VITAMIN D STATUS AND BREAST CANCER IN SAUDI ARABIAN WOMEN: CASE CONTROL STUDY

By

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LIST OF ABBREVIATIONS

25(OH)D	25-hydroxyvitamin D
1,25(OH)D	1,25-dihydroxyvitamin D
BMI	body mass index
WHO	World Health Organization
CI	confidence interval
FFQ	food frequency questionnaire
OR	odds ratio
VDR	vitamin D receptor
UVB	Ultraviolet B radiation
KFH	King Fahad Hospital
IARC	The International Agency for Research in Cancer
HPLC	high performance liquid chromatography
KFCMR	King Fahd Center for Medical Research
DRI	Dietary Reference Intakes
IOM	The Institute of Medicine
KSA	Kingdom of Saudi Arabia
PTH	parathyroid hormone

ABSTRACT:

Vitamin D is an essential nutrient in the human diet. A unique property of vitamin D is that it can be produced by endogenous synthesis in the skin following sufficient Ultraviolet B (UVB) radiation. In fact, our understanding of this compound has changed, such that it is no longer consider a true vitamin, but rather a steroid hormone. De-identified data for this analysis were derived from women residing in Jeddah, Saudi Arabia who completed routine medical visits in the summer of 2009 at King Fahad Hospital (KFH). In Chapter 1,"THE ASSOCIATION BETWEEN VITAMIN D STATUS IN NORMAL WEIGHT VERSUS OBESE WOMEN RESIDING IN WESTERN SAUDI ARABIA" we evaluate the relationship between body size and serum 25(OH)D concentrations including the association between change in body size during adulthood and vitamin D status. This study examines whether the current weight and weight change since age 18 years are associated with vitamin D status. This study found that neither current weight nor adult weight gain were associated with vitamin D status in Saudi Arabian women. In chapter 2,"IS AVOIDING SUN EXPOSURE VIA SUN PROTECTION PRACTICES ASSOCIATED WITH LOW VITAMIN D STATUS IN SAUDI ARABIAN WOMEN?" we investigate whether women who avoid UV exposure have lower 25(OH)D concentrations than women who do not avoid exposure. UV exposure was defined by time in outdoor activities, use of protective clothing and sunscreen. This study demonstrated that avoiding UV exposure via indoor activity and the use of sunscreen or/and wearing protective clothing was not associated with vitamin D status. Chapter 3, "VITAMIN D STATUS AND BREAST CANCER IN SAUDI ARABIAN WOMEN: A CASE CONTROL STUDY" we examine if vitamin D status as assessed by serum concentrations of 25(OH)D would be lower in breast cancer cases as compared to controls. This study demonstrated that there is a significant

relationship between higher serum concentrations of 25(OH)D and lower risk of breast cancer. Chapter 4, "IMPLICATIONS AND FUTURE DIRECTIONS" is presented a summary of key findings from the three studies in this dissertation to determine avenues of further research. The appendices consist of materials related to the dissertation work.

CHAPTER 1
THE ASSOCIATION BETWEEN VITAMIN D STATUS IN NORMAL WEIGHT VERSUS OBESE WOMEN RESIDING IN WESTERN SAUDI ARABIA

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Abstract

Low concentrations of circulating 25-hydroxyvitamin D [25(OH) D] are common among adults, particularly those diagnosed as overweight or obese. Female Saudi Arabian residents are at risk of developing vitamin D insufficiency related to cultural practices that reduce exposure to Ultraviolet B (UVB) radiation. During the past 10 years, there has been a dramatic increase in obesity and overweight over time in the Kingdom of Saudi Arabia (KSA) and the rates remain high. The objective of this study is to examine whether current weight and weight change since 18 years can alter vitamin D status among healthy Saudi women. We hypothesized that serum 25(OH)D is significantly higher in women who maintained stable weight over time than in those who gained weight. We conducted this study among 120 healthy women with a mean age 47.9 years; range (18–75) years, and a mean body mass index (BMI) of 29.7 kg/m^2. Height, weight, serum 25(OH)D concentrations, and questionnaires about medical history, health behaviors were measured at one time. Approximately 89% of women were 25(OH) D deficient (≤ 10 ng/mL), and 11% were sufficient (≥ 20 ng/mL). The adjusted logistic regression model shows no relationship between BMI and serum 25(OH)D (p-value= 0.085). The odds ratios (CI 95%) are 0.69 (0.19, 2.5) and 3.99 (0.90, 17.6) for the overweight and obese categories, respectively. Our results suggest that neither current weight nor adult weight were associated with vitamin D status in Saudi Arabian women. This lack of association is likely related to the high prevalence of deficiency at all weight classifications. Saudi Arabian women may represent a specific subgroup at high risk for deficient vitamin D status.

Introduction

According to the World Health Organization (WHO), the worldwide incidence of obesity has increased over the past decades (1). Obesity has also increased in the Arabian Gulf region in the last decade due to the discovery of oil and related increases in economic growth, access of food and a more convenience-oriented lifestyle (2). Obesity can be defined as a medical condition with excess adiposity or as a body mass index (BMI) above 30 kg/m^2 (3).

Obese individuals demonstrate abnormal metabolic and endocrine function (4). In addition, body size has been shown in several studies to be inversely associated with circulating concentrations of 25-hydroxyvitamin D [25(OH)D], a widely used clinical indicator for vitamin D status (5-9). This inverse correlation has been hypothesized to be related to sun avoidance in those with a higher BMI, or to sequestration of 25(OH)D in adipose tissue (10,11). Previous studies have found that obese individuals have higher circulating serum concentrations of 1, 25-dihydroxyvitamin D [1,25(OH)D], the active form of vitamin D, which can be stored in adipose tissue (12-14). This elevation may be associated with weight gain because 1,25(OH)D is a fat soluble hormone and may play a role in predisposing individual to greater intracellular Ca^{2+} concentrations, predominantly in adipocytes (15-17).

The actions of 1,25(OH)D are mediated by binding to the vitamin D receptor (VDR), a member of the steroid hormone receptor subfamily (18), which has been found in >30 cell types, including fat cells (5). Some studies suggest that 1,25(OH)D and VDR influence adipocyte differentiation (19,20).

Vitamin D not only regulates bone metabolism, but also has an important role in adipogenesis and in diseases such as osteoporosis, diabetes, and cancer (21-23). The main source of vitamin D is by production through solar Ultraviolet B (UVB) radiation (280–315nm) that penetrates the skin 7-dehydrocholesterol, which is located in the epidermal layer of the skin, is converted to previtamin D_3, which is then rapidly converted to vitamin D_3 (17,21). Vitamin D_3 can also be found in a few foods such as fatty fish and vitamin D supplements and fortified foods (24).

Vitamin D deficiency has increased recently worldwide and can vary according to skin pigmentation, age, adiposity, geography, and other factors (5,25). Although, UV sunlight in Saudi Arabia is high throughout the year, Saudi Arabian people, especially women, demonstrate vitamin D deficiency (26). More than 30 % of healthy young women in Saudi Arabia were found to be in a deficient state (27). A study done in Saudi citizens between 1982 and 1992 demonstrated that vitamin D deficiency is lower in Saudi citizen compared to the rest of the world (28).

In this study we examined whether the current weight and weight change since age 18 years are associated with vitamin D status. In this study of healthy Saudi Arabian women, we sought to evaluate the relationship between body size and serum 25(OH)D concentrations including the association between change in body size during adulthood and vitamin D status.

Subject and Method

Study Population

The study was conducted between June-August 2009in Jeddah, Saudi Arabia, (Latitude 21.4500 degrees North and Longitude 39.8167 degrees East). All participants were seen at King Fahd Hospital (KFH) in Jeddah, Saudi Arabia for regular clinical visits. One hundred twenty women aged 18 – 75 years with BMI ≤ 40 kg/m^2, all in good health and residents of Saudi Arabia for at least 5 years, were included. The exclusion criteria included presence of chronic diseases that could affect vitamin D metabolism, the presence of renal or hepatic endocrine, and autoimmune disease or a history of other serious medical conditions. This analysis of the de-identified data for epidemiological study was determined to be exempt according to the Human Subjects Committee at the University of Arizona, Tucson, Arizona.

Eligible women completed the consent process and signed a written informed consent. The de-identified dataset provided specific information on weight and height measurement and blood draws at one time. General questionnaires pertaining to lifestyle, demographic information, medical history, smoking habits, and food and usual dietary intake for prior 12-month period, sun exposure, use of sun protection, and style of dress were also assessed. Vitamin D deficiency was defined as serum 25(OH)D levels below 12 ng/mL. Insufficiency was defined as 12–19 ng/mL. Vitamin D sufficiency is defined as serum 25(OH)D ≥20 ng/mL to meet the needs of the non-endocrine pathway (129). Stable weight was defined as <10% change from weight at age 18 years to current weight, and weight gain was defined as ≥10% from weight at age 18 years to current weight.

Anthropometry

Weight was measured to the nearest 0.1 kg with a balance beam scale (Weigh-Tronix, New York, NY) with no shoes, socks, and with light clothing and empty pockets. Height was measured to the nearest 0.1 cm with no shoes using a wall-mounted stadiometer (Holtain, Crosswell, Wales). BMI was calculated as weight in kilograms divided by height in meters squared.

Blood Sample Collection and Measurement of 25(OH)D By HPLC

Blood samples were collected from participants (n=120) to measure total serum 25(OH)D. Participants were not required to fast for blood collection. Blood was collected via venipuncture, allowed to clot, centrifuged, and then stored at -80^{o}C until thawed for analyses by high performance liquid chromatography (HPLC) in King Fahd Center for Medical Research (KFCMR). The method used has been described in detail elsewhere (30). Briefly, the method utilizes a reversed- phase HPLC technique that shows a clear resolution of 25-hydroxyvitamin D_2 [25(OH)D_2] and 25(OH)D_3. The mobile phase is an acetonitrile extract of serum by solid phase extraction C18/OH cartridges. HPLC was preformed using a Shimadzu LC-10 system with Shimadzu LC-10AT pump (Corporation, Kyoto, Japan).

Statistical Analysis

All analyses were conducted using the STATA statistical software package (version 11.0, Stata Corporation, College Station, TX). Demographic and clinical characteristics were evaluated using descriptive statistics mean including standard deviation and frequencies. Backwards variable selection was used to determine variables for the full model, using a p-value of 0.05 to select covariates that remained in the model. All variables that have been demonstrated to be significant covariates in research evaluating the relationship between BMI and 25(OH)D levels, even if not statistically significant, were forced into the full models. This included logistic regression was also used to compute odds ratios for the association between body mass index and vitamin D status. Vitamin D status was converted to a dichotomous outcome variable; less than 20ng/ml = 0, greater than or equal to 20ng/ml = 1. Potential confounding variables were assessed using linear regression modeling. Confounders were defined as variables, which changed the point estimates by 10% or greater; the covariates used in the full model did not significantly change the point estimates. The covariates used in the final adjusted model have been described as confounders in earlier research evaluating the association of BMI and vitamin D concentrations thus included in the adjusted model. Variables included in the final adjusted model were age, BMI, physical activity, education, parity, smoking, and income.

