2009

A determination of vitamin D status and intake of pregnant and non-pregnant Saudi Arabian women in Mecca, Saudi Arabia

Wedad Azhar

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A Determination of Vitamin D Status and Intake of
Pregnant and Non-Pregnant Saudi Arabian Women in Mecca, Saudi Arabia

by

Wedad Azhar

Thesis
Submitted to the Department of Dietetics and Human Nutrition
School of Health and Sciences
Eastern Michigan University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

in

Human Nutrition

Thesis Committee:
George Liepa, PhD, Chair
Yousif Khodari, PhD

September 2009

Ypsilanti, Michigan
DEDICATION

This thesis is dedicated to all women in my country, Saudi Arabia.
ACKNOWLEDGMENTS

First, I would like to express my deepest gratitude to my chair member and advisor Professor. George Liepa. He was the most helpful and supportive person throughout my program and the course of this thesis. He was very understanding of my concerns and was generous with his time and advice. It has been a great pleasure to work under his guidance.

I would like to thank my committee member, Dr. Yousif Khodari, for his help and support at the Al-Noor Specialist Hospital laboratory and for his mentorship.

A special thanks to Dr. John Knapp for his statistical guidance on this thesis. He was very generous with his time.

Sincere thanks to Osama AbuFarraj for being a great supportive husband and friend. I would like to thank him for his patient help and encouragement throughout this thesis.

I would like to thank my wonderful family for all that they did for me from the beginning of my life until now. My thanks go to my father, Mr. Fouad Azhar, and my mother, Mrs. Inaam Sabbag, for their support and encouragement to do my best. I would like to thank my brothers and sisters for their constant support.

I would also like to acknowledge my daughter Dialla and my son Nomai for their patience throughout my graduate studies.

I would like to thank the people from the ministry of health. Dr. Khaled Zafar, Dr. Zamel Attar, Mr. Salman Alshareef, and Mr. Fouad Azhar for their help and support.
Special thank you to Mrs. Nada Althyab for her collaboration with me in this thesis and for being supportive.

I would like to acknowledge Mrs. Lydia Kret and Mrs. Nancy Parker for helping me working with the NutriBase software.

I also would like to thank the people who helped me at the Maternity and Children's Hospital in Mecca: Dr Abdulhakeem Banjar, Dr Husni Rayes, Dr. Abeer Matar, Mrs. Azizah Aljahdaly, and Mrs. Esraa Dahwah.

A special thank for Dr. Yousif Khodari, Mrs. Hawaa Barnawi, and Jawaher Sabbag for their help at the Al-Noor Specialist Hospital.

Finally, special thanks to those women who participated in this study. I would like to thank them for their cooperation in answering all the questions and in providing blood samples for analysis.
The objective of the present study was to determine serum 25(OH)D and PTH concentrations as well as vitamin D and Ca dietary intake in healthy pregnant (P) (n=48) and non-pregnant (NP) (n=70) Saudi Arabian women who reside in Mecca- Saudi Arabia. Age, occupation, skin color, smoking status, use of sunscreen, use of supplements, and sun exposure were correlated to vitamin D status. Dietary and supplement intake was assessed. Among the participants, 50.8% (35% P, 65% NP) women had 25(OH)D concentration that were ≤ 11 ng/mL. PTH concentrations were normal in 60.2% and high in 37% of the participants. Dietary vitamin D intake was normal, whereas dietary Ca intake was extremely low. Positive correlations were shown to exist between serum 25(OH)D concentrations and the use of supplements, avoidance of smoking, and the length of exposure to sunlight. Vitamin D deficiency appears to be prevalent among P and NP Saudi women.
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Chapter 1: Introduction

Vitamin D deficiency or insufficiency is normally not an expected to be a problem in a country like Saudi Arabia because of the abundance of sunlight throughout the year. However, studies have shown a high prevalence of vitamin D deficiency in Saudis of both genders and all age groups (1-7). Data regarding vitamin D status of pregnant (P) and non-pregnant (NP) women who reside in Mecca (Makkah) – Saudi Arabia are absent. It is critical that this type of information be obtained since vitamin D deficiency is a worldwide epidemic among both children and adults (8).

