**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/m2. Height, weight, serum 25(OH)D concentrations, and questionnaires about medical history, health behaviors were measured. Results reported that 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. 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 noradult 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.

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**1. Introduction.**

Obesity defined as a medical condition with excess adiposity or as a body mass index (BMI) above 30 kg/m2, obese individuals demonstrate abnormal metabolic and endocrine functions**(Seidell and Flegal, 1997)**. Incidence of obesity has increased over the past decades **(WHO, 2000)**. In the Arabian Gulf region obesity has increased 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 **(Al-Rethaiaa *et al*., 2010)**. OO

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 **(Martins *et al*., 2007 and Mason *et al*., 2011)**. 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 **(Wortsman *et al*., 2000)**. 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 **(Wortsman *et al.*, 2000 and Holick, 2002).** 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 Ca2+ concentrations, predominantly in adipocytes **(Parikh *et al*., 2004 and Holick, 2009).**

The actions of 1,25(OH)Dare mediated by binding to the vitamin D receptor (VDR), a member of the steroid hormone receptor subfamily, which has been found in >30 cell types, including fat cells **(Kato, 2000)**. Vitamin D not only regulates bone metabolism, but also has an important role in adipogenesis and in diseases such as diabetes, and cancer **(Mathieu and Badenhhjoop, 2005 and Davis and Dwyer, 2007)**. The main source of vitamin D is by production through solar Ultraviolet B (UVB) radiation (280–315nm) that penetratesthe skin 7-dehydrocholesterol, which is locatedin the epidermal layer of the skin, is converted topre-vitamin D3, which is then rapidly converted to vitamin D3 **(Davis and Dwyer, 2007**). Vitamin D3 can also be foundin a few foods such as fatty fish and vitamin D supplements and fortified foods **(Solomons, 2008).**

Vitamin D deficiency has increased recently worldwide and can vary according to skin pigmentation, age, adiposity, geography, and other factors **(Holick, 2007)**. Although, UV sunlight in Saudi Arabia is high throughout the year, Saudi Arabian people, especially women, demonstrate vitamin D deficiency,more than 30 % of healthy young women in Saudi Arabia were found to be in a deficient state **(Sadat *et al*., 2008)**. 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 BMI and serum 25(OH)D concentrations including the association between change in BMI during adulthood and vitamin D status.

**2. Subjects and Method**s.

**Study population.**

The study was conducted between (June-August 2009)at King Fahd Hospital (KFH) in Jeddah, Saudi Arabia. Women (n=120) aged (18 – 75 year) with BMI ≤ 40 kg/m2, 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 and smoking habits were 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 **(Hollis, 2005)**. 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 wasmeasured 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.**

Blood samples were collected from participants 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 -80oC until thawed for analyses by high performance liquid chromatography (HPLC) in King Fahd Center for Medical Research (KFCMR). The method used for the determination according to (**Rapuri and Gallagher, 2004).** Briefly, the method utilizes a reversed- phase HPLC technique that shows a clear resolution of 25-hydroxyvitamin D2 [25(OH)D2] and 25(OH)D3. 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.

**3.Results.**

Table (1) represented the demographic, anthropometric and lifestyle characteristics of Saudi Arabia women. 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/m2 and current BMI of 29.6 ± 6.04 kg/m2 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**)**. Regardless of weight, serum 25(OH)D concentration was well below recommended levels, 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± 18.3ng/mL), *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*= 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).

The distributions of circulating 25(OH)D concentrations in Saudi Arabian women as shown in (Figure 1) indicated that approximately 89% of women were 25(OH)D(insufficient and deficient) (≤ 20 ng/mL), and 11% were (optimum and sufficient) (≥ 20 ng/mL) with a mean concentration of circulating 25(OH)D of (15.4 ± 12.2 ng/mL). Figure (2) showed 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/m2) had the lowest 25(OH)D ascompared to overweight and normal weight women, these differences were not statistically significant (*p*=0.78).Table (3) showed crude and adjusted odds ratios across BMI categories. The normal BMI category is the referent. The crude odds ratios (CI 95%) are 0.67 (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 0.69 (0.19, 2.5) and 3.99 (0.90, 17.6) for the overweight and obese categories, respectively.