Results

A total of 120 women were included in this study with a mean age of 47.9 ± 13.6 years. The average BMI at age 18 of 22.8±4.6 kg/m^2 and current BMI of 29.6 ± 6.04 kg/m^2 were included in the analytical sample. Half of the participants were smokers (51.7 %), approximately 35.1 % has some college education, and 85.8 % reported having had children. The mean serum 25(OH)D concentration was 15.4±12.31 ng/mL (**Table 1**). Regardless of weight, serum 25(OH)D concentration was well below recommended levels (31), and most of participants were obese (n=58) had serum 25(OH)D concentrations 13.5±9.2 ng/mL compared to normal weight (n=33) with serum 15.7±10.1 ng/mL and overweight (n=29) with serum 25(OH)D 18.3± 18.3, *p*-value(0.78), (**Table 2**). Weight change as a categorical variable have shown in table 2; the mean serum 25(OH)D levels for three categories were established for weight change: <10%, 10-20% and >20%. Mean serum 25(OH)D levels (19.8±15.6ng/mL) were significantly higher in women with weight change of <10%. Compared to 10-20% change and the >20% change with concentrations of 12.6±11.6 ng/mL and 14.5±10.5ng/mL respectively, however, this trend was not statistically insignificant (p-value= 0.13). There were no differenced in women how gained weight in serum 25(OH)D levels for women since age 18 years (n=86) compared to women who had stable weight since that age (n=34).

Notably, the distributions of circulating 25(OH)D concentrations in Saudi Arabian women as shown in (**Figure 1**) indicate that approximately 89% of women were 25(OH)D deficient (≤ 20 ng/mL), and 11% were sufficient (≥ 20 ng/mL) with a mean concentration of circulating 25(OH)D of 15.4 ± 12.2 ng/mL. **(Figure 2)** shows the vitamin D concentration across the different BMI categories, with higher serum 25(OH)D in overweight comparing to the other categories

In (**Figure 3)**, obese women (BMI >30kg/m^2) had the lowest 25(OH)D as compared to overweight and normal weight women with mean 25(OH)D concentrations of 13.5 ± 9.1 ng/mL 18.3 ± 18.3 ng/mL, and 15.7±10.1 ng/mL, respectively, these differences were not statistically significant (p-value=0.78).

Table 3 shows crude and adjusted odds ratios across BMI categories. The normal BMI category is the referent. The crude odds ratios (CI 95%) are .67 (0.19, 2.3) and 3.05 (0.79, 11.7) for the overweight and obese categories respectively. After adjusting for age, education, physical activity, smoking and income, the odds ratios are .69 (0.19, 2.5) and 3.99 (0.90, 17.6) for the overweight and obese categories respectively.

Discussion

Obesity has been a public health concern in many countries, and is a risk factor for vitamin D deficiency (19). Previous studies have reported an inverse relationship between circulating concentrations of 25(OH)D, BMI and body fat mass (32). Peak serum level of vitamin D is achieved after exposure of the skin to the sun, however, circulating 25(OH)D remain inversely associated with BMI even among obese individuals with UV exposure (33).

The present study is the first study to address the relationship between body weight and weight change since age 18 years and vitamin D status in Saudi Arabian women. In the present study, we found that serum 25(OH)D concentrations were lower in obese women as compared to those who were overweight and normal weight but the difference was not statistically significant. Further, in this study women who maintain stable weight over time (n=34), and women who gain weight (n=86) the mean serum 25(OH) D is (15.7±14.4, 15.2±11.4 ng/mL), respectively, thus in both groups they were vitamin D deficient. The absence of association between BMI and level of vitamin D status could be attributed to high rates of vitamin D deficiency in our sample but also could be due to genetic differences or other factors that affect cutaneous production of vitamin D. Women in this country traditionally cover their entire body while out in public, thus reducing the exposure to the sunlight. Other factors that may contribute to inadequate vitamin D are multiple pregnancies and diet. In our study population, the average woman reported having three children. Diet may also contribute to lower vitamin D status because there are few food sources of vitamin D in the typical Saudi diet and intake is limited (24,34). Most importantly the majority of women in the study were obese, which may affect the bioavailability of vitamin D, although this remains controversial (35).

A recent study by Mason et al., in postmenopausal women (n=439) found that greater adiposity is associated with lower serum of 25(OH)D (5). On the other hand, weight loss could increase circulating 25(OH)D concentrations due to a decrease in peripheral sequestration (5). The release of vitamin D from body stores may incur vitamin D intoxication and hypercalcemia from deposition in body fat (36). However, others demonstrate that vitamin D concentrations that have been found in adipose tissue are not necessarily high, thus people who lose weight may not become vitamin D intoxicated (37).

Obese and overweight individuals have a higher prevalence of vitamin D deficiency than normal weight individuals (38-40), thus, it is reasonable to assume that serum 25(OH)D is dramatically decreased as BMI increases. Yet, the influence of vitamin D status in weight loss during lifespan is not known.

The main potential mechanism of action for the association between body size and vitamin D is that vitamin D is a fat-soluble vitamin, which can be sequestered in adipose tissue and stored in subcutaneous fat for later use as confirmed from animals studies given high doses (41-43). Vitamin D can be stored in adipose tissue for approximately two months (44). The bioavailability of cutaneous vitamin D is decreased in obese people by more than 50 % (13). Additionally, vitamin D storage in fat tissue has been demonstrated in humans as well (46). Vitamin D supplementation with 50,000 IU dose of ergocalciferol (D_2) could increase circulating 25(OH)D in individuals of normal weight compared to obese individuals as shown by Worstman et al (13).It should also be emphasized that obese people are less active and spend less time outdoors, and therefore, have lower exposure to UV radiation. There are many factors that also can decrease the bioavailability, and can be associated with lower serum of 25(OH)D in overweight and obese individual such as age, latitude, time of day and year, renal function, skin pigmentation, and use of sunscreen (25). In addition to all of these factors, sun protection policy has increased in the recent years in an effort to reduce skin cancer and may cause a decrease in serum vitamin D (47).

Approximately one billion people worldwide may have vitamin D deficiency (25). However, the optimal level of vitamin D is still controversial. Circulating concentrations of 25(OH)D below 20 ng/mL have been defined as vitamin D deficiency (31) but others have indicated that the cutoff should be higher (48). Individuals with relatively low circulating concentrations of 25(OH)D demonstrate high levels of 1,25(OH)D and PTH

(49-52). Moreover, increase PTH promotes an increase intracellular calcium concentration within adipocytes, which can inhibit lipolysis (45). Thus, suppressing 1,25(OH)D with higher 25(OH)D would be predicted to inhibit adiposity and promote weight loss (45). In our women neither PTH nor 1,25(OH)D were measured.

A previous study in 26 adult females indicated that weight loss of 10% was associated with a significant increase in vitamin D concentrations of up to 34% as compared to baseline from 15.4 to 18.3 ng/mL, with $p < 0.05$ (53). In agreement to that there was a study done in male and female gastric bypass patients (n=123) with serum 25(OH)D concentrations of 22.7 ± 9.9 ng/mL before surgery (56). However, after gastric bypass surgery the mean circulating 25(OH)D concentration was 29.7 ± 14.1 ng/mL with p-value < 0.001 (56). In contrast, among black women who had gastric bypass surgery, at the baseline the mean serum 25(OH)D concentrations increased after 1 month (p=0.004), and then decreased after 2 years of follow up (p=0.02) (57). A similar study in premenopausal women (12 wk of weight reduction program) showed that the concentrations of vitamin D increased by 2.9 ng/mL (from 30.3 to 33.2 ng/mL) (54). These studies support the idea that vitamin D has been linked with body weight, and BMI. In our women (n=12) who reported reduction in weight in adulthood, serum 25(OH)D concentrations was 7.24±3.08 ng/mL, compared to 15.4 ± 12.2 ng/mL, which was the average value for women in this study, (data not shown).

To the best of our knowledge, only one other study has assessed vitamin D status in healthy Saudi Arabian people. In this study young Saudi males and females were matched on age and gender. Vitamin D deficiency was significantly higher in obese (19

%) as compared to controls (15.8 %). Mean vitamin D concentrations were 33±12 nmol/L in obese people and 40.4±19.3 nmol/L in lean control group (*P*-value=0.004). Additionally, among both groups vitamin D was higher in males compared to females (58).

In contrast, in the present study of all women vitamin D concentrations were markedly lower, possibly related to differences in gender, age, body fat and place of residency. Both studies applied HPLC to assess vitamin D status; however previous study not only measured serum 25(OH)D, but also measured serum calcium, inorganic phosphorus, intact parathyroid hormone, serum insulin, fasting glucose, renal and liver function tests (58).

In our participants the vast majority of women (almost 89%) were identified as vitamin D deficient (below 20 ng/mL). These data are consistent with another research showing that the Asian population also has an extremely high prevalence of vitamin D deficiency (59). In western Saudi Arabia vitamin D deficiency is widely spread in both genders especially females (28). In a study among Saudi students attending universities (n=17) the mean level of vitamin D was below 27 ng/mL (60). In young Saudi women more than 52% demonstrated severe hypovitaminosis D, with 25(OH)D levels below 8 ng/mL (61). Further, the dietary intake of vitamin D in Saudi women is below the recommended level of (15µg daily) (62,31). In our study the percent distribution for the amount of milk intake and fortified food were consumed at sub-optimal levels for dietary vitamin D (data not shown).

Jeddah is a sunny place with relatively high temperature, so being outdoors is very rare (63,64). Women try to avoid the sun as much as they can and/or use a sunscreen with a sun protection factor SPF >15, which may reduce vitamin D synthesis by more than 99.5% (25,48). Additionally, clothing practices in the Arabian Gulf, including Saudi Arabia, contribute to vitamin D deficiency (61,65). In one study deficiency of vitamin D was more common in veiled women compared with nonveiled women (66).

Strengths of our study include a focus on Saudi women who are at risk for vitamin D deficiency, sample size, and collection of all samples during summer months. However, our study has weaknesses, in that we included only one serum sample from each study participant, limited diet and physical activity data, and were unable to measure of PTH and 1,25(OH)D. Importantly, this analysis suggests that Saudi Arabian women are at great risk for vitamin D deficiency, but that body size is not as strong associated as with other populations. Our work suggests increased efforts should be made to assess vitamin D status as well as evaluate the effectiveness of vitamin D supplementation in the Saudi Arabian population. We recommend a larger cohort with longitudinal design and repeated sampling for serum 25(OH)D status and weight assessment. Additionally, we suggest evaluation of the effect of vitamin D supplementation on status in normal weight and overweight/obese Saudi Arabian women, and evaluation of the relationship between circulating vitamin D and weight loss in Saudi Arabian adults. Based on the logic that there is widespread vitamin D deficiency with known health consequences, this paper gives further evidence of this in A Saudi Arabian population, and there should be a public health mechanism to address this.