Vitamin D is an essential fat-soluble vitamin that is required for the maintenance of good health. It is obtained either through synthesis in the skin with exposure to ultraviolet B (UVB) radiation or through dietary sources. Season, latitude, skin pigmentation, aging, sunscreen use, obesity, and smoking all influence the production of vitamin D (9, 10). There are two main types of vitamin D [$D_2$ (1,25-dihydoroxyvitamin D) and $D_3$ (25-hydroxyvitamin D)]. Vitamin D status is measured by determining serum 25-hydroxyvitamin D [25(OH)D] concentrations. Vitamin D deficiency not only causes rickets among children, but it also causes bone diseases among adults. Furthermore, it is associated with decreased immune function and an increased risk of cardiovascular disease, type 1 and type 2 diabetes, polycystic ovary syndrome (PCOS), and many types of cancer such as breast, colon, and prostate cancers (11,12).

The objective of the present study is to determine the serum vitamin D and parathyroid hormone (PTH) status of Mecca women who were either P or NP and to correlate these results with exposure to sunlight, smoking habits, use of supplements, and dietary intake.
Chapter 2: Review of Literature

In recent years, vitamin D deficiency or insufficiency has been shown to be a common international problem (8, 11). Humans and other mammals can produce vitamin D when exposed to ultraviolet blue (UVB) light, which is provided by sunlight. Three additional sources of vitamin D include (a) foods that contain vitamin D, (b) foods that are fortified with vitamin D, and (c) vitamin D supplements. Saudi Arabia has ample sunlight for adequate vitamin D production, and yet there is a high prevalence of vitamin D deficiency across all age groups (13).

Vitamin D Metabolism

Vitamin D is an essential fat-soluble vitamin that acts as a pro-hormone in the body. There are two main types of vitamin D (D₂ and D₃). The skin of humans can convert 7-dehydrocholesterol to pre-vitamin D₃ when exposed to UVB radiation; this compound can then be converted into vitamin D₃ (9). Other sources of vitamin D include dietary intake of foods that contain or are fortified with vitamin D. Vitamin D₂ is made from ergosterol by yeast when it is exposed to ultraviolet irradiation. Vitamin D₂ is also manufactured by the food industry from lanolin, via exposure to ultraviolet irradiation (9, 14). Both vitamin D₂ and D₃ are used as supplements.

Both types of vitamin D, when consumed, are metabolized in the liver and are converted to Calcidiol [(25-hydroxyvitamin D) (25(OH)D)], which is the storage and circulating form of vitamin D. Calcidiol, which is also known as 25-hydroxycholecalciferol, is then transported to the kidneys and converted to Calcitriol (1,25-dihydroxyvitamin D) by 25-hydroxyvitamin D-1α–hydroxylase (CYP27B1) (9, 14). Calcitriol [1,25-(OH)₂ D] is the most active form of vitamin D in the body. It plays a primary role, along with PTH, in the regulation of serum calcium (Ca) (11, 14). Long-term vitamin D deficiency can lead to
increased PTH concentrations and decreased serum 1,25(OH)\(_2\) D concentrations which can, in turn, lead to the development of osteomalacia and other diseases (9).

**Measurement of Vitamin D**

Vitamin D status is best established by measuring serum 25(OH)D concentrations since this value is reflective of both oral intake and subcutaneous vitamin D production. A consensus has not been reached at the present time as to what should be considered as "insufficient" or "adequate" vitamin D intake.