**Table (1) Demographic, anthropometric and lifestyle characteristics of Saudi Arabia women (N=120).**

|  |  |  |
| --- | --- | --- |
| **Characteristic** | Mean | ± SD |
| **Age** (year) | 47.9 | 13.6 |
| **BMI** (kg/m2)  **BMI at age18** (kg/m2)  Difference in BMI (kg/m2) | 29.6  22.8  6.7 | 6.04  4.6  6.5 |
| **Weight** (kg)  **Height** (cm) | 75.0  159.1 | 14.8  4.8 |
| **Serum 25(OH)D** (ng/mL) | 15.4 | 12.31 |
| **Lifestyle** | N | % |
| **Smoking**  Yes  No  **Education**  High School or Less  Post High School  Some College  College Graduate  **Parity**  Nuliparous  Have children  **Monthly Income**  <5000 SR  5000 SR and more | 62  58  35  26  39  11  17  103  59  61 | 51.7 %  48.3 %  31.5 %  23.4 %  35.1%  9.9 %  14.2 %  85.8 %  49.2 %  50.8 % |

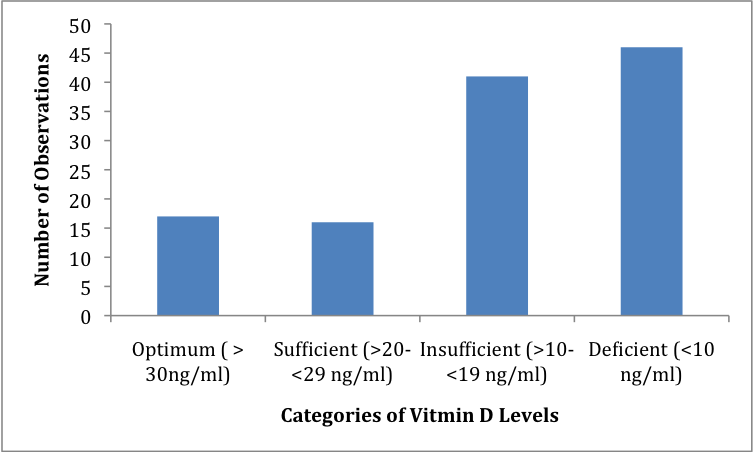
1 NS differences when comparing women reporting weight gain vs. stable weight since age 18 years.

**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.**

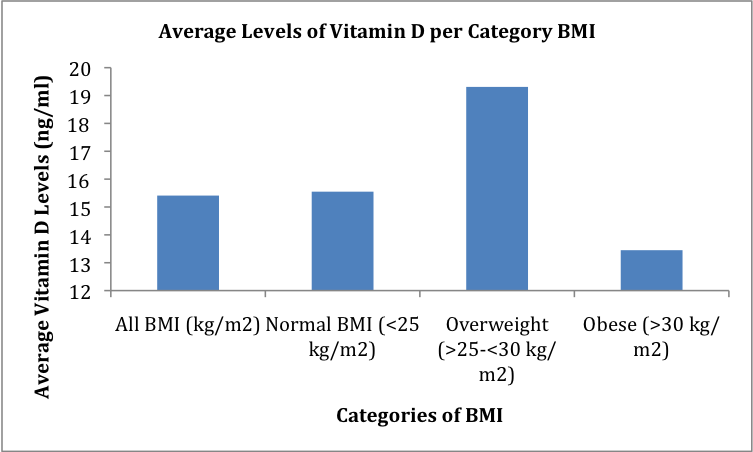
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| --- | --- | --- | --- | --- |
| **Current BMI** | **N** | **Serum 25(OH)D**  (ng/ml) (Mean ± SD) | **Weight change**  (Since age 18 years) | **Serum 25(OH)D**  (ng/ml) (Mean ± SD) |
| **Normal weight**  (18.5-24.9) | 33 | 15.7±10.1 | <10% | 19.8±15.6 |
| **Overweight**  (25-29.9) | 29 | 18.3 ± 18.3 | 10-20% | 12.6±11.6 |
| **Obese1**  (≥30) | 58 | 13.5 ± 9.1 | >20% | 14.5±10.5 |
| ***P-trend*** |  | 0.78 |  | 0.13 |
| **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.



