.9)
Osteoporosis prevention**	193 (75.7)
Hair growth	87 (34.1)
Immunity**	51 (20.0)
Skin softness	21 (8.2)
Vision health	14 (5.5)
Factors that can decrease the amount of vitamin D[a]	
Avoid of sun exposure**	199 (78.0)
Sunscreen**	66 (25.9)
Age**	58 (22.7)
Skin color**	33 (12.9)
Fatty diet	24 (9.4)

Note: Participants were able to choose more than one option therefore percentages do not add up to 100%. ** Indicate the correct answer. KAMC; King Abdul-Aziz Medical City.

TABLE 3

Associations between vitamin D knowledge score and demographics variables among Saudi premenopausal women aged 20-50 years attending the primary health care center at KAMC Jeddah (n=257).

Variables value	N	Vitamin D knowledge score Mean + SD	*P*
Age			
20-30	153	4.3 +1.1	0.686[a]
31-40	73	4.5 + 0.9	
41-50	31	4.4 + 1.3	
Marital status			
Unmarried (single/separated/divorced)	145	4.5 + 1.2	0.052[b]
Married	112	4.2 + 1.0	
Education status			
Less than college	74	3.9 + 1.2	< 0.001[a]*
College graduate	163	4.6 + 0.9	
More than College	20	4.6 + 1.1)	
Employment status			
Unemployed	124	4.2 +1.2	0.041[b]*
Employed	133	4.5 + 0.9	
Income level (SR per month)			
< 15000	143	4.2 +1.1	0 .008 [b]*
≥15000	89	4.6 +0.9	

*Abbreviation: KAMC; King Abdul-Aziz Medical City; [a.] One way analysis of variance test was performed; [b] t tests was performed; * indicates significance at the 0.05 level.*

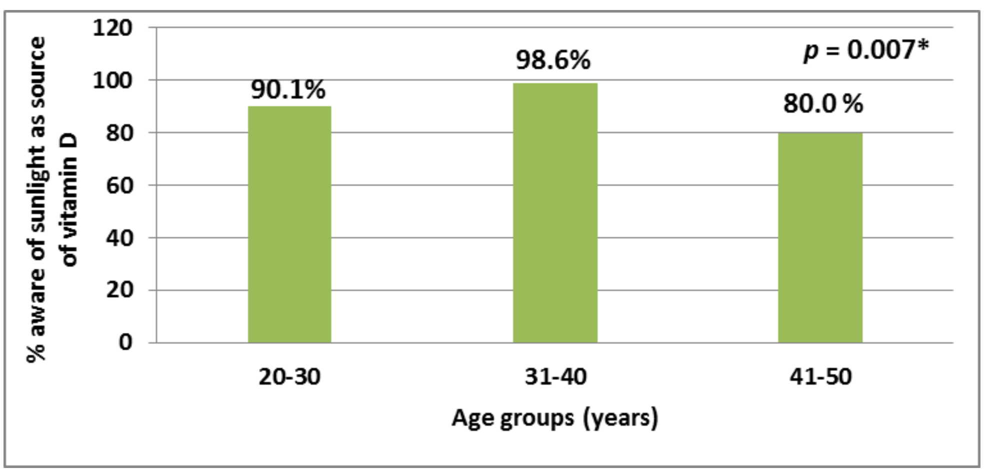


FIGURE 1. Percentage of women aware of sunlight as main source of vitamin D stratified by age Chi square tests was performed;* indicates significance at the 0.05 level.

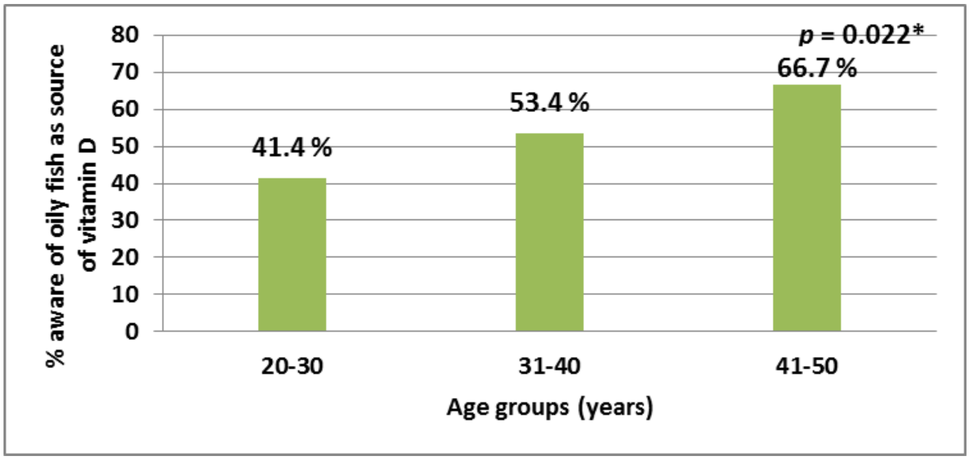


FIGURE 2. Percentage of women aware of oily fish as source of vitamin D stratified by age. Chi square tests was performed;* indicates significance at the 0.05 level.