The current recommendation regarding vitamin D intake [Dietary Reference Intakes (DRI)] for vitamin D in the United Status is 400 IU (International Unit) / day; however, it has been suggested that this recommendation does not result in adequate vitamin D concentrations in the body (15). Serum 25-hydroxyvitamin D [25(OH) D] concentrations that are between 25 and 37.5 nmol/L are considered to be inadequate, whereas concentrations that are \(\geq 75\) nmol/L are within the normal range, and serum concentrations that are \(\geq 500\) nmol/L are considered to be toxic (Table1).
<table>
<thead>
<tr>
<th>ng/mL**</th>
<th>nmol/L**</th>
<th>Health status</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;11</td>
<td>&lt;27.5</td>
<td>Associated with vitamin D deficiency and rickets in infants and young children.</td>
</tr>
<tr>
<td>&lt;10-15</td>
<td>&lt;25-37.5</td>
<td>Generally considered inadequate for bone and overall health in healthy individuals.</td>
</tr>
<tr>
<td>≥30</td>
<td>≥75</td>
<td>Proposed by some as desirable for overall health and disease prevention, although a recent government-sponsored expert panel concluded that insufficient data are available to support these higher concentrations.</td>
</tr>
<tr>
<td>Consistently &gt;200</td>
<td>Consistently &gt;500</td>
<td>Considered potentially toxic, leading to hypercalcemia and hyperphosphatemia, although human data are limited. In an animal model, concentrations ≤400 ng/mL (≤1,000 nmol/L) demonstrated no toxicity.</td>
</tr>
</tbody>
</table>

* Serum concentrations of 25(OH)D are reported in both nanograms per milliliter (ng/mL) and nanomoles per liter (nmol/L).

** 1 ng/mL = 2.5 nmol/L. (16)

New studies (12) suggest that the optimal serum concentrations of 25(OH)D should be between 90 and 100 nmol/L. These concentrations cannot be reached by consumption of the current DRI recommendations. Studies (11, 12) seem to suggest that when Americans consume approximately 4000 IU/day of vitamin D the risk of breast cancer is reduced by approximately 50%. Holick (17) recommends the following vitamin D intake pattern: (a) a
supplement intake of 1000 IU/day of vitamin D, (b) an additional 400 IU of vitamin D/day from multivitamins, and (c) 300 IU/day from fortified milk. He also suggests that additional vitamin D should be obtained from daily sun exposure with minimal use of sunscreen (17).

Recent studies suggested that the DRI recommendation of vitamin D should be changed (11, 12, 17).

**Vitamin D Status of Pregnant / Non-Pregnant Saudi Women**

A number of studies (1-7) have shown that Saudi Arabian women of all age groups are deficient in vitamin D. In a study by Fonseca et al. (1), the median serum concentration of 25(OH)D in 31 adult Saudi Arabian women was shown to be 6 ng/mL. Vitamin D concentrations were significantly lower in those subjects who lived in apartments and whose average exposure to sunlight was less than 30 minutes/day. Elidrissy et al. (2) determined serum 25(OH)D concentrations of 36 mothers of rachitic infants who resided in the central region of Saudi Arabia. Serum 25(OH)D concentrations were shown to be extremely low (5.2 ng/mL) in all participants. In a study by Serenius et al. (3), vitamin D nutritional status was analyzed in 119 pregnant women who resided in the central region of Saudi Arabia. Serum 25(OH)D concentrations were reported to be very low (5.7 ng/mL); however, women of higher socioeconomic backgrounds were more likely to take vitamin D supplements and had higher serum concentrations. In a recent study by Al Faraj et al. (4), vitamin D status of Saudi women who experienced low back pain and who lived in a central province of Saudi Arabia was determined. Before and after treatment with vitamin D supplements, serum calcium, phosphate, and 25-(OH)D concentrations, as well as alkaline phosphatase activity were measured. Results showed that 83% of the patients had abnormally low concentrations of vitamin D before treatment. When vitamin D supplements were provided, clinical improvements in symptoms were noted. In a research study that was conducted in a western
province of Saudi Arabia by Khoja et al. (5), the influence of diet on bone health in healthy pre-postmenopausal Saudi women between the ages of 20-60 years was analyzed. Measurements of weight, height, spine, hip, and calcaneal bone mass were also determined. All subjects were interviewed in order to determine their dietary intake, physical activity levels, and general lifestyle. Seventy-four percent of the women had serum 25-(OH)D concentrations that were deficient (≤12 ng/mL), and 26% had 25(OH)D concentrations that were adequate (≥12 ng/mL). In addition, significant negative correlations were shown to exist between 25(OH) D and PTH (r = -0.23; P < 0.02). In a study by Siddigui et al. (6), adolescent school girls aged 12-15 years who resided in a western province of Saudi Arabia were shown to have a high prevalence of vitamin D deficiency and rickets. Thirty-two percent of the girls had very low concentrations of vitamin D, 49% had inadequate concentrations, and 19% had adequate concentrations. Eighty-nine percent of the participants had normal serum calcium concentrations. A positive correlation was shown to occur between low calcium in the diet and decreased exposure to sunlight. In a study by Al Turki et al. (7), vitamin D status of healthy Saudi women who lived in the eastern part of the country was analyzed. Women who were 25-35 years old were placed in group 1, and women who were 36-50 years old were placed in group 2. A clinical examination as well as laboratory tests (serum calcium, phosphorous, parathyroid hormone, 25(OH)D, and alkaline phosphatase activity) were performed. Furthermore, data regarding lifestyle, dietary intake, and demographics were collected. Serum 25(OH)D was defined as being reflective of a deficiency when values were ≤ 20 ng/mL, insufficient when they were between 23-29 ng/mL, and normal when they were ≥ 75 ng/mL. It was concluded that 70% of the group 1 subjects had normal 25(OH)D concentrations and 30% were deficient. In group 2, 45% had normal 25(OH)D concentrations and 55% were deficient. Exposure to sunlight and consumption of dairy products were below the required amount.
Factors Influencing Vitamin D Status