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TABLE 1 Demographic, anthropometric and lifestyle characteristics of Saudi Arabia women (n=120) [1]

Characteristic	Mean	SD
Age, years	47.9	13.6
BMI (kg/m^2)	29.6	6.04
BMI at age18 (kg/m^2)	22.8	4.6
Difference in BMI (kg/m^2)	6.7	6.5
Weight (kg)	75.0	14.8
Height (cm)	159.1	4.8
Serum 25(OH)D (ng/mL)	15.4	12.31
Lifestyle	**N**	**%**
Smoking		
Yes	62	51.7 %
No	58	48.3 %
Education		
High School or Less	35	31.5 %
Post High School	26	23.4 %
Some College	39	35.1%
College Graduate	11	9.9 %
Parity		
Nuliparous	17	14.2 %
Have children	103	85.8 %
Monthly Income		
<5000 SR	59	49.2 %
5000 SR and more	61	50.8 %

[1] NS differences when comparing women reporting weight gain vs. stable weight since age 18 years

TABLE 2 Serum 25(OH)D (ng/mL) concentrations among Saudi Arabian women stratified by BMI categories and stable vs. increasing weight over adulthood (n=120)[2]

Current BMI	Serum 25(OH)D (ng/ml) (mean±SD)	Weight Change since age 18 years	Serum 25(OH)D (ng/ml) (mean±SD)
Normal weight 18.5-24.9 ***N*=33**	15.7±10.1	<10%	19.8±15.6
Overweight 25-29.9 ***N*=29**	18.3 ± 18.3	10-20%	12.6±11.6
Obese[1] ≥30 ***N*=58**	13.5 ± 9.1	>20%	14.5±10.5
PTrend	.78		.13
		[Serum vitamin D] [1] (mean±SD)	
Stable weight <10% change (n=34)		15.7 ± 14.4	
Weight gain ≥10% change (n=86)		15.2 ± 11.4	

[1] Non significant difference
[2] adulthood= current measured weight minus self-reported weight at age 18 y.

TABLE 3 Crude[1] and adjusted[2] odds ratios (95% Confidence Intervals) for deficiency serum 25(OH)D concentration (< 10 ng/ml) by BMI categories

Category of BMI	**Obese (>30 kg/m^2)**	**Overweight (>25 - <30 kg/m^2)**	**Normal BMI (Referent) (>18 and <25 kg/m^2)**	**P-trend**
Total (n)= 120	58	29	33	
Crude Odds Ratios[1] (95% CI)	3.05 (0.79, 11.7)	0.67 (0.19, 2.28)	1.00	0.087
Adjusted Odds Ratios[2] (95% CI)	3.99 (0.90, 17.6)	0.69 (0.19, 2.49)	1.00	0.085

[1]Logistic regression models with no adjustments.
[2]Logistic regression models adjusted for age, physical activity, parity, education, smoking, and income.

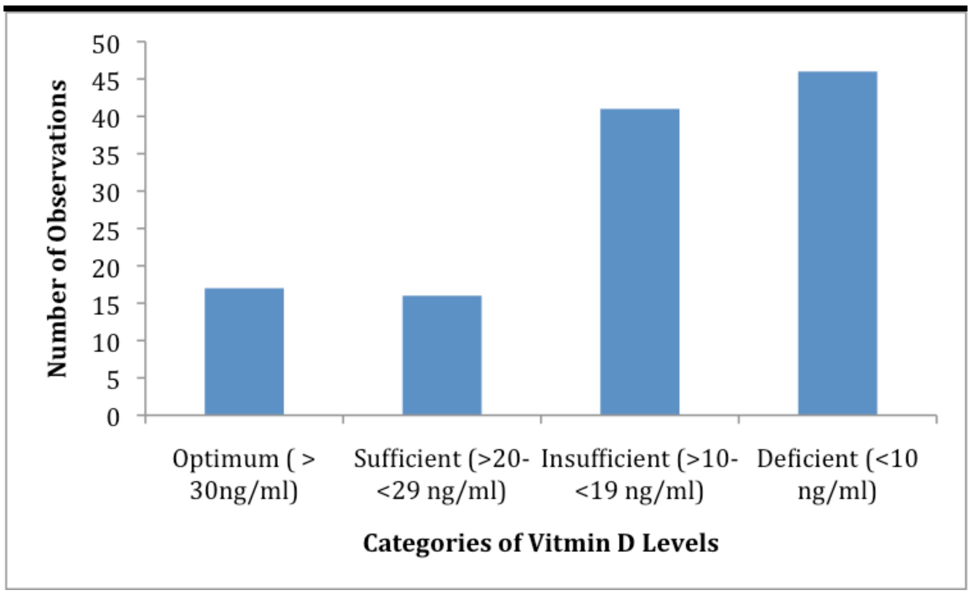

FIGURE 1 Distribution of circulating serum 25(OH)D(ng/mL) concentrations in Saudi Arabian women (n=120)

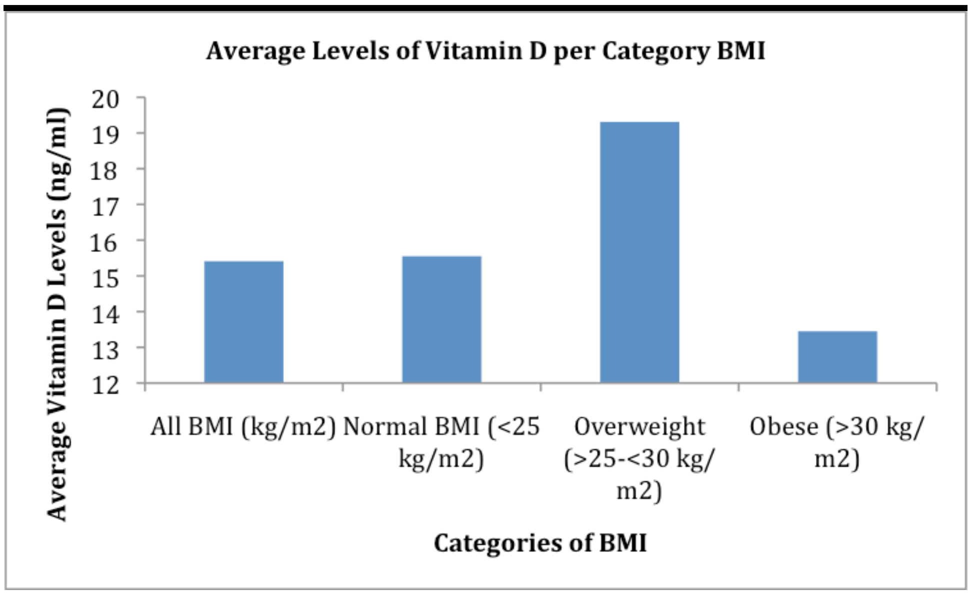

FIGURE 2 Serum 25(OH)D concentration based on BMI categories

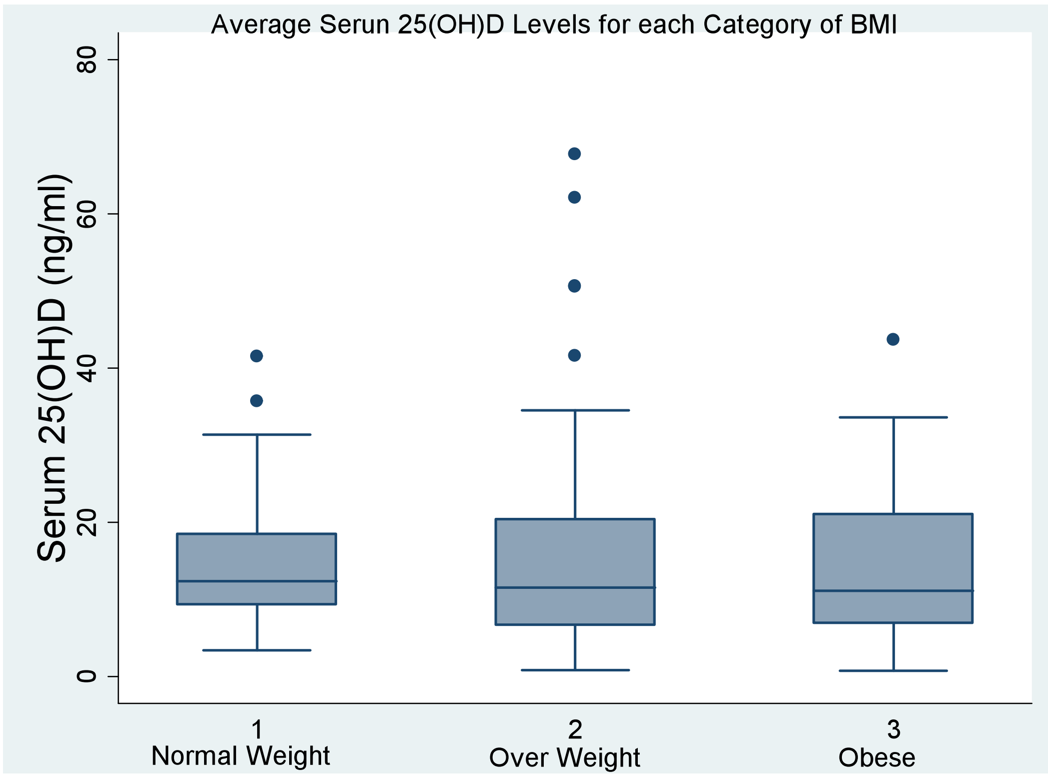

FIGURE 3 The average serum 25(OH)D concentrations (ng/mL) in normal weight, overweight, and obese were (15.7±10, 18.3 ± 18.3, and 13.5 ± 9.1 ng/mL, respectively) in Saudi Arabian women (n=120)

CHAPTER 2

IS AVOIDING SUN EXPOSURE VIA SUN PROTECTION PRACTICES ASSOCIATED WITH LOW VITAMIN D STATUS IN SAUDI ARABIAN WOMEN?

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Abstract

Background: Although Saudi Arabia enjoys a sunny climate year round; most of the population has little or no exposure to direct sunlight due to cultural practices. This is especially true for women.

Objective: The purpose of this study was to investigate whether women who avoid UV exposure have lower 25-hydroxyvitamin D [25(OH)D] concentrations than women who do not avoid exposure.

Method: This study assessed vitamin D status in 120 healthy women age 18-75 years who attended King Fahad Hospital (KFH) for regular healthcare visits. Participants provided blood samples for analysis of serum 25(OH)D concentrations, and completed a questionnaire on physical activity, sun protection and sun exposure habits. A one-way ANOVA analysis was performed to compare the mean vitamin D levels for the categories of sunlight exposure. Independent t-tests were performed to compare mean vitamin D levels for the variables sun protection and length of physical activity

Result: There was no relationship between mean serum 25(OH)D concentrations and outdoors activity. The mean serum 25(OH)D concentrations for participants who exercised greater than 30 minutes versus less than 30 minutes were 14.9±2.08 ng/ml and 15.6±1.34 ng/ml, respectively ($p = 0.79$). The ANOVA analysis also showed no relationship between exposed skin to sun exposure and mean serum 25(OH)D concentrations (p-value=0.55).