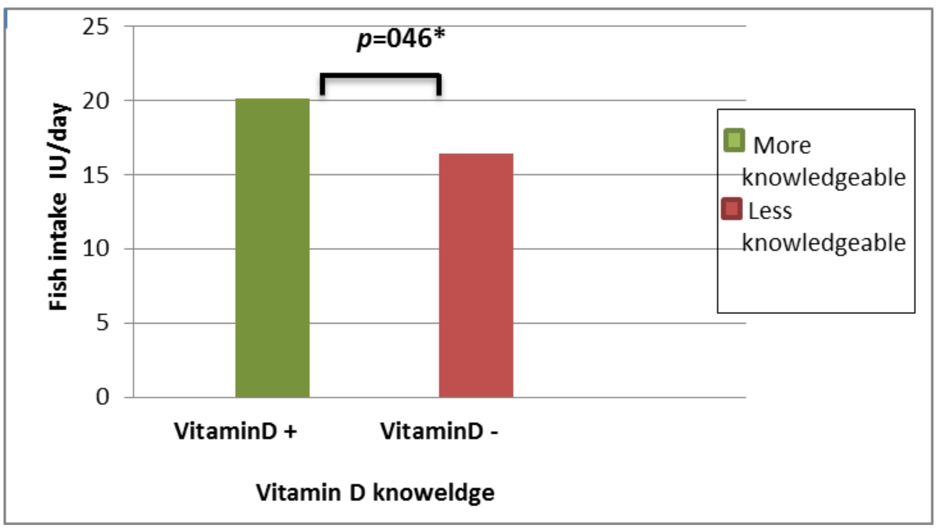


FIGURE 3. Median fish intake IU/day by vitamin D knowledge groups. Mann-Whitney *U* test was performed. * indicates significance at the 0.05 level.

TABLE 4-a
Regression analysis of vitamin D intake , age and years of formal education according to vitamin D knowledge for Saudi premenopausal women attending the primary health care center at KAMC, Jeddah

	Unstandardized Coefficients		Standardized Coefficients			Collinearity Statistics	
	B	SE	Beta	t	P	Tolerance	VIF
Intercept	2.096	0.448		4.677	0.000*		
Total vitamin D intake	0.000029	0.000076	0.023	0.380	0.705	0.955	1.048
Age	0.013	0.009	0.088	1.443	0.150	0.935	1.069
Years of education	0.123	0.021	0.352	5.899	0.000*	0.975	1.026

Note: Dependent variable* = knowledge of vitamin D. *N = 257. B = Unstandardized regression coefficients. SE = standard error. Beta = Standardized regression coefficients. t = t-statistic. p = p-value. * indicates significance at the 0.05 level.

TABLE 4-b
Regression analysis of serum 25(OH) D levels, age and years of formal education according to vitamin D knowledge for Saudi premenopausal women attending the primary health care center at KAMC, Jeddah

	Unstandardized Coefficients		Standardized Coefficients			Collinearity Statistics	
	B	SE	Beta	t	P	Tolerance	VIF
Intercept	2.289	0.443		5.173	0.000*		
Serum vitamin D levels	0.003	0.003	0.051	0.820	0.413	0.914	1.094
Age	0.008	0.009	0.059	0.928	0.354	0.896	1.116
Years of education	0.115	0.021	0.341	5.596	0.000*	0.965	1.036

Note: Dependent variable = knowledge of vitamin D. N = 250. B = Unstandardized regression coefficients. SE = standard error. Beta = Standardized regression coefficients. t = t-statistic. p = p-value. * indicates significance at the 0.05 level.

TABLE 5
Attitudes and behavior toward sunlight exposure among Saudi premenopausal women aged 20-50 years attending the primary health care center at KAMC Jeddah (n=257).

Sun exposure questions	Responses	Frequency (%)
1. I believe sunlight exposure is good for my health	Always	148 (57.6)
	Sometimes	108 (42.0)
	Never	1 (0.4)
2. I like going outside in the sun	Often	80 (31.1)
	Rarely	129 (50.2)
	Never	47 (18.3)
3. I enjoy spending time outside (courtyards) in the sun	Often	25 (9.7)
	Rarely	87 (33.9)
	Never	46 (17.9)
4. I use sunscreen when I go outside	Always	38 (14.8)
	Sometimes	51 (19.8)
	Rarely	53 (20.6)
	Never	115 (44.7)
5. I wear colored *abaya* when I go outside in the sun	Always	19 (7.4)
	Sometimes	59 (23.0)
	Never	179 (69.6)
6. I spend time in the sun (in private area) in order to get a tan	Often	21 (8.2)
	Rarely	75 (29.2)
	Never	161 (62.6)
7. I seek a suntan from artificial tan such as tanning bed	Often	1 (0.4)
	Rarely	16 (6.2)
	Never	239 (93.0)
8. The primary reason for avoiding the sun is	Custom/religion	22 (8.6)
	Public health messages	4 (1.6)
	Specific health reasons	34 (13.2)
	Hot weather	20 (7.8)
	To avoid dark skin	101 (39.3)
	Do not avoid the sun	108 (42.0)
9.I would spend more time in the sun if [a]	I wasn't worried about skin cancer	24 (9.3)
	I had more time	106 (41.0)
	I had more privacy	76 (29.6)
	I would not spend more time in the sun	98 (38.1)

Note: For "I like going outside in the sun" and "I seek a suntan from artificial tan such as tanning bed", participants were excluded from the analysis if answering "Don't know" (n = 255). For "I enjoy spending time outside (courtyards) in the sun", participants were excluded from the analysis if answering "No courtyards" (n= 154). [a] Participants were able to choose more than one option therefore percentages do not add up to 100%. Abbreviation: KAMC; King Abdul-Aziz Medical City;