A wide variety of factors affect vitamin D status. Factors that are associated with vitamin D status in Saudi Arabia are often related to Saudi customs. Generally, vitamin D status is affected by things that have an impact on the dermal synthesis rate of vitamin D.

The dermal production of vitamin D₃ is influenced by many factors. In many parts of the world, UVB radiation does not penetrate the atmosphere during winter months, and this prevents the production of vitamin D₃ by the skin. In addition, dermal vitamin D production also depends on UVB intensity, which in turn depends on latitude, season of the year, and time of day. In latitudes above 35° [i.e. New York City (40°), Paris (48°), and Moscow (55°)], winter sunlight lacks adequate amounts of UVB radiation, making the skin unable to produce vitamin D₃ (10). Air pollution, which is usually associated with urban settings, also reduces the amount of UVB reaching the earth's surface (18). Covering the skin with clothing due to lifestyle/ cultural practices can minimize or completely block vitamin D production. Moreover, some studies have condemned the daily use of sunscreen since, while this product protects against sun burn, it also reduces or blocks the synthesis of vitamin D (9, 19). However, other studies have shown that sunscreen use does not cause vitamin D deficiency or insufficiency (18, 20). The elderly are particularly at risk of vitamin D deficiency or insufficiency because they often have decreased mobility or are institutionalized and, consequently, have decreased concentrations of skin 7-dehydrocholesterol (7-DHC), which is the precursor for cutaneously synthesized vitamin D. In general, conditions that decrease exposure to sunlight also lead to decreased renal production of 1,25(OH)₂ D(18, 21).

Other factors which lead to decreased vitamin D syntheses include age, skin pigmentation, obesity, and smoking. The skin's ability to synthesize vitamin D decreases by 50 ng/mL between the ages of 20 and 70 years (21). The degree of skin pigmentation has
also been correlated with an increased risk of vitamin D deficiency because melanin in the skin competes with 7-DHC to absorb UVB radiation. Pigmented and non-pigmented skins both have the same capacity to make vitamin D₃; however, pigmented skin requires longer exposure times (22). Obese individuals have been shown to have lower concentrations of circulating 25(OH) vitamin D since vitamin D is stored deep in adipose tissue and is therefore not as available for secretion into the blood (17, 23). Studies have shown a negative association between low serum concentrations of 25(OH)D and smoking (24-26). This association may be caused by a direct effect of tobacco smoke on vitamin D metabolism or on the effect tobacco smoke has on genetic factors such as polymorphism within the vitamin D receptor VDR gene (24, 25, 27).