Conclusions: Neither outdoors activity nor sun exposure or sun protection practice was associated with vitamin D status in Saudi Arabian women residing in Jeddah.

Introduction

Excessive sun exposure has been associated with an increased risk of skin cancer (67). However, exposure to solar Ultraviolet B (UVB) radiation (280-315 nm) stimulates the conversion of 7-dehydrocholesterol to vitamin D_3 in the skin ultimately contributing more than 50-90 % of circulating vitamin D (25-hydroxyvitamin D [25(OH)D]) after it is metabolized by the liver (68,69). The double hydroxylation of 7-dehydrocholesterol to vitamin D_3 has been shown to protect against the development of rickets in children, and osteoporosis, fracture, and osteomalacia in adults (67-70). An adequate level of serum 25(OH)D in the blood may also prevent certain cancers such as colon, breast, and prostate cancer, as well as diabetes and multiple sclerosis (71,72). While foods such as fish, egg yolks, and fortified foods (25) contain vitamin D, no more than 20 % of vitamin D is obtained from the diet; thus, avoidance of sun exposure may contribute to vitamin D deficiency worldwide (69,25). It is agreed that one-third of the minimal erythemal dose (MED) is required to achieve maximum vitamin D production (73). Once vitamin D production is achieved, further sun exposure has no additional benefit (73-75). It is estimated that exposure of 15% of the body (i.e. the face, arms and hands) to the sun at least two to three times a week is adequate to produce sufficient levels of vitamin D (30 ng/mL) for optimal overall health (48,73, 74). Following adequate exposure to UVB radiation, serum 25(OH)D concentrations increase 5 to 10 fold within 24 to 48 hours (76). Given the role of UV exposure in promoting optimal vitamin D status it would be unexpected that climates with higher numbers of sunny days would demonstrate higher rates of vitamin D deficiency. Yet, studies conducted in Saudi Arabia show a significant proportion of women have low vitamin D status (60-63,77). Interestingly, studies conducted within Saudi Arabian cities where the level of UV radiation is high such as AL-Khobar, Riyadh, and Jeddah, have also reported a high prevalence of vitamin D deficiency, especially among older populations (≥ 50 y) and women (77-79).

Physical inactivity affects more than 66.8% of the Saudi Arabian population aged 30-70 years (89,90). Despite, the fact that physical inactivity is more prevalent in Saudi women (89,90) it has not received the same attention and has therefore been overlooked as a public health concern (89,90). Poor physical performance has been associated with low vitamin D status in Europe and the USA (80-82). However, not all studies support these results (83,84). Studies have shown a strong relationship between vigorous physical activity and vitamin D status (85,87). This correlation has been attributed to an increase in sun exposure during vigorous physical activity, or that might be physical activity contributing to the maintenance of adequate vitamin D status (86,88). In addition to the potential lack of sun exposure, it is well known that a sedentary lifestyle is associated with numerous adverse conditions including heart disease, diabetes mellitus, breast, and colon cancers (27). On the other hand, physical activity is correlated with numerous health benefits including the reduction of cardiovascular risk factors (91,92).

While it is probable that the use of sunscreen with a sun protection factor (SPF) of 15 reduces vitamin D synthesis by more than 99.5% (67,93), some studies found that the use of sunscreen does not cause vitamin D deficiency (94-96). Additionally, wearing protective clothing that largely covers the body may also reduce vitamin D production in the skin (97).

The purpose of this study was to investigate whether women who avoid UV exposure have lower 25(OH)D concentrations than women who do not avoid exposure. UV exposure was defined by time in outdoor activities, use of protective clothing and sunscreen use. We hypothesized that avoidance of sun exposure in Saudi Arabian women would be associated with lower concentrations of 25(OH)D.

Subject and Method

Study Population

This study was conducted in King Fahd Hospital in Jeddah, Saudi Arabia (Latitude 21.4500 degrees North and Longitude 39.8167 degrees East) where the average temperature is 36.7°C during the day (98). All participants (n=120) were females and between the age of 18 and 75 years. In order to minimize variation of UV exposure, all data were collected during summer months. Non-fasting blood samples were collected from the participants for analysis of 25(OH)D. The de-identified dataset provided specific information on height and weight. Body mass index (BMI) was calculated as weight in kilogram divided by height in meters squared, women were grouped into three categories: normal (BMI <25 kg/m^2), overweight (BMI=25–29.9 kg/m^2), or obese (BMI ≥ 30 kg/m^2). All the participants completed a self-administrated sun exposure questionnaire about the following factors: lifestyle, demographic information, smoking ,time spent outdoors, physical activity, duration physical activity (≤ 15 min/ day, ≤ 30 min/day, ≤ 1h/day), which part of the body was exposed to the sun (face, hand, face and hand, both arms, both legs, completely covered), use of sun protection, and usual dietary intake of food for prior 12-month period. Vitamin D deficiency as defined as serum 25(OH)D levels below 12 ng/mL and insufficiency was considered at concentratins12–19 ng/mL. Sufficient levels of vitamin D were defined as serum 25(OH)D ≥20 ng/mL to meet the needs of the non-endocrine pathway (129). This analysis of the de-identified data set for epidemiological study was determined to be exempt of human subjects investigation according to review by the Human Subjects Committee at the University of Arizona Tucson, Arizona.

Blood Sample Collection and Measurement of 25(OH)D By HPLC

Blood for analysis was collected from participants (n=120) to measure total serum 25(OH)D. Blood samples were collected via venipuncture by trained phlebotomists allowed to clot, centrifuged, and then stored at -80^{o}C until thawed for analyses by high performance liquid chromatography (HPLC) in King Fahad Center for Medical Research, the method used has been adopted by Rapuri (30). Briefly, the method utilizes a reversed- phase HPLC technique that shows a clear resolution of 25-hydroxyvitamin D_2 [25(OH)D_2] and 25(OH)D_3. The mobile phase is an acetonitrile extract of serum by solid phase extraction C18/OH cartridges. HPLC was preformed using a Shimadzu LC-10 system with Shimadzu LC-10AT pump (Corporation, Kyoto, Japan).

Statistical Analysis

All statistical analysis was performed using the STATA 11.0 statistical software (College Station, Texas). Anthropometric and lifestyle characteristics of 120 participants were evaluated using descriptive statistics including mean, standard deviation and frequencies. A one-way ANOVA analysis was performed to compare the mean vitamin D levels for the categories of sunlight exposure. Independent t-tests were performed to compare mean vitamin D levels for the variables sun protection and length of physical activity. Variable selection using the Akaike's Information Criterion (AIC) was used to ascertain predictors to formulate the best fit model to predict the relationship between outdoor activity and serum 25(OH)D concentrations. To examine the independent relationships between vitamin D status and UV exposure, multiple linear regression models were used to regress physical activity and sun protection variables on vitamin D status, while controlling for education, calcium supplementation, menarche, weight, and breastfeeding.

Results

Table 4 displays the anthropometric and lifestyle characteristics of 120 women were included in these analyses. Mean serum 25(OH)D concentration was 15.4$\pm$12.31ng/mL. Physical activity was preformed less than 30 minutes by (n=82) women and more than 30 minutes by (n=38) women. Only 27,7% of women did their physical activity outdoors while 66.6% of women spend more than 30 minutes a day of their times outdoors. More than 49.1% the participants were completely covered, (**Table 4)**. The box-plots in (**Figure 4)** shows more than 98% of participants has 25(OH)D levels $\leq$ 20ng/mL, and as such were vitamin D deficient and only 5 women had an optimal level of vitamin D more than 30ng/mL.

There was no relationship between the mean serum 25(OH)D and women who exercise less than 30 minutes (15.6+1.34 ng/mL) and women who exercise greater than 30 minutes (14.9+2.08 ng/mL) (p-value=0.79) (**Table 5)**. Also, this Table shows that comparison of mean circulating serum 25(OH)D for women who use sun protection (n=68) and who do not (n=52). No relationship was observed between sunscreen use and the mean circulating serum 25(OH)D, with mean concentrations of 15.1±1.52 ng/mL for sunscreen users and 15.7±1.68 ng/mL for non-users, respectively (p-value=0.79). **(TABLE 6)** shows a one-way ANOVA analysis that examines the mean serum 25(OH)D by exposed of specific body part to the sun. Women who only had the hands exposed had mean serum levels of 13.9±11.92 ng/mL and women who had their hands and face exposed their mean serum 25(OH)D was 15.3±12.57ng/mL. However, women who were completely covered had higher serum 25(OH)D concentration17.3±12.38 ng/mL compared to women who had of specific body part exposed to the sunlight. Finally, (**Table 7)** shows the covariates that are considered the best predictors of serum 25(OH)D concentrations; the covariates include education, menarche, breastfeeding, weight, and calcium supplementation. All are statistically significant with a R^2 value of 0.14. The final best fit model explains 14 percent of the variability in serum 25(OH)D concentrations. (**Figure 5**) shows the relationship between length of exposure to sunlight and serum 25(OH)D concentrations in different times spent outdoors. In this figure most of the participants spend 30 minutes outdoors (66%) at the most with average serum 25(OH)D 15.2ng/mL. Only (n=1) woman spent more than 60 minutes outdoors.

Discussion

Vitamin D deficiency is a major health problem in developed and developing countries (100), especially in the Arabian Gulf countries where vitamin D deficiency is common among women (101), even though, Saudi Arabia has a greater number of days with sunshine than most places in the world(102). It is known that vitamin D deficiency can result from inadequate sun exposure (100). While the exact cause of vitamin D deficiency in Saudi Arabia is not clear, cultural practices, dietary and lifestyle patterns as well as limited time outdoors (97,101, 103) are likely involved (104).

Most of studies of Saudi Arabian women have shown that they are at significant risk for vitamin D deficiency in all age groups (102,105-107). A previous study done in the eastern province of Saudi Arabia has shown that serum 25(OH)D concentrations ≤ 20 ng/mL affect more than 30% of the population between 25-35 years (108), and more than 55% of the population aged 36-50 years(108). As shown in a small study by Fonseca et al., the average serum 25(OH)D concentrations in Saudi Arabian women (n=31) was 6 ng/mL (102). Additionally, this study found that vitamin D status was significantly lower in women who lived in apartments and women who were exposed to sunlight < 30 min/d (102). Another study determined vitamin D status in 360 women who had lower back pain. At baseline 83% of the women (n= 275) presented with serum 25(OH)D concentrations below 10 ng/mL (107) which was responsive to vitamin D supplementation (5000 –10,000 U/day for 3 months).

In the present study serum samples were collected from participants between June and August, when vitamin D levels would likely be at the highest level due to intense sun exposure; however, we found that serum 25(OH)D concentrations were, on average, deficient (15.4±12.31.ng/mL) . In this study population, avoidance of sunlight exposure when they are in public was reported by 49.1% of women, which may have contributed to the high prevalence of vitamin D deficiency.