**Figure (1): Distribution of circulating serum 25(OH)D(ng/mL) concentrationsin Saudi Arabian women (n=120)**



**Figure (2): Serum 25(OH)D concentration based on BMI categories (kg/m2).**

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**Figure (3): The average serum 25(OH)D concentrations (ng/mL) in normal, overweight, and obese Saudi Arabian women (n=120).**

**Table (3): Crude1 and adjusted2 odds ratios (95% Confidence Intervals) for deficiency serum 25(OH)D concentration (< 10 ng/ml) by BMI categories.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Category of BMI** (kg/m2) | **Obese**  (>30) | **Overweight**  (25-29.9) | **Normal**  (Referent) (18.5-24.9) | ***p*-trend** | | **Total** (N=120) | 58 | 29 | 33 |  | | Crude Odds Ratios1 (95% CI) | 3.05 (0.79, 11.7) | 0.67 (0.19, 2.28) | 1.00 | 0.087 | | Adjusted Odds Ratios2 (95% CI) | 3.99 (0.90, 17.6) | 0.69 (0.19, 2.49) | 1.00 | 0.085 | |

1Logistic regression models with no adjustments.

2Logistic regression models adjusted for age, physical activity, parity, education, smoking, and income.

**4. Discussion.**

Obesity has been a public health concern in many countries, and is a risk factor for vitamin D deficiency. Previous studies have reported an inverse relationship between circulating concentrations of 25(OH)D, BMI and body fat mass **(Parikh *et al*., 2004)**. 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 **(Holick, 2002)**. 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*vs.* 15.2±11.4 ng/mL, respectively), thus in both groups they were vitamin D deficient.

The absence of association between BMI and vitamin D status could be attributed to high rates of vitamin D deficiency in our sample, but also could be due to genetic differences **(Heaney, 2004),** or other

factors that affect cutaneous production of vitamin D **(Sahota *et al*., 2004).** 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 the present 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 **(Solomons, 2008 and Elsammak, 2010)**. Most importantly the majority of women in the study were obese, which may affect the bioavailability of vitamin D, although this remains controversial **(Heaney *et al*., 2011).**

A recent study by **Mason *et al*. (2011)** in postmenopausal women (n=439) found that greater adiposity is associated with lower serum of 25(OH)D, on the other hand, weight loss could increase circulating 25(OH)D concentrations due to a decrease in peripheral sequestration. The release of vitamin D from body stores may incur vitamin D intoxication and hypercalcemia from deposition in body fat **(Heaney *et al*., 2008)**. 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 **(Heaney *et al*., 2009)**. Obese and overweight individuals have a higher prevalence of vitamin D deficiency than normal weight individuals 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, vitD 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 **(Blum *et al*., 2008 and Reid, 2008).** Vitamin D can be stored in adipose tissue for approximately two months **(Jones, 2008)**. The bioavailability of cutaneous vitamin D is decreased in obese people by more than 50 % (13). Vitamin D supplementation with 50,000 IU dose of ergocalciferol (D2) could increase circulating 25(OH)D in individuals of normal weight compared to obese individuals as shown by **Worstman *et al*. (2000)**. 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 **(Holick, 2007)**. In addition, 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 **(Lagunova *et al*., 2011)**.

Approximately one billion people worldwide may have vitamin D deficiency **(Holick, 2007)**. 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 **(Ross *et al*., 2011)** but others have indicated that the cutoff should be higher. A 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 baselinefrom 15.4 to 18.3 ng/mL (*p*< 0.05) (**Papadopoulou *et al*., 2010).** 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) **(Holecki *et al*., 2007)**. These studies support the idea that vitamin D has been linked with 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* =0.004). Additionally, among both groups vitamin D was higher in males compared to females **(Al-Sultan *et al*., 2011)**. 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.

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 **(Shaw and Pal, 2002)**. In western Saudi Arabia vitamin D deficiency is widely spread in both genders especially females **(Sedrani *et al*., 1992)**. In a study among Saudi students attending universities (n=17) the mean level of vitamin D was below 27 ng/mL **(Kumosani, 1996)**. In young Saudi women more than 52% demonstrated severe hypovitaminosis D, with 25(OH)D levels below 8 ng/mL **(Ghannam *et al*., 1999 )**. Further, the dietary intake of vitamin D in Saudi women is below the recommended level of (15μg daily) **(Ross *et al*., 2011).** Jeddah is a sunny place with relatively high temperature, so being outdoors is very rare **(Fida, 2003)**. As mentioned, 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%. Additionally, clothing practices in the Arabian Gulf, including Saudi Arabia, contribute to vitamin D deficiency.

This studyreported that, Saudi Arabian women are at great risk for vitamin D deficiency, but body size is not as strong associated factors. 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 and weight assessment. Additionally, evaluation vitamin D supplementation effect on status in normal and overweight/obese Saudi women, and determine the relationship between circulating vitamin D and weight loss in adults.

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