Factors Influencing Vitamin D Status in Saudi Arabia

In addition to all the previously mentioned factors, people in Saudi Arabia have unique conditions that influence vitamin D status. Saudi Arabia is sun-drenched throughout the year, and temperatures often rise above 50 °C (122 °F) in the summer months. Therefore, Saudis usually limit the time they spend outdoors during day time. Moreover, women block sunlight by the use of dark veils that cover their bodies for cultural and religious reasons. Some women do not reveal any parts of their body, and others reveal their faces or their hands.

Dietary Intake

Most foods do not contain enough vitamin D to maintain a healthy body. Fatty fish such as salmon, tuna, sardines, kanad, shaour, and shrimp provide 200 – 350 IU of vitamin D/100 g. Kanad, shaour, and sardines are consumed mostly in the eastern province of Saudi Arabia, whereas they are not popular in the western province cities such as Mecca. Eggs contain approximately 20 IU of vitamin D/egg. Typical vitamin D fortified foods include
milk, buttermilk, and yogurt. Each of these dairy products is found in Saudi Arabia and provide 400 IU of vitamin D/cup. Cereal products provide normally 40-50 IU of vitamin D/cup.

**Supplement Intake**

Two forms of vitamin D supplements that are commonly consumed are ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). Studies have shown that vitamin D₂ is as effective as vitamin D₃ in maintaining 25-hydroxyvitamin D status (9, 28, 29). Usually vitamin D supplements are sold in combination with Calcium (Ca). These supplements are best absorbed when consumed prior to a meal that contains some type of dietary fats.

**Calcium, Phosphorus, Parathyroid Hormone and Bone Metabolism**

One of the main functions of vitamin D in the human body is to work in tandem with PTH in order to maintain normal Ca and Phosphorus (P) concentrations in the blood. Only 10-15% of dietary Ca and 60% of P is normally absorbed when an insufficient amount of vitamin D is present in the body (9). Low concentrations of Ca and P in the blood stimulate the parathyroid gland to secrete PTH. This condition is known as hyperparathyroidism. Increased PTH concentrations in the blood stimulate the kidney to produce 1, 25(OH)₂ D₃, which causes changes in gene expression. These changes lead to normalization of Ca concentrations in the blood by increasing intestinal Ca absorption, renal re-absorption, and mobilization of Ca from bone (9, 16). Increases in serum PTH also cause a decrease in the serum concentration of P. If serum P is not available in sufficient quantities, bone cannot be mineralized and osteomalacia will develop (16).
Common Diseases Associated with Vitamin D Deficiency

Vitamin D status has been associated with diseases such as rickets, osteomalacia, and osteoporosis (10, 30). The discovery of VDR, in more than 30 body tissues, has provided evidence that vitamin D may play a role in the prevention and development of a number of other diseases (11, 14). Recently epidemiological investigations have shown that vitamin D also plays a role in the development of a variety of immune system disorders, cardiovascular disease, type 1 and type 2 diabetes, PCOS, and many types of cancer (i.e. breast, colon, and prostate) (10-12).

Immunity Related Disorder

The effects of vitamin D status on immune function depend on the nature of the immune challenge and the calcium status of the host (9). The identification of VDRs in peripheral blood mononuclear cells sparked an early interest in vitamin D's role as an immune system regulator (31). Since 1,25(OH)2D3 suppresses the development of various autoimmune diseases and prolongs allograft survival, it has been suggested that vitamin D functions as an immunosuppressive hormone (31).

Cardiovascular Disease

Clinical studies have shown cross-sectional associations between low serum vitamin D concentrations and plasma rennin activity, blood pressure, coronary artery calcification, and cardiovascular disease (29, 32). Vitamin D receptors have a broad cardiovascular tissue distribution that includes vascular smooth muscle cells, endothelial cells, and cardiomyocytes (32). Additionally, ecological studies have reported higher rates of coronary heart disease and hypertension with increasing distance from the equator, which is a
phenomenon that has been attributed to the higher prevalence of vitamin D deficiency in regions with less exposure to sunlight (29).