Our data also confirmed results of previous studies indicating a high prevalence of vitamin D deficiency among women in Jeddah (59,60,109). In a study by Khoja et al., 74% of women had serum 25(OH)D concentrations ≤ 12 ng/mL (109) as compared to 38.3% in our sample. Other studies provide similar rates of deficiency in Arabian women (110-11). In our study we found that low education, early menarche, calcium supplementation used, increased weight, and history of breastfeeding less than 6 months were best predictors of decreased serum 25(OH)D.

In the current study, there is no relationship between serum 25(OH)D concentrations and outdoors activity. Women who spent most of their time indoors had mean serum 25(OH)D concentrations of (16.3+ 12.8 ng/ml). Conversely, women who are outdoors more often (n=1) had serum 25(OH)D concentrations of (13.0+ 10.4ng/ml). A study done in elderly people (n=1234) over 3 years found that there is a positive relationship between serum 25(OH)D concentrations below 20 ng/ml and poorer physical performance (117). The correlation between high serum 25(OH)D and physical activity might be due to spending more time outdoors, which can lead to higher endogenous vitamin D productions than physical inactivity (113,116,118). The relationship between of physical activity and vitamin D metabolism is unclear (114,115,119). These studies had some important limitation that make them unlikely to end the controversy surrounding vitamin D status and sun exposure due to outdoor physical activity such as small sample size, inadequate sun exposure measurement, or lack of vitamin D intake and exposure in control groups.

Increasing outdoor activity may not only increase the level of serum vitamin D, but could also improve overall health. Even though it is well known that vitamin D plays an important role in maintaining skeletal health (103) the relation to physical performance is still undetermined unknown.

Vitamin D synthesis can be affected by days of sunshine as well as latitude, time of day, sunscreen, aging, protective clothing, glass, and skin pigmentation (120,121). As mentioned previously, Saudi Arabia is a sunny place and temperatures can be dangerously hot, rising above 50 C° (122 F°) in the summer months. Consequently, it is common for Saudi people to avoid spending time outdoors in the middle of the day. Another important factor that may impact vitamin D status in Saudi Arabia, is that it is customary for Arab women to completely cover their entire body when they are in public. In addition, very conservative women wear a face and hand cover, which can further block sun exposure by covering their entire bodies with dark veil for cultural or religious reasons. Previous studies have shown that veiled Arabian women have lower serum 25(OH)D concentrations than non-veiled women (111,112). However, in the present study we found that women who had their entire body covered showed higher serum 25(OH)D concentrations (17.3±12.38 ng/mL) than women who obtained some sun exposure such as face and hand (15.3±12.57ng/mL); however both groups were vitamin D deficient and the differences between them was not statistically significant (*p*-value=0.55). Likewise, a study done on Emirati women who also covered their entire body or exposed their face and hands, found significantly lower circulating serum 25(OH)D levels compared to non Arab women who dressed in Western clothing (103). That may be explained by increased skin pigmentation of the participants or/ and limited skin exposure due to covering clothing. Other studies done in Turkish women showed that there is a correlation between extent of body clothing and low serum of 25(OH)D (122,123). However, the current study was comparable to study has been reported in Jordanian women (97). Matsuoka showed that vitamin D levels could be affected by sunscreen use; however, this effect was dissipated after 7 days (117) Moreover, some studies have criticized the use of sunscreen protection against sun burn, which can block vitamin D synthesis (25,124). In contrast, other studies have shown that no difference in circulating levels of 25(OH)D between sunscreen use versus placebo (125,126).

In terms of public health, we must fight skin cancer by raising the awareness of UV radiation risk due to excessive sun exposure. We should find a balance between avoiding skin cancer but also allows sufficient vitamin D production. Also, the sun protection message needs to shift away from avoiding sun exposure altogether.

It remains difficult to find a sufficient level of UV exposure due to the limitations of existing research. Additionally, this is issue is complicated because of skin type, age, race and related clothing practice, which can affect vitamin D absorption through UV exposure (73,130).

Finally, there is an urgent need for public health education in the vital role of vitamin D in overall health, by increasing dietary enrichment or supplementation with vitamin D with more emphasis on outdoors activity and sunlight exposure, especially for women within the framework of their religious and cultural commitments. Prolonged exposure to UV light is not necessary; just exposed 25% of body (both hands, both legs, and back) 5-10 min between 10 AM and 3 PM at least twice a week in order to produce enough vitamin D (25). However, the relation between vitamin D and outdoors activity is not fully elucidated, thus more studies are needed.

Acknowledgments

The author's responsibilities were: "FMY (principal investigator, writing of manuscript); PTK (data analysis, writing of manuscript); RMA (performed statistical analysis); FMY, JMY (hands-on conduct of the experiments and data collection); TAK (provided essential materials for the research, made all clinical decisions); FMY, ETJ (had primary responsibility for final content and provision of significant advice or consultation); FMY, CAT (project manger, development of overall research plan and design of the experiment).

TABLE 4 Baseline anthropometric and lifestyle characteristics in Saudi Arabia women (n=120)

Anthropometric (N=120)	**Mean± SD**	**[1]P-Value**
Age (y)	47.9±13.6	NS
BMI (kg/m^2)	29.5±6.1	NS
Height (cm)	159.2±4.8	NS
Weight (kg)	75±14.8	NS
Serum 25(OH)D (ng/ml)	15.4±12.3	NS
Lifestyle	**N (%)**	
Physical activity <30 minutes >30 minutes	 82 (68.3) 38 (31.6)	NS
Outdoor Activity (Yes)	33 (27.5)	NS
Time Spent Outdoors 15 minutes 30 minutes 60 minutes	 39 (32.5) 80 (66.6) 1 (00.8)	NS
Sunlight Exposure Hand Hand and Face Completely Covered	 33 (27.5) 59 (49.1) 28(23.3)	NS
Sun Protection (Yes)	52 (43.3)	NS
Calcium Supp (Yes)	6 (5.00)	NS
Vitamin D Supp (Yes)	4 (3.3)	NS
Multivitamin use (Yes)	2 (1.6)	NS
Smoking (Yes)	62 (51.6)	NS

Education		NS
High school or Less	30 (25)	
Post High School	27 (22.5)	
Some College	39 (32.5)	
College Graduate	24 (20)	
Marital Status		NS
Married	105(87.5)	
Divorced	14 (11.6)	
Widowed	0 (0)	
Single	1(0.8)	
Parity		NS
0	17 (14.1)	
1	1 (0.8)	
2	14 (11.6)	
3	14 (11.6)	
4	74 (61.6)	

[1]p-value was statistically insignificant=NS (>0.05)

TABLE 5 Independent t-tests to compare mean serum 25(OH)D by categories of time in outdoor activity and use of sunscreen protection in (n=120) women in Saudi Arabia

Outdoor activity	N	Serum 25(OH)D (ng/ml) (mean±SD)	Sun Protection	N	Serum 25(OH)D (ng/ml) (mean±SD
<30 minutes	82	15.6±1.34	Yes	52	15.1±1.52
>30 minutes	38	14.9±2.08	No	68	15.7±1.68
P-Value		0.79	P-Value		0.79

TABLE 6 Relationship between body parts exposed to sunlight and serum 25(OH)D concentrations of Saudi Arabian women (n=120)

Sunlight Exposure By Body part	**N**	**Serum 25(OH)D ng/mL (mean±SD)**	**P-value**
Hand exposed	33	13.9+11.92	0.55
Hand and face exposed	59	15.3+12.57	
Completely covered	28	17.3+12.38	

TABLE 7 Covariates that best predict the relationship between outdoor activity and serum 25(OH)D concentrations[1]

Predictors	Coefficient	P-Value	R^2
Education	**-3.15**	**0.003**	**0.14**
Calcium	**-10.08**	**0.041**	
Breastfeeding	**-1.18**	**0.042**	
Weight	**-0.16**	**0.034**	
Menarche	**-1.32**	**0.032**	

[1]Variable selection using Akaike's Information Criteria

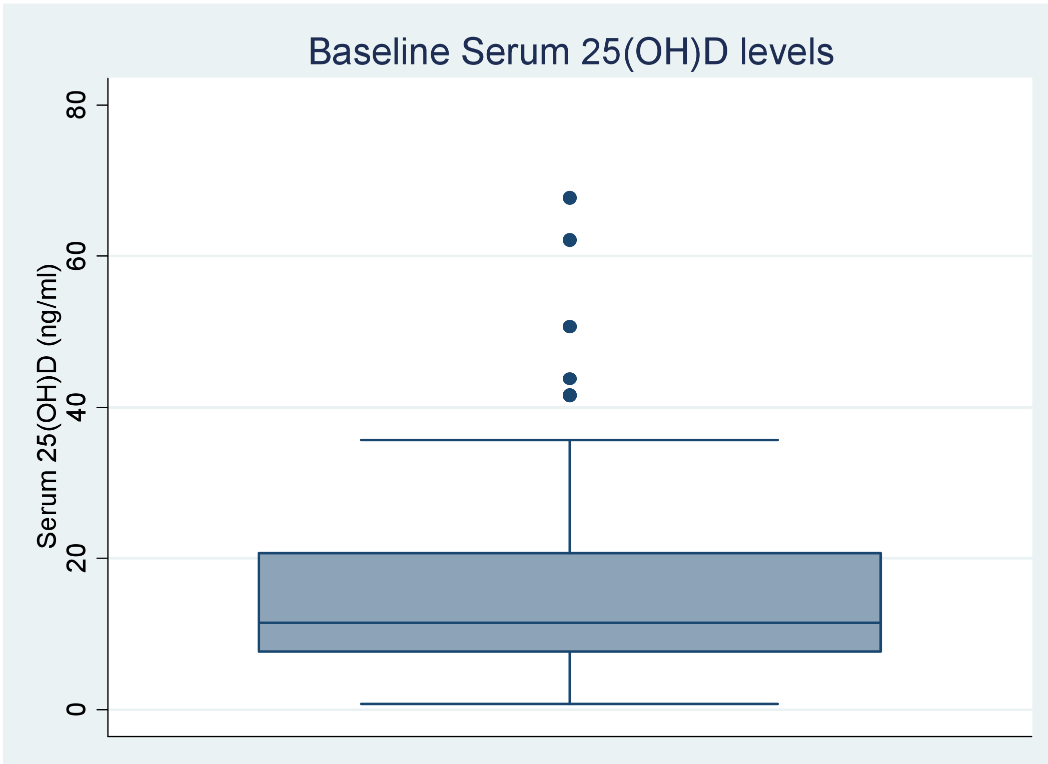

FIGURE 4 Box-plot of serum 25(OH)D concentrations in women (n=120) in Saudi Arabia