TABLE 6
Attitudes and behavior toward sunlight exposure and serum vitamin D level among Saudi premenopausal women aged 20-50 years attending the primary health care center at KAMC Jeddah (n=250)

		Serum vitamin D level		
		Deficiency	**Sufficiency**	**p-value**
1. I believe sunlight exposure is good for my health	**Always**	**114 (58.8)**	**30 (53.6)**	**0.642**
	Sometimes	**79 (40.7)**	**26 (46.4)**	
	Never	**1 (0.5)**	**0**	
2. I like going outside in the sun	**Often**	**55 (28.4)**	**24 (43.6)**	**0.072**
	Rarely	**100 (51.5)**	**25 (45.5)**	
	Never	**39 (20.1)**	**6 (10.9)**	
3. I enjoy spending time outside (courtyards) in the sun	**Often**	**19 (15.3)**	**6 (20.0)**	**0.465**
	Rarely	**67 (54.0)**	**18(60.0)**	
	Never	**38 (30.6)**	**6 (20.0)**	
4. I use sunscreen when I go outside	**Always**	**24 (12.4)**	**14 (25.0)**	**0.138**
	Sometimes	**40 (20.6)**	**8 (14.3)**	
	Rarely	**41 (21.1)**	**12 (21.4)**	
	Never	**89 (45.9)**	**22 (39.2)**	
5. I wear colored *abaya* when I go outside in the sun	**Always**	**12 (6.2)**	**7 (12.5)**	**0.016***
	Sometimes	**40 (20.6)**	**19 (33.9)**	
	Never	**142 (73.2)**	**30 (53.6)**	
6.I spend time in the sun (in private area) in order to get a tan	**Often**	**12 (6.2)**	**8 (14.3)**	**0.121**
	Rarely	**60 (30.9)**	**13(23.2)**	
	Never	**122 (62.9)**	**35 (62.5)**	
7.I seek a suntan from artificial tan such as tanning bed	**Often**	**0**	**1 (1.8)**	**0.165**
	Rarely	**14 (7.2)**	**2 (3.6)**	
	Never	**179 (92.3)**	**53 (94.6)**	

Note: For "I like going outside in the sun" and "I seek a suntan from artificial tan such as tanning bed", participants were excluded from the analysis if answering "Don't know" (n= 249). For "I enjoy spending time outside (courtyards) in the sun", participants were excluded from the analysis if answering "No courtyards" (n= 154). Numbers in parentheses are %. Serum vitamin D 25(OH) D deficiency was defined according to Institute of Medicine as 25(OH) D < 50 nmol/L, sufficiency as 25(OH) D ≥ 50 .* indicates significance at the 0.05 level.

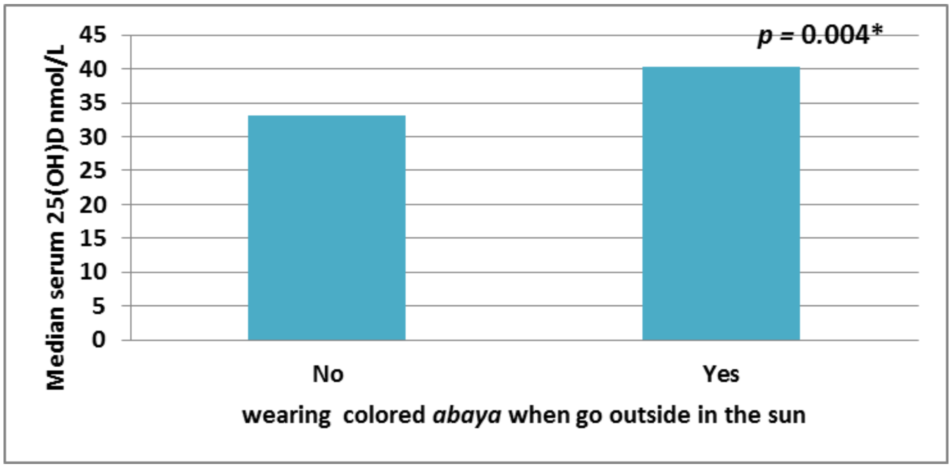


FIGURE 4. Association of wearing colored *abaya* with median serum 25(OH) D levels. Mann-Whitney *U* test was performed. * indicates significance at the 0.05 level.

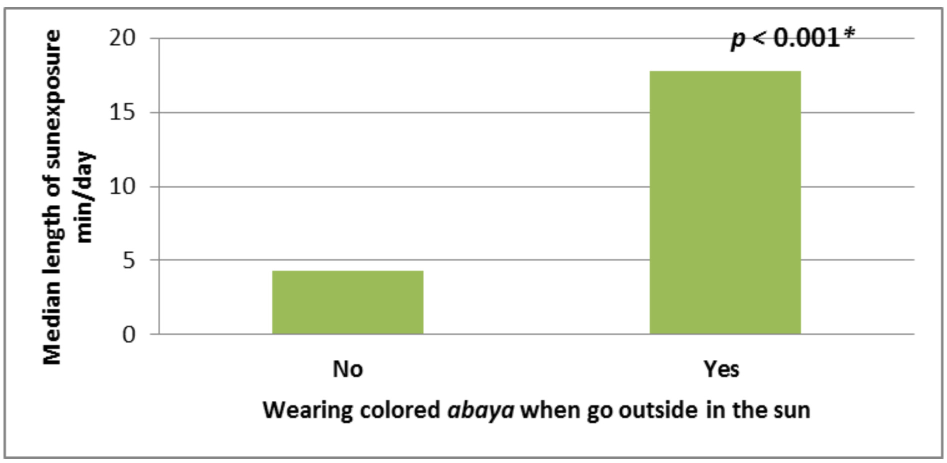


FIGURE 5. Association of wearing colored *abaya* with median length of sun exposure min/day. Mann-Whitney *U* test was performed. * indicates significance at the 0.05 level.