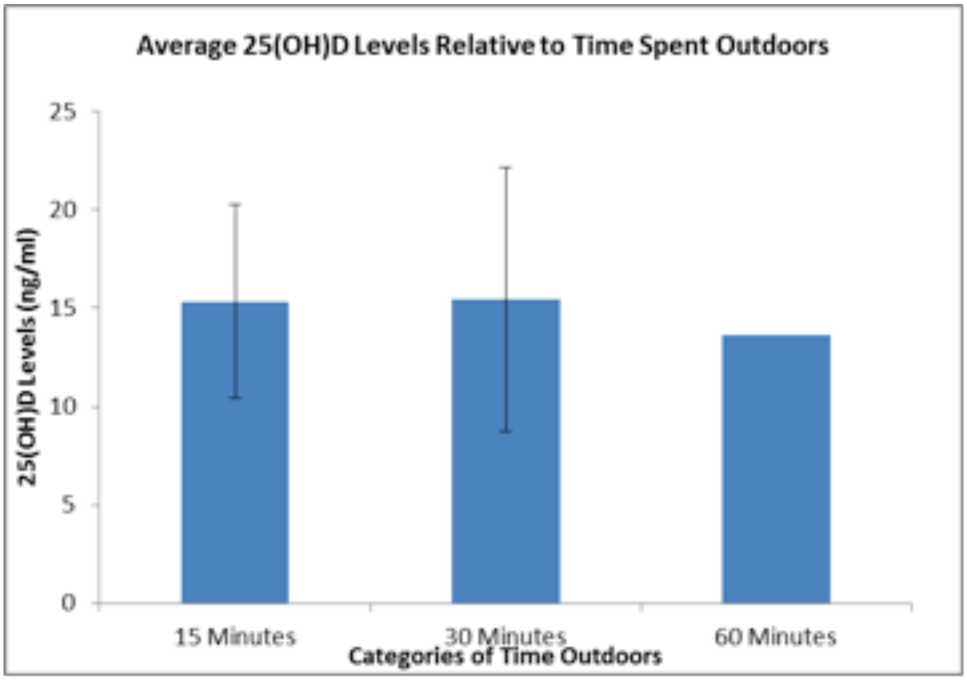

FIGURE 5 Relationship between length of exposure to sunlight and serum 25(OH)D concentrations of Saudi Arabian women (n=120)

CHAPTER 3
VITAMIN D STATUS AND BREAST CANCER IN SAUDI ARABIAN WOMEN: CASE CONTROL STUDY

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ABSTRACT

Background: The role of vitamin D in breast cancer prevention is equivocal. Saudi Arabian women are thought to be at greater risk for vitamin D deficiency due to darker skin type and greater likelihood for reduced UVB radiation exposure. However, there is a lack of data regarding the vitamin D status of Saudi Arabian women and how this may relate to breast cancer risk.

Objective: The purpose of this research was to evaluate the association between circulating concentrations of 25(OH)D and breast cancer risk in Saudi Arabian women.

Design: A case-control study was conducted among 120 breast cancer cases and 120 controls. The study population was drawn from patients admitted to KFH in Jeddah, Saudi Arabia from June to August 2009. Participants completed questionnaires on diet and medical history and serum samples were collected from all women to measure circulating 25(OH)D concentrations.

Results: The mean age of study participants was 47.8 years with a mean BMI of 30.2 kg/m^2. Breast cancer cases had significantly lower serum concentrations of 25(OH)D (9.4 $\pm$ 6.3 ng/mL) compared to controls (15.4 $\pm$ 12.3 ng/mL; *p*-value 0.0001). Compared to those in the highest category of vitamin D status for this population ($\geq$ 20 ng/ml), the adjusted OR (95% CI) was 6.5 (2.6, 15.8) for those with concentration of 25(OH)D of <10 ng/mL and 4.0 (1.6, 10.2) for $\geq$10 and <20 ng/mL, (p-trend=0.0001).

Conclusion: Our findings support an inverse association between serum 25(OH)D concentrations and risk of breast cancer in Saudi Arabian women.

Introduction

According to the Saudi Arabian National Cancer Registry, in 2007 breast cancer was the most frequent cancer among women, accounting for 26 % of all newly diagnosed ale cancers (131,132). In Saudi Arabia breast cancer is commonly diagnosed in women under the age of 40 years compared with only 6.5% in the United States (133). Epidemiological studies have suggested that there is an inverse association between serum 25-hydroxyvitamin D [25(OH)D] levels and risk of breast cancer (134,135), though these results vary by study design. There is evidence of an association between high levels of vitamin D and decreased risk of breast cancer as has been concluded by The International Agency for Research in Cancer (IARC); nevertheless, there is not sufficient evidence to conclude that a causal effect exists (174). Several mechanistic studies have identified specific targets for vitamin D in cancer prevention including such functions as anti-proliferation (136), pro-differentiation (137), and cell cycle stabilization (138). However, whether these mechanisms translate to breast cancer risk reduction remains unclear. In 2010 the Institute of Medicine (IOM) considered vitamin D deficiency as 25-hydroxyvitamin D [25(OH)D] levels below 12 ng/mL and insufficiency was considered 12–19 ng/mL. A sufficient level of vitamin D was defined as serum 25(OH)D ≥20 ng/mL. Since vitamin D is acquired predominantly through endogenous synthesis in the skin following ultraviolet B (UVB) radiation exposure, its deficiency is common in many parts of the world where exposure to sunlight is limited due to cultural beliefs, clothing, and public health recommendations (139). Furthermore, while vitamin D is found in foods such as fortified milk, fatty fish, and cod liver oil, other food sources of vitamin D are very limited (139). After vitamin D is synthesized or ingested, it is hydroxylated in the liver to form 25(OH)D, the primary biomarker for vitamin D status. A second hydroxylation in the kidney is necessary to convert 25(OH)D to the active form 1, 25-dihydroxyvitamin D [1,25(OH)D]. 1, 25(OH)D binds to the nuclear vitamin D receptor (VDR), which is found in a number of cells including both normal and cancerous breast cells (139,144). In vitro and animal studies have both shown that 1,25(OH)D promotes cell differentiation and apoptosis, and inhibits cell proliferation (100).

Saudi women are thought to be at greater risk for vitamin D deficiency because of their darker skin type and the likelihood of reduced UV exposure (145, 146). However, data in Saudi Arabian populations are limited (145,147,148). The objective of this study was to evaluate the association between circulating concentrations of 25(OH)D and breast cancer risk using data collected from a case-control study in Saudi Arabian women.

Subjects and Methods

Study Population

Participants were recruited from King Fahad Hospital (KFH) in Jeddah, Saudi Arabia, using a case-control study design to evaluate the relationship between breast cancer risk and 25(OH)D concentrations. Jeddah is a city located in the western region of Saudi Arabia (Latitude 21.4500 degrees North and Longitude 39.8167 degrees East), and is the second most populated city (population 2.1 million) in Saudi Arabia with population representing mostly native-born Saudis. The study was performed during the summer months; the city experiences an average of 9 hours of sunlight daily with a mean maximal temperature 36.7°C. The de-identified dataset was provided for this analysis and was approved for epidemiological study by the Human Subjects Committee at the University of Arizona.

Cases were 120 female patients at KFH with newly diagnosed with stage I through stage IV breast cancer, and were between the ages of 18 and 75 years. The control group included 120 women same ages as cases with no history of breast cancer who visited the women's clinic at KFH for regular clinical visit. A single medical doctor recruited all control women during preventive care visits at the clinic.

Inclusion and Exclusion Criteria

Eligibility criteria were chosen in order to reduce variance in vitamin D status associated with environment, culture behaviors and diet and included: 1) women between the age of 18 and 75 years, with BMI ≤ 40 kg/m^2, 2) a native of Saudi Arabia or a resident of Saudi Arabia for more than 5 years; and, 3) absence of chronic diseases that could affect vitamin D metabolism, including renal or hepatic endocrine, and autoimmune disease. All women were identified by physicians at the hospital and were provided care at this facility during the summer of 2009. Participants were also willing to complete all study-related activities such weight and height measurements and blood draws, and were willing to sign a written informed consent document.

Data Collection

A lifestyle and medical history questionnaire was administered to each participant during their clinic visit to determine participant characteristics such as overall health status, tobacco use and medical history including medication use, history of cancer or benign breast cancer, family history of breast cancer and menstrual history (normal, or abnormal), breastfeeding history, parity, oral contraception, age at first birth, education, and socioeconomic status.

A short Food Frequency Questionnaire (FFQ) was administered that consisted of 15 questions regarding amount and frequency of intake of milk, dairy products, eggs, organ meats such as liver, other meats (beef, chicken, salmon, tuna, fish, and seafood), fruits, and vegetables. The questionnaire asked respondents to report the frequency of daily intake per food item with responses ranging from ≥ 3-times/ day to rarely/ never. Participants were asked about their diet for the 12-month period prior to the study, including use of supplemental vitamin D and calcium. Sun exposure (face, hand, face and hand, both arms, both legs, and completely covered), use of sun protection, and style of dress was also assessed. Women were also queried about frequency of physical activity at enrollment including question regarding sedentary (sitting, standing, casual walking), moderate (regular walking or swimming) and vigorous activity (brisk daily jogging).

Blood Sample Collection and Measurement of 25(OH)D By HPLC

From June through August 2009, a single blood sample was collected from 240 women (120 cases and 120 controls) to assess total serum 25(OH)D. Approximately 2 ml of blood were collected via venipuncture. All tubes were protected from light, and the specimens were centrifuged at 3000 rpm for 10 min. The serum was separated and stored at -80°C until analyzed by high performance liquid chromatography (HPLC) in King Fahd Center for Medical Research (KFCMR). The method used has been described in detail elsewhere (30). Briefly, the method utilizes a reversed- phase HPLC technique that shows a clear resolution of 25-hydroxyvitamin D_2 [25(OH)D_2] and 25(OH)D_3. The mobile phase is an acetonitrile extract of serum by solid phase extraction C18/OH cartridges. HPLC was preformed using a Shimadzu LC-10 system with Shimadzu LC-10AT pump, Corporation, Kyoto, Japan).

Statistical Analysis

All statistical analysis was performed using STATA 11.0 statistical software (College Station, Texas). Demographic and clinical characteristics of cases and controls were evaluated using descriptive statistics: mean, standard deviation as well as frequencies. For comparisons of baseline characteristics p-values were calculated with chi-squared analyses for categorical variables and regression analyses for continuous variables. Backwards variable selection was used to determine variables for the full model, using a p-value of 0.05 to select covariates that remained in the model. These variables include age, BMI, history of cancer, parity, family history of cancer, exercise, location of exercise, multivitamin use, presence of breast cancer in daughters, benign cancers, menopause, and breastfeeding. Variables such as age, exercise, and breastfeeding have been demonstrated to be significant covariates in early research evaluating the association between vitamin D and breast cancer while not significant in our sample were forced into the adjusted models.

Potential confounding variables were assessed using logistic regression modeling. Confounders were defined as variables which changed the point estimates 10% or greater. However, the covariates used in the full model did not significantly change the point estimates. Therefore, the covariates used in the final adjusted model have been labeled as confounders in earlier research evaluating the association of Vitamin D status and breast cancer. Variables included in the final adjusted model were age, body mass index (BMI), physical activity, parity, education, and history of breastfeeding. Logistic regression modeling was employed to assess the relationship between serum 25(OH)D concentrations and risk for breast cancer.

Results

Data from 240 women including 120 breast cancer cases and 120 controls were included in this analysis. The characteristics of the study population are shown in **(Table 8)**. The mean age was 47.9 years for cases and 47.7 years for controls. Age at menarche, height, and body weight did not differ between cases and controls. The mean averaged BMI in both groups was 30 kg/m^2 indicating the study sample met criteria for obesity. Family history of breast cancer was more common in cases (31.7%) than in controls (10.8%) (*P*-value =0.0001). Moreover, the cases demonstrated lower education, fewer live births, were more likely to report breastfeeding greater than > 6 months, and reported lower use of oral contraceptive agents than controls. However, controls were more likely to report current smoking history than cases.

As shown in **(Table 9)** serum 25(OH)D concentrations were significantly lower in cases as compared to controls in this sample of women residing in Saudi Arabia. The mean serum concentration of 25(OH)D was 9.4± 6.35ng/mL, and 15.4±12.31 ng/mL in cases and controls, respectively, (*P*-value=0.002). Notably, 60.8% of cases and 38.3% of control women demonstrated circulating 25(OH)D concentrations below 10 ng/mL; while the current recommended concentration for adequacy is above 20 ng/ml (129). Concentrations of 25(OH)D of ≥ 10 and < 20 ng/mL were present in 32.5% and 34.2% of cases and controls, respectively. Only 6.7% of cases and 27.5% of controls demonstrated 25(OH)D concentrations of more than 20 ng/mL (Table 2).

In **(Table 10)** presents the odds ratios for breast cancer in the total sample population by category of vitamin D status; < 10 ng/mL > 10 and < 20 ng/mL and > 20 ng/mL category as our referent. The unadjusted and adjusted odds ratios show statistically significant risk for breast cancer across decreasing 25(OH)D concentrations (*P* -value=0.0001). After adjusting for age, BMI, physical activity, parity, education, and history of breastfeeding and compared to those women with 25(OH)D concentrations >20 ng/ml, the odds ratios (95% CI) for breast cancer risk was 4.06 (1.6, 10.2), and 6.51 (2.6, 15.8), for women with 25(OH)D concentrations of ≥ 10 and < 20 ng/mL and < 10 ng/mL, respectively.

Discussion

The risk of breast cancer has been observed to be greater in geographic areas with lower amounts of sunlight during the year (150), and also others have demonstrated a protective association between UV exposure earlier in life and breast cancer risk, mostly during breast development (151), leading to the hypothesis that vitamin D may be associated with reduced risk for breast cancer (152). Some studies support this hypothesis, but not all effect estimates were statistically significant (150,152-157) both in relation to UV exposure/ time spend outdoors (158,159) and vitamin D status (152,160), but epidemiological results are inconsistent (135, 141). Moreover, vitamin D status has been inversely associated with breast cancer stage, recurrence and mortality (161). The majority of studies of vitamin D and breast cancer to date have been conducted in predominantly non-Hispanic white women. The current study is, to our knowledge, the first study to evaluate the association between serum 25(OH)D and breast cancer in women residing in Saudi Arabia, an area of high UV sunlight exposure, but potentially low vitamin D status related to skin type and cultural practices of dress. In support of our findings (152) Abbas and colleagues found a protective association of interest vitamin D concentrations below 30 nmol/L was association with increase risk of breast cancer and a possible threshold effect was shown at above 50 nmol/L (152). An exploratory analysis in pre and separately post-menopausal women in our sample showed increased risk in both groups, but confidence intervals were large, with ORs (95% CIs) of 3.6 (0.43, 30.1) and (18.1(2.2, 149.8) for premenopausal and postmenopausal women, respectively. In a case-control analysis conducted by Abbas et al, risk of breast cancer (n=289) was reduced in women within the highest category (≥ 24 ng/mL) of 25(OH)D compared to lowest category (< 12 ng/mL) with p-trend=0.0006 (156).This study was different from our study in that the participants were only postmenopausal women; two controls were matched per cases on years of birth, and study region to the cases (156). Further, the blood samples were collected prior to breast cancer diagnosis for the cases whereas our sampling was conducted at the time of diagnosis. Study by Rossi et al., 2009 found strong association between vitamin D intake (>190 IU/day) and 64% reduced risk of breast cancer among women living in Southern Italy, but this study was attenuated and not significant among women who lived in North of Italy (142). Importantly, in each of the studies higher vitamin D was shown to be protective whereas in our study the overall lower 25(OH)D status of the population inferred greater risk for breast cancer; protective concentrations were not identified.

The Institute of Medicine recently recommended a dietary allowance of vitamin D intake of 600 IU/d (or 15μg/d) for women age 1 to70 years and 800 IU/d for age 71 years and older (162). In our study population, dietary vitamin D intake was low with less than 34% of cases and 39% of controls consuming more than a single serving of vitamin D-rich foods fish, dairy (data not shown). In addition, the food supply in Saudi Arabia is not fortified with vitamin D as it is in the U.S.

Sun exposure can serve as the primary strategy for meeting vitamin D requirements, particularly in people with modest dietary intake of vitamin D. However, this recommendation is conditional to skin type, latitude, and other factors (163-165). Time of the day is also an important factor because the angle of the sun changes throughout the day, so it is more difficult to produce vitamin D in the early morning or late afternoon (163). Thus, for sufficient vitamin D synthesis in the skin, at least 25 percent of the body should be exposed to the sun (without sunscreen) to enable the body to make enough vitamin D (163).

Women in Saudi Arabia, as well as in other Arab populations, have a high prevalence of vitamin D deficiency (148, 166-168). Among healthy Saudi women, rates of vitamin D deficiency are highly prevalent at premenopausal and postmenopausal women (148). In our population, despite the fact that women reside in an area where the UV light levels are high year-around, we found mean concentrations of serum 25(OH)D to be quite low. Even with the relatively low circulating levels, in this study the level of vitamin D concentrations was higher in women without breast cancer as compared to women diagnosed with breast cancer. In addition, Jeddah is a place where UV light is present almost every day and at relatively high latitudes. Given the associated heat, residents practices sun avoidance. Spending time outdoors is very rare in this culture (169,170). Moreover, socio-cultural practices related to dress play an important role in ensuring that women are not overexposed to the sun. Housing design and lifestyle choices (i.e., living in an air-conditioned apartment and avoiding sun exposure) also can contribute to greater time indoors, thus leading to increased risk for vitamin D deficiency.

Strengths of this study include focus in Saudi Arabian women, a group at greater risk for vitamin D deficiency and who demonstrate low concentrations of 25(OH)D. The short window for biosampling thus reduced seasonal effects was an additional strength as was the availability of demographic and lifestyle data during a face-to-face visit with each woman. Limitations include the lack of biosamples prior to diagnosis, and ideally, more than one measure to 25(OH)D would be the beast to measure the long- term average 25(OH)D, further, lacked detailed dietary intake. However, there is sparse evidence that dietary vitamin D alone significantly modifies circulating concentrations in depleted individuals (171) and our emphasis on objective measures versus self-report dietary sources is the strength of the study design. Further, our study did not afford an opportunity to evaluate the association between vitamin D status and specific breast cancer subtypes as has been suggested by others (172). Additionally, we were not able to complete genotype analysis to determine the role of vitamin D polymorphisms and risk of breast cancer, as has been suggested by McCullough et al, in a nested case-control study (173).

In summary, the area of vitamin D and breast cancer is a field of intense study and there are many aspects of this association that require further investigation. Here we provide evidence of a significant association between low levels of circulating 25(OH)D and higher risk of breast cancer using a case-control study design of women residing in Saudi Arabia. Importantly, mean, serum 25(OH)D concentrations were very low in the study sample overall.

Conclusion and Future Directions

The results of this case control study show a high prevalence of vitamin D deficiency in women in Jeddah, Saudi Arabia. Low vitamin D status is associated with greater risk for breast cancer. These findings will inform clinical care for women. Efforts to more routinely assess vitamin D status and possibly provide supplementation to correct depletion in this at-risk group should be evaluated. Finally, the association between vitamin D and breast cancer risk require further investigation.

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The author's responsibilities were: "FMY (principal investigator, writing of manuscript); PTK (data analysis, writing of manuscript); RMA (performed statistical analysis); JMY (hands-on conduct of the experiments and data collection); TAK (provided essential materials for the research, made all clinical decisions); ETJ (had primary responsibility for final content and provision of significant advice or consultation); CAT (project manger, development of overall research plan and design of the experiment).

TABLE 8 The Characteristics Descriptions of Saudi Arabian Women Case-Control (n=240)

aracteristic	**All (n=240)**	**Cases (n=120)**	**Controls (n=120)**	***P*-value**
	Mean± standard deviation			
Age (y)	47.7	47.9 ± 13.6	47.6 ± 11.02	.8
Age at menarche (y)	12.9	12.9 ± 1.7	12.9 ± 1.6	.8
Height (cm)	158.6	158.1 ± 3.9	159.1 ± 4.8	.06
Weight (kg)	75.4	75.8 ± 9.4	75 ± 14.8	.6
BMI (kg/m^2)	30.0	30.3 ± 4.05	29.6 ± 6.04	.2
Percentage				
Parity				
% Nuliparous	7.9 %	1.7 %	14.2 %	.000
% Have children	92.1 %	98.3 %	85.8 %	.000
% Family history of BC	21.3 %	31.7 %	10.8 %	.000
% Mother history of BC	7.5 %	5.0 %	10.0 %	.141
% Sister History of BC	12.5 %	3.3 %	21.7 %	.000
% Benign BC	18.8 %	32.5 %	5.0 %	.000
% Used oral contraceptives	16.7 %	1.7 %	31.7 %	.000
% Breastfeeding				
<6 months	62.1 %	53.3 %	70.8 %	.005
>6 months	37.9 %	46.7 %	29.2 %	.005
% Education				
High school or less	37.8 %	43.7 %	31.5 %	.17
Post high school	21.3 %	19.3 %	23.4 %	.000

Some college	35.2 %	35.3 %	35.1 %	. 010
College graduate	5.7 %	1.7 %	9.9 %	.000
%Annually income				
≤ 5000 SR	49.2 %	49.2 %	49.2 %	.000
≥ 5000 SR	50.8 %	50.8 %	50.8 %	.000
% Smoking	44.2 %	36.7 %	51.7 %	.000

TABLE 9 Circulation serum 25(OH)D concentration in Saudi Arabian women, Cases (n=120), Controls (n=120)

Variables	**Cases (n=120)** *N* %	**Controls (n=120)** *N* %	***P*-value**
Serum 25(OH)D (ng/mL)[1]	**Mean±SD**		
	9.4±6.35	15.4±12.31	0.002
<10 ng/mL	73 (60.8)	46 (38.3)	0.42
≥10 - < 20ng/mL	39 (32.5)	41 (34.2)	0.51
≥ 20ng/mL	8 (6.7)	33 (27.5)	0.17

[1]Deficient is defined as circulating concentrations of 25(OH)D of less than <10 ng/ml; Insufficient as ≥10 and < 20 ng/ml; Optimal is ≥20 ng/ ml.