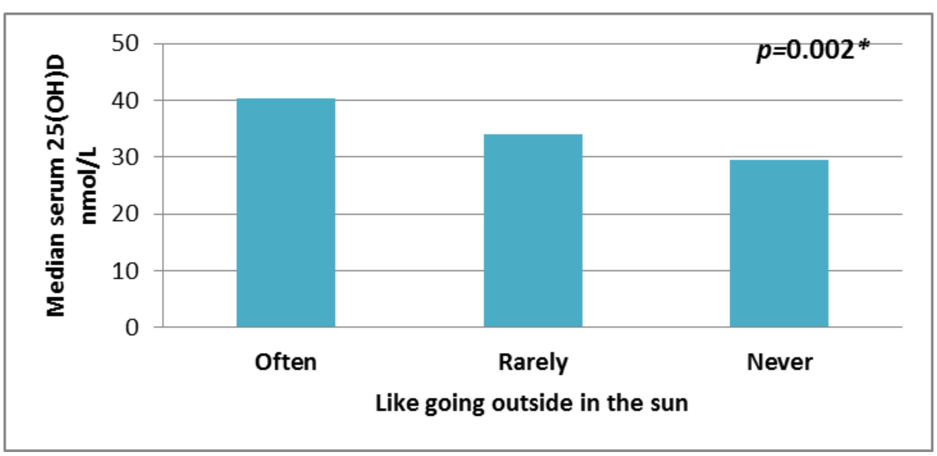


FIGURE 6. Association of attitude toward sun exposure with median serum 25(OH) D levels. Kruskal-Wallies test was performed; * indicates significance at the 0.05 level.

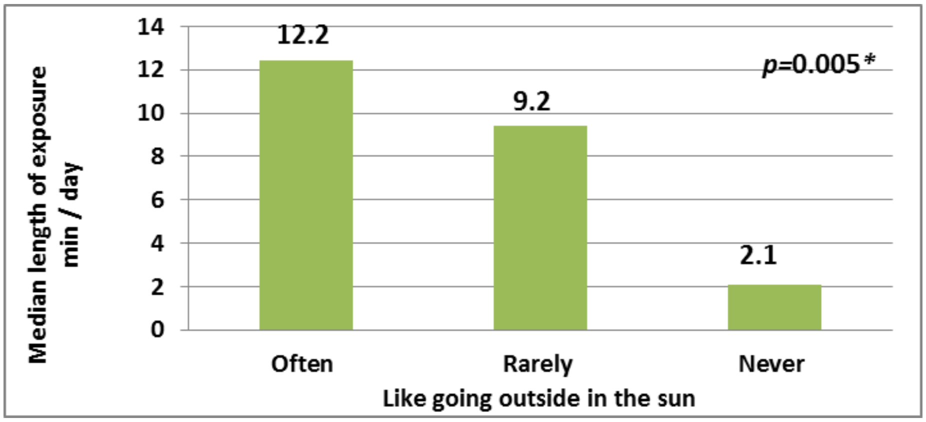


FIGURE 7. Association of attitude toward sun exposure with median length of sun exposure min/day . Kruskal-Wallies test was performed; * indicates significance at the 0.05 level.

Chapter 5: Summary and Conclusions

Despite the growing interest in vitamin D research in Saudi Arabia, this study is the first to provide new data on realistic vitamin D intake estimates based on vitamin D content from both food and supplement sources that make the biggest contributions among premenopausal women in Jeddah and among the subgroup most vulnerable to insufficient vitamin D intake. This study is one of few to include data on bone mineral density (BMD) in young Saudi women, and is the first to assess young women's dietary intake of vitamin D and calcium as it relates to BMD.

The present study focused on 20-50 year old premenopausal woman attending the primary care clinic at King Abdul-Aziz Medical City in Jeddah, Saudi Arabia. It aims to fill gaps in the existing literature by examining predictors that are associated with vitamin D status, including dietary vitamin D and calcium intake, sunlight exposure, skin color, and anthropometric measures. The study also assessed the adequacy of vitamin D intake among premenopausal women in Jeddah as compared to the U.S. Estimated Average Requirement (EAR) of 400 IU/day, investigated the sources of vitamin D in their diet, and examined potential determinants of vitamin D intake. Furthermore, it evaluated the association between dietary calcium, vitamin D intake, serum vitamin D levels (25(OH)D), and serum parathyroid hormone levels (PTH) as they related to BMD in a subgroup of premenopausal women in Jeddah. Finally, it examined the women's overall knowledge of vitamin D, their attitudes toward sun exposure, and associations between these factors and the women's serum vitamin D levels.

Our results confirm that vitamin D deficiency is highly prevalent (77.6%) among premenopausal Saudi women in Jeddah. Young women, and women with low vitamin D intake from food and supplemental sources, have been identified as at risk of vitamin D deficiency. More importantly, we have also shown that vitamin D deficiency is associated with increased risk of elevated PTH levels in this sample. These results are of particular concern given the increase we observed in the subjects' PTH levels which, together with decreasing 25(OH) D levels in young women, which suggests that these women are at risk of impaired bone mineralization and bone loss. The prevalence of osteopenia in one-third of our DXA (double energy X-ray absorptiometry) subsample reveals the need for intervention and educational campaigns to reduce occurrences of low bone mass in young women, because if left untreated; they may lead to osteoporosis and fractures, which are growing public health and economic issues in Saudi Arabia. Saudi Arabian studies have shown that the prevalence of osteoporosis among postmenopausal women ranges from 23% to 44.5% across different regions of the country (214, 215, 216). In a retrospective study of all patients admitted to King Fahd University Hospital in Khobar, Saudi Arabia between January 2001 and December 2006 (217), forty-three patients were admitted with hip fractures, which included 23 males and 20 females with an average age of 72 years and total hospital stays of 760 days. The direct cost of treating the hip fractures was SR2.09 million (US$ 557,333.00) at the rate of SR48,712 (US$ 12,9890.90) per patient. Early screenings of serum vitamin D level and parathyroid hormone in young women would be an important step toward reducing the risk of vitamin D deficiency and its complications in this age group, which is especially important given the increased costs of treating fractures and the reduced quality of life experienced by women with osteopenia and osteoporosis.

Insufficient intake of vitamin D and calcium is a growing concern for premenopausal women in Jeddah. The study showed that this sample of 20-50 year old Saudi women did not consume sufficient amounts of dietary vitamin D and calcium to meet the EAR for this age group. Our data provides additional evidence that sufficient vitamin D intake from food and supplements is important; we found a statistically significant, and positive, association between vitamin D intake and serum 25(OH)D levels. In cases of low vitamin D intake, it is important to consider supplementation as an effective approach to help ensure sufficient vitamin D levels and to address vitamin D deficiency among women. Calcium intake insufficiency was more pronounced in women with low lumbar spine bone mass than in women with normal bone mass, and we observed a statistically significant relationship between calcium intake and lumbar spine BMD.

This study supports the call for intense public health strategies to improve nutrition among young women. Such strategies should start in adolescence, before peak bone mass is reached, to help provide diets rich in calcium and vitamin D and promote physical activity. Dairy products provide the most calcium and vitamin D in the average diet; it is important for girls to meet the recommended intake of dairy products during peak height velocity (227). Healthcare professionals must have more nutritional knowledge in order to educate young women regarding the importance of sufficient dietary vitamin D and calcium intake to optimize bone health and decrease their risk of fractures. We suggest that young women should be encouraged to select products fortified with vitamin D. Furthermore, lactose intolerant women should be educated to make appropriate dietary substitutions, such as well-tolerated dairy products like Laban and yogurt.

By analyzing the various vitamin D sources, we have shown that women have a high consumption rate of fortified dairy products, which explains the role of dairy products, particularly Laban and yogurt, as major sources of vitamin D. In 2014, the Saudi Food and Drug Authority (SFDA) issued a mandatory circular regarding vitamin D fortification that manufacturers were to adopt within a six-month period and continue to adhere to. The standards for this vitamin D fortification policy were developed by the Gulf Standard Organization and mandated that vitamin D should be added to whole milk, skimmed milk, and Laban at a level of 400 IU/L and to yogurt at a level of 40 IU/g (244). However, our product label analysis indicated that there are still several companies that have not fortified their products with vitamin D despite of SFDA policy. In light of this, we recommend that the SFDA implement an appropriate monitoring program to ensure the fortification policy is followed. In addition, although the SFDA policy directed that flavored yogurt be fortified with vitamin D at a level of 40 IU/g, our product label analysis indicated that flavored yogurt is not being fortified with vitamin D in Saudi Arabia. Therefore, it would be beneficial to introduce new vitamin D-fortified products to the Saudi market, especially flavored yogurt, which is regularly consumed by young women like those in our study population. Toward this goal, we recommend that the SFDA encourage manufacturers to fortify such new products, which may help young women achieve adequate levels of vitamin D. In addition we recommend that the SFDA website provide education on products fortified with vitamin D and the benefits of maintaining adequate nutrition. However, in spite of the known benefits of vitamin D fortification to the health of the general population, consumers of fortified products should be studied to identify any negative health effects that excessive vitamin D consumption might cause. Therefore, we strongly recommend future studies on the effects of this vitamin D fortification scheme on health outcomes in the general population (245, 246).

There is conflicting knowledge regarding the benefits of sunlight as a source of vitamin D, and a negative attitude towards sun exposure is present among many of the young women who are at the greatest risk of vitamin D deficiency. Our analysis supports the need for clear, accessible, and reliable information concerning the effects of exposure to the sun to be available to the most vulnerable portion of the population: young women with a low level of education and low household income. Educational programs should emphasize the importance of sunlight and provide information concerning the best times of day for obtaining adequate sun exposure, particularly during winter. Healthcare professionals should be informed and updated about the guidelines for sun exposure in order to better educate the community, and help prevent vitamin D deficiency throughout the country. It would also be beneficial to encourage women who have private courtyards to spend 15-30 minutes in the sun without veils between 8:30 am to 10:30 am and /or between 2:00 pm to 4:00 pm three times every week (240). In addition, we recommend an increase in the availability of private areas in cities for those who don’t have their own private space by designating areas in neighborhood parks and work places, such as private gardens, for women to take in sunlight.