Table 10 Crude[1] and Adjusted[2] Odds Ratios (95% Confidence Intervals) for the Association Between Circulating Concentrations of 25(OH)D and Breast Cancer.

Category of 25(OH)D concentration (ng/ml)	**Deficient (<10 ng/ml)**	**Insufficient (≥10 and <20ng/ml)**	**Sufficient (≥ 20 ng/ml)**	**P-trend**
All cases and controls Total (n)	119	80	33	
Crude OR[1] (95% CI)	6.5 (2.8, 15.4)	3.9 (1.6, 9.5)	1.00 (referent)	< .0001
Adjusted OR[2] (95% CI)	6.5 (2.6, 15.8)	4.0 (1.6, 10.2)	1.00 (referent)	< .0001

[1] Conditional logistic regression models with no adjustments.

[2] Conditional logistic regression models adjusted for age, BMI, physical activity, parity, education, history of breastfeeding, and oral contraceptive use.

CHAPTER 4
IMPLICATIONS AND FUTURE DIRECTIONS

Summary of Dissertation Work

Because vitamin D is stored in adipose tissue, it was important to assess the association between serum 25(OH)D concentrations and body size. We found that the lowest mean serum 25(OH)D concentrations in obese group compared to overweight and normal weight women with mean serum 25(OH)D concentrations of 13.5 ± 9.1 ng/mL, 18.3 ± 18.3 ng/mL, and 15.7±10.1 ng/mL, respectively. We then compared women with stable body weight since age 18 years to women who gained weight since that age. In this study, women who maintained a stable weight over time (n=34) as well as women who gained weight (n=86) had no significant difference in mean circulating 25(OH)D 15.7±14.4, 15.2±11.4 ng/mL, respectively, thus both groups were vitamin D deficient. These differences were not statistically significant. This lack of association between BMI or weight gain and vitamin D status is likely related to the high prevalence of vitamin D insufficiency at all weight classifications. Further epidemiological studies should assess the relationship between the active form of vitamin D, 1,25(OH)D and PTH in obese individuals as compared to overweight and normal weight individuals.

Outdoors activity and sun protection did not appear to significantly contribute to vitamin D status in our study sample. Surprisingly, we found that women who had their entire body covered showed higher serum 25(OH)D concentrations (17.3±12.38 ng/mL) than women who obtained some sun exposure such as face and hand (15.3±12.57 ng/mL). Yet both groups demonstrated sub-optimal status. The lack of significant differences may be explained by the minimal time spent outdoors with UV exposure regardless of coverage, use of sunscreen or moderately darker skin tone of Saudi Arabian people.

In this dissertation we concluded that there is a strong association between high circulating of 25(OH)D concentrations and decrease risk of breast cancer in Saudi Arabian women. Higher mean serum 25(OH)D shows in healthy women (15.4±12.3 ng/mL) as compared to women who have a diagnosis of breast cancer (9.4 ± 6.3 ng/mL; *p*-value 0.0001). After adjusting for age, BMI, physical activity, parity, education, and history of breastfeeding and compared to those women with 25(OH)D concentrations >20 ng/ml, the odds ratios (95% CI) for breast cancer risk was 4.06 (1.6, 10.2), and 6.51 (2.6, 15.8), for women with 25(OH)D concentrations of ≥ 10 and < 20 ng/mL and < 10 ng/mL, respectively. In this study 82.3% of the participants were below the referent level. This suggests that assessment of vitamin D status during regular health check up for Saudi Arabian women is warranted. While this observed association between serum 25(OH)D and breast cancer is compelling, a well-designed randomized controlled trial to establish the therapeutic benefit is needed before specific recommendations can be made in regards to routine use of vitamin D supplementation for breast cancer prevention. Moreover, additional observational studies conducted in Saudi Arabia are needed to assess the association between decrease risk of breast cancer and sun exposure as primary source of vitamin D.

The results suggest that the Kingdom of Saudi Arabia should consider more regular evaluation of vitamin D status and dietary intake. Fortification of food as well as increased availability of supplementation should be considered. The current DRI of 600

IU/d for those 1-70 years of age is likely inadequate in Saudi Arabian woman given the very depleted vitamin D status. Thus, future work should determine an adequate DRI, especially designed to meet the needs of this depleted population. Alternately, Saudi women might be encouraged to increase their exposure to sunlight through outdoor activity during the daytime. In addition, efforts to investigate the potential role of vitamin D and many other diseases such as diabetes, cardiovascular disease and bone health, should be enhanced.

Summary of Findings

The results of our study suggest that there is no associations were observed between stable weight since age 18 years, current weight and serum 25(OH)D concentrations. The high prevalence of vitamin D deficiency among Saudi Arabian women is common, independent of BMI. The majority of our samples are obese, and this maybe a significant contributor to low vitamin D status. Nevertheless, we found no association between serum 25(OH)D and outdoor activity nor with time spend outdoors in this population of Saudi Arabian females. Finally, vitamin D status is associated with increased breast cancer risk in Saudi Arabian women.

Future Direction

Additional epidemiological studies are needed to further evaluate relationship between serum 25(OH)D concentrations and risk of breast cancer. These analysis should not only measure of 1,25(OH)D, but also characterization of vitamin D receptor, stage of diagnosis, menopausal status, and time spending outdoor. This will be very an important area of research, since breast cancer is the leading type of cancer among Saudi Arabian women (131).

Moreover, longitudinal studies are needed to determine the potential efficacy of vitamin D supplementations or/and increased sun exposure and the prevention of breast cancer. Independent of this work, public health awareness should be improved to educate the population on the benefits of vitamin D in relation to health as well as guidance to optimize vitamin D status.

Furthermore the development of public policies to increase outdoors physical activity for women or increase exposure to the sun could be considered. However, religious beliefs and traditional clothing practices may preclude such efforts. Alternatively, creation of special areas for women to get UV exposure such as clubs established in neighborhoods around the city may be warranted.

Additionally studies are required during multiple periods of life to assess the association between vitamin D status and direct sunlight exposure and/or dietary intake of vitamin D from food and/or supplement use. Future studies to evaluate all of theses factors also should take into consideration gene-environment interactions such as polymorphisms in vitamin D receptor gene or vitamin D binding protein gene and vitamin D status.

Conclusion

Despite the emerging body of literature suggesting that high circulating serum 25(OH)D concentrations may decrease risk of breast cancer in Saudi Arabian women.

The results of the totality of evidence internationally still in inconsistent, and there are still many gaps in our knowledge of these associations. Hence, to make reference to a causal association is not possible at this time. This dissertation work demonstrates an inverse association between serum 25(OH)D concentrations and risk of breast cancer in Saudi Arabian women. This works also suggest that in generally depleted population in which only 2.9% of women had vitamin D levels > 40ng/mL, factors that have been previously associated with risk (i.e. outdoors or total physical activity and BMI) for vitamin D deficiency do not show significant associations in our analysis.

Authors' contributions

The author's responsibilities were: "FMY (principal investigator, writing of manuscript); FMY and PTK (data analysis, writing of manuscript); RMA (performed statistical analysis); FMY, JMY (hands-on conduct of the experiments and data collection); FMY, TAK (provided essential materials for the research, made all clinical decisions); FMY (had primary responsibility for final content and provision of significant advice or consultation); FMY, ETJ, and CAT (project manger, development of overall research plan and design of the experiment).

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APPENDIX A – QUESTIONNAIRE

Age (Years): It should be between (18 – 75)

--

Weight (kg):________ Height (cm):________

What was your weight when you were 18 years old?

How old were you when you started your menarche?

Do you have a cancer?

Yes	1
No	2

Have any of your family's members had a cancer?

Yes	1
No	2
I don't know	3

If so, what kind of cancer do you have?

Breast	1
Ovarian	2
Colon	3
I don't know	4

Had your sister(s) had breast cancer?

Yes	1
No	2
I don't know	3

Had your mother had breast cancer?

Yes	1
No	2
I don't know	3

Had your daughter(s) had breast cancer?

Yes	1
No	2
I don't know	3

Have you ever done BRCA1or BRCA2 test?

Yes	1
No	2
I don't know	3

Have you ever had benign breast diseases?

Yes	1
No	2

Are you currently taking birth control pills?

Yes	1
No	2

How old were you when you gave first birth?

How many children have you given birth to?

1	1
2	2
3	3
≥ 3	4
No kids	5

How many babies did you breast feed?

How long?

Less than 6 months	1
More than 6 months	2
No kids	0

Have you gone through menopause (Have you stopped having menstrual periods)?

Before age 55	1
After age 55	2
No	3

What is your academic status?

High school or less	1
Post high school	2
Some college	3
College graduate	4

What is your marital status?

Currently married	1
Divorced	2
Widowed	3
Single	4

Do you smoke or are you used to smoke?

Yes	1
No	2
Smoke in the past	3

Do you consume vitamin D Supplement?

Yes	1
No	2

Do you consume Calcium Supplement?

Yes	1
No	2

Do you consume Multivitamins Supplement?

Yes	1
No	2

How much time do you spend outdoors in the daytime?

≤15m	1
≤ 30m	2
≤ 1h	3

Which parts of your body get exposed to the sun?

Face	1
Hand	2
Face and hand	3
Both arms	4
Both legs	5
Completely covered	6

Do you use sunscreen whenever you are outside?

Yes	1
No	2

What is your monthly income?

Less than 5000	1
More than 5000	2

In a typical day, do you walk?

Less than 30min	1
More than 30min	2

If so, where do you walk?

Indoors	1
Outdoors	2

Do you drink Milk?

≥ 3	Rarely	Never
1	2	3

Do you eat Orange Vegetable?

≥ 3	Rarely	Never
1	2	3

Do you eat Green Vegetable?

≥ 3	Rarely	Never
1	2	3

Do you eat Fruit?

≥ 3	Rarely	Never
1	2	3

Do you eat Grain?

≥ 3	Rarely	Never
1	2	3

Do you eat Eggs?

≥ 3	Rarely	Never
1	2	3

Do you eat Cheese?

≥ 3	Rarely	Never
1	2	3

Do you eat Chicken?

≥ 3	Rarely	Never	≥ 3
1	2	3	1

Do you eat Red Meet?

≥ 3	Rarely	Never	≥ 3
1	2	3	1

Do you eat Fish?

≥ 3	Rarely	Never	≥ 3
1	2	3	1

Do you eat Fast Food?

≥ 3	Rarely	Never
1	2	3

Do you eat Fried Food?

≥ 3	Rarely	Never
1	2	3

Do you eat Tuna?

≥ 3	Rarely	Never	≥ 3
1	2	3	1

Do you eat Liver?

≥ 3	Rarely	Never	≥ 3
1	2	3	1

Stage of breast cancer

I	1
II	2
III	3
IV	4

Duration since diagnosis

Tamoxifen or Raloxifene for 5 years or more

Yes	1
No	2

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