Protective traditional clothing (black *abaya*) was identified as the most commonly used sun protection measure in Saudi Arabia. Seventy percent of the women studied wore the traditional black *abaya* whereas 7% of the women usually wore colored *abayas* and 23% of the women wore colored *abayas* only occasionally. The women who wore colored *abayas* in the sun had significantly higher serum vitamin D levels when compared to those women who wore black *abayas*, and this association remained significant after adjusting for demographic factors, vitamin D intake, and minutes per day of sunlight exposure. Additional studies researching the magnitude of sunlight attenuation by *abayas* of different colors and fabrics are needed to determine whether specific colors and/or fabrics directly affect the photoproduction of vitamin D.

This study has some limitations. A causal relationship between predictors and serum vitamin D levels cannot be established from this cross-sectional study design. The sample was derived from the primary health center at King Abdul-Aziz Medical City in Jeddah, and therefore, may not be generalizable to all women residing in Jeddah or in the other regions of Saudi Arabia. The use of Caucasian normative data for peak adult bone mass in Saudi Arabia may have led to the over-diagnosis of the prevalence of osteopenia. Our data reveals the need for future research to develop reference values for BMD which are specific to Saudi women or Jeddah in particular, to ensure reliable interpretation of individual DXA data and to identify women who are at risk.

The association between adequate vitamin D and calcium intake and bone health among young women has been evaluated, and some at-risk groups have been identified. However, prospective studies on this topic in Saudi Arabia are needed to develop new and effective strategies for improving nutrition among women across the country, to provide a clearer picture of their long-term bone health, and to determine if BMD measurements should be commonly practiced among at-risk groups. These prospective studies should also investigate whether nutrition interventions, particularly with regard to vitamin D and calcium intake, are effective in increasing BMD in young Saudi women. Further studies are also needed to assess whether educational interventions about food high in calcium and vitamin D, specifically its importance in preventing osteoporosis, can improve vitamin D and calcium intake for this age group.

Appendices

SECTION 1. DEMOGRAPHIC QUESTIONNAIRE

Date …………………………………………… Subject ID………………

1. What is your date of birth ………………**? (dd/mm/year)**

2. **How many years of formal education do you have? ....**

3. What is your current work situation?

Employee ☐ Student ☐ House wife ☐ unemployed ☐

4. Which of the following categories best describes your total household income in Saudi Riyals (SR) per month?

Less than 4,000 ☐ 4,000- 7,999 ☐ 8, 000- 14,999 ☐
15,000 24, 999 ☐ 25,000 or above ☐ I don't know ☐

5. What type of home do you live in?

Rented apartment ☐ Owned apartment ☐ Rented villa ☐ Owned villa ☐

Others (specify)………

6. What is your current marital status?

Single (never married) ☐ Married ☐ Divorced ☐ Widowed. ☐ Separated ☐

If you have never married skip the following questions and go to section 2.

7. How many living children do you have?...................... or ☐ No kids

8. Have you ever had pregnancy that did not go to term?

Yes ☐ No ☐

If yes how many months before the pregnancy terminated? ....

9. How many babies did you breast feed? ……..

10. Have you ever taken vitamin D supplement while you are breastfeeding?

Yes ☐ No ☐ Don't know ☐

SECTION 2. HEALTH INFORMATION

1. Have you ever been told by a doctor or a physical professional that you have any of the following conditions?

a.	Diabetes	□ Yes	□ No
b.	Osteoporosis	□ Yes	□ No
c.	Bone disease	□ Yes	□ No
d.	Cancer	□ Yes	□ No
e.	Renal diseases	□ Yes	□ No
f.	Liver disease	□ Yes	□ No
g.	Hyperthyroidism	□ Yes	□ No
h.	Hypothyroidism	□ Yes	□ No
i.	Glucose or gluten intolerance	□ Yes	□ No
j.	Malabsorption	□ Yes	□ No

2. Have you ever had a bone fracture or broken bone during adulthood?

Yes ☐ No ☐

3. Are you taking any medications (prescription and non-prescription)?

Yes ☐ No ☐

If yes, what medication? __________________________

4. When was your last period? ……………

If you are currently married, please answer the following questions (5-7); if not please go to section3

5. Are you taking an oral contraceptive?

Yes ☐ No ☐

6. Are you currently pregnant?

Yes ☐ No ☐ Do not know ☐

7. Are you currently breastfeeding?

Yes ☐ No ☐

SECTION 3. LIFESTYLE QUESTIONNAIRE

I. Smoking

1. Have you ever smoked?

Yes ☐ No ☐

If you answered "No" go to section 3

2. Do you currently smoke?

Yes ☐ No ☐

3. If yes, what type of smoking?

Cigarette ☐ Shisha (Hookah) ☐ Both ☐

5. How many cigarettes per day? ....

6. How many shisha (approximately how many per month).........

II. Physical Activity (International Physical Activity Questionnaire-Short form)

The questions are about the time you spent being physically active in the last 7 days. They include questions about activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Please answer each question even if you do not consider yourself to be an active person.

In answering the following questions,

- **vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal.
- **moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

1a. During the last 7 days, on how many days did you do vigorous physical activities like aerobics, or fast bicycling,?

Think about *only* those physical activities that you did for at least 10 minutes at a time.

________ days per week ⇨

or

None ☐

1b. How much time in total did you usually spend on one of those days doing vigorous physical activities?
_____ hours ______ minutes

2a. Again, think *only* about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

__________ days per week ⇨

or

None ☐

2b. How much time in total did you usually spend on one of those days doing moderate physical activities?
_____ hours ______ minutes

3a. During the last 7 days, on how many days did you walk for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.

________ days per week ⇨

or

None ☐

3b. How much time in total did you usually spend walking on one of those days?

_____ hours ______ minutes

The last question is about the time you spent sitting on weekdays while at work, at home, while doing course work and during leisure time. This includes time spent sitting at a desk, visiting friends, reading traveling on a bus or sitting or lying down to watch television.

4. During the last 7 days, how much time in total did you usually spend *sitting* on a week day?
____ hours ______ minutes

SECTION 4. SUN EXPOSURE QUESTIONNAIRE

The next set of questions is about your exposure to the sun in the past 30 days. For each question, please select one answer that best describes you.

1. **During the past 30 days, on average weekdays, how much time did you spend outdoors between 10 am and 4 pm?**

None ☐ …………..mintues/day ☐ …………hours/day ☐

2. During the past 30 days, on average weekends, how much time did you spend outdoors between 10 am and 4 pm?........................

None ☐ …………mintues/day ☐ …………hours/day ☐

3. Do you currently have a courtyard in your home?

Yes ☐ No ☐

*If you answered "**No**" go to question 5*

4. **In the past 30 days, on an average week, how much time did you spend in your courtyards between 10 am and 4 pm? ....**

None ☐ ………mintues/day ☐ …………..hours/day ☐

For the following questions think about what you do when you are outdoors:

5. In the past 30 days, did you use sunscreen protection?

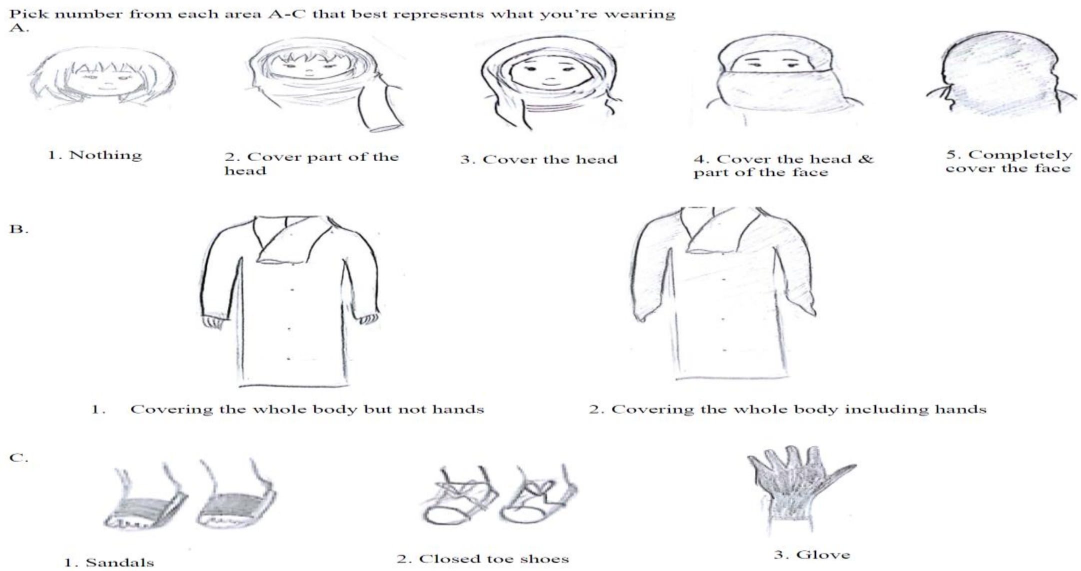
Yes☐ No ☐

If you answer "No" go to question 7.

6. Where did you apply the sunscreen?

On all exposed skin ☐ On face and hands ☐ On face only☐

7.



SECTION 5. KNOWLEDGE ABOUT VITAMIN D & ATTITUDE TOWARD SUN EXPOSURE QUESTIONNAIRE

I. <u>Knowledge about vitamin D</u>

1. Have you ever heard about vitamin D?

Yes ☐ No ☐

If you answered 'No', please go to section 6.

2. Do you think vitamin D is important for your health?

Yes ☐ No ☐ Do not know ☐

3. What is the single most important source of vitamin D?

Natural food sources ☐ Sunlight ☐ Fortified food products ☐ Supplements ☐

Do not know ☐ other (please specify)…………

4. Name dietary sources of vitamin D (Please check all that apply)

Green leafy vegetables ☐ Citrus fruits ☐ Red meat ☐ Oily fish (e.g., Tuna¸ Sardines) ☐
Fortified cow's milk ☐ Do not know ☐ other (please specify)……

5. Have you heard about vitamin D fortification in food?

Yes ☐ No ☐

6. **What the most important effect of vitamin D**

Don’t know the effects ☐ Immunity ☐ Bone Health ☐

Hair Growth ☐ Vision Heath ☐ Skin softness ☐ Osteoporosis prevention ☐

Other ☐ (please specify)………

7. **Factors that can decrease the amount of vitamin D an individual can get are…**

Skin color ☐ Age ☐ Sunscreen ☐ Fatty diet ☐ Avoid of sun exposure ☐

Don’t know ☐ Other ☐ (please specify)………

II. Attitudes and behavior toward sunlight exposure and sun protection:

1. I believe sunlight exposure is good for my health.

Always ☐ Sometimes ☐ Never ☐ Don’t know ☐

2. I like going outside in the sun

Often ☐ Rarely ☐ Never ☐ Don’t know ☐

3. I enjoy spending time outside (courtyards) in the sun.

Often ☐ Rarely ☐ Never ☐ Don’t have courtyard ☐

4. I use sunscreen when I go outside.

Always ☐ Sometimes ☐ Rarely ☐ Never ☐

5. I wear colour- *abaya* when I go outside in the sun.

Always ☐ Sometimes ☐ Rarely ☐ Never ☐

6. I spend time in the sun (in private area) in order to get a tan?

Often ☐ Rarely ☐ Never ☐ Don’t know ☐

7. I seek a suntan from artificial tan such as tanning bed.

Often ☐ Rarely ☐ Never ☐ Don’t know ☐

8. My primary reason for avoiding the sun is:

Custom or religion ☐ Public health messages ☐ Specific health reasons ☐

To avoid dark skin ☐ Do not avoid the sun ☐ Other ☐(please specify)………………..

9. I would spend more time in the sun if:

I wasn’t worried about skin cancer ☐ I had more time ☐ I had more privacy ☐

I would not spend more time in the sun ☐ Other ☐(please specify)…………

SECTION 6. SEMI QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

Date: ____________________ Subject ID _________________

These questions are designed to estimate the amount of vitamin D and calcium you get from the foods, drinks, supplements you usually consume. Thank you for taking the time to answer these questions.

Please list Brand name of **products** used in past 30 days:

DAIRY PRODUCTS	BRAND NAME
Milk: whole, 2%, 1% or Skim	
Flavoured milk chocolate , strawberry : whole, 2%, 1% or skim	
Drink Laban (Butter milk) whole, 2%, or Skim	
Flavoured butter milk	
Yogurt whole, 2%, 1% or Skim	
Flavoured Yogurt	
Soya milk	
Milk in coffee or tea	
Cheese; hard	
Cheese; soft	
Butter or margarine /oil	
Orange Juice	
Cereal fortified with vitamin D	

Please list Brand name of **Nutritional supplement** (multivitamin / vitamin D /calcium tablets, cod liver oil, fish oil) used in past 30 days.

BRAND NAME OF SUPPLEMENT	NUTRIENTS	AMOUNT	FREQUENCY (day or week)

Types of Food or Drink	Never or less than 1 per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	**Medium Serving**	Your serving size S	M	L
Milk: whole, 2%, 1% or Skim fortified with vitamin D										1 Cup (250 mL)			
Milk: whole, 2%, 1% or Skim NOT fortified with vitamin D										1 Cup (250 mL)			
Drink Laban (Butter milk) whole, 2%, or Skim fortified with vitamin D										1 Cup (250 mL)			
Chocolate Milk: whole, 2%, 1% or skim										1 Cup (250 mL)			
Soy Milk: plain or flavored										1 Cup (250 mL)			
Other milk										1 Cup (250 mL)			
Milk on cereal (if not included above)										½ Cup (125 mL)			
Milk in coffee or tea										1 Table spoon			
Milk shake										1 Cup			
Milk dessert (ice cream, Mohallabia (budding) Cream caramel Cream custard										½ Cup one scoop, 1container			
Arabic dessert Knafeh b'jiben Knafeh b'kasta										1 Slice 113g			
Yogurt whole, 2%, 1% or Skim										½ Cup (125 g , 1 container)			
Yogurt (fruited or flavored)										½ Cup (125 g , 1 container			
Labnah Cream										1table spoon			
Cheese; white										2 slices 57 g			
Cheese; hard Cheddar, Swiss										2 slices 57 g			

Types of Food or Drink	Never or less than 1 per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 Per week	1 per day	2+ per day	Medium Serving	Your serving size S	M	L
Cheese: soft or Spread										1table spoon			
Haloom cheese										2 slices 57 g			
Fatayer with /Cheese with /Labnah with /Zatar										1 slice 100g			
White bread pita, roll, bun,										2 slices , small roll, 35 g			
Brown bread pita , roll										2 slices, small roll, 35g			
Cereal Corn flakes fortified with Vitamin D										1 Cup (30g)			
Waffle, pancake, French toast										1 piece (1/4 waffle) 70g			
Cake										1 slice 58g			
Butter (in any foods eaten)										1 teaspoon			
Margarine (in any foods eaten)										1 teaspoon			
Cheese pizza										1 slice 66g			
Macaroni with cheese, lasagna										1 Cup 250g			
Canned salmon										2 tablespoon 35g			
Canned tuna										2 tablespoon 35g			
Canned sardines										2 fish (1/2 can) 48g			
Salmon steak Tuna										90 g			
Fish with sauce										90 g			
Other fish: oily										90g			
Cream soups made with milk										1 Cup (250 mL)			

Types of Food or Drink	Never or less than 1 per month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	**Medium Serving**	Your serving size S	M	L
Eggs: eaten alone or in other food										1 large egg(56 g)			
Potatoes; mashed with milk and margarine										½ Cup (one scoop) (105g)			
Orange juice; fortified with calcium										1 Cup (250 mL)			
Orange juice; fortified with calcium and vitamin D										1 Cup (250 mL)			
Broccoli, kale, okra, cabbage and raw cabbage slow										1 Cup raw or 1/3 Cup cooked (60g)			
Fresh mints Parsley										1 Cup raw (25g)			
Hummus										1 tablespoon			
Seafood: shrimp, prawn, lobster, crab										90 g			
Shellfish: mussels, oyster										½ Cup			
Meat Beef/ Steak										90 g			
Lamb										90 g			
Liver										114 g			
Chicken										90 g			
Almonds Mixed nuts										¼ Cup 35g			
Halawa Tahiniya										2 slices 60g			
Dates										3 dates 40 g			

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