**Type 1 and 2 Diabetes**

Vitamin D deficiency is involved in the pathogenesis of both type 1 and type 2 diabetes. Inadequate vitamin D concentrations have been shown to be associated with impaired insulin synthesis and secretion in humans and in animal models (27). Furthermore, epidemiological studies suggest that a link exists between vitamin D deficiency in early life and the later onset of type 1 diabetes (14, 17). In some populations, type 1 diabetes has been associated with certain polymorphisms within the vitamin D receptor gene (9, 33). Studies have also shown that children who have a vitamin D intake of at least 2,000 IU/day have a corresponding 80% reduction in the incidence of type 1 diabetes (27, 33).

**Polycystic Ovary Syndrome (PCOS)**

Vitamin D deficiency appears to occur frequently in women who have been diagnosed with PCOS and may be a contributing factor to some of the biochemical abnormalities that are associated with this condition. Studies have shown that when women are provided with adequate amounts of vitamin D and Ca they are more likely to have normal menstrual cycles (17, 34). Moreover, Insulin resistance and central obesity are prominent features of PCOS, and increased body fat has been associated with alterations in vitamin D and PTH metabolism. Despite the fact that obese individuals have a larger total surface area and, therefore, greater exposure to sunlight, it has been shown that serum 25(OH)D concentrations are decreased in obese subjects (35). It has been suggested that this relationship occurs because subcutaneous fat, which has been shown to store vitamin D, sequesters larger amounts in obese persons (29, 35)
Cancer

Vitamin D is one of the most potent cell growth regulating hormones. Calcitriol has been shown to inhibit proliferation and induce differentiation of normally functioning cells (36). Studies have shown that 1,25(OH)₂D regulates cell growth and prevents cancer progression by (a) reducing angiogenesis, (b) increasing cell differentiation and apoptosis of cancer cells, and (c) reducing cell proliferation and metastases (37). The antiproliferative and prodifferentiative activity of VDR ligands was noted almost 3 decades ago (38). Several recent studies have shown that breast, colon, and prostate cancer cells as well as osteosarcomas and melanomas are responsive to the antiproliferative effects of 1,25(OH)₂D (9, 38). Epidemiological studies have also shown that higher 25(OH)D concentrations are associated with reduced cancer incidence and decreased cancer-related mortality (37-39).
Chapter 3: Methodology

The present study was designed to determine the serum concentrations of 25(OH) Vitamin D and PTH as well as the vitamin D and Ca intake of healthy P and NP Saudi Arabian women in Mecca (Saudi Arabia) and correlate these values to other factors that are related to vitamin D metabolism.

Subjects

One hundred and eighteen Saudi women between the ages of 20 and 60 years were selected to participate in the present study. Both P (n=48) and NP (n=70) women who were patients at the Obstetrician/ Gynecologist Clinic at the Maternity and Children's Hospital in Mecca agreed to take part in this study. Files of all of the selected participants were checked to confirm that they had not been diagnosed with hepatic or renal disease, metabolic bone disease, malabsorption, sterility, oligomenorrhea, type II diabetics, hypercortisolism or malignancy. In addition, women who were immobile for more than one week or who were being treated for any bone disorders were excluded from the study. Prior to taking part in the study, all participants had an informed consent statement [Appendix A- English, Appendix B- Arabic] read to them if they were illiterate or were allowed to read it on their own. The College of Health and Human Services Human Subject Review Committee at Eastern Michigan University approved the present study (Appendix C).

Research Design

The present research study was conducted between the months of July and August (2008). Participants in the study came to the Maternity and Children's Hospital in Mecca for a follow-up examination or a physical examination. All participants provided a fasting blood sample that was stored at -30 C° and analyzed for 25(OH)D and PTH at the Al-Noor
Specialist Hospital laboratory (Mecca). Participants also completed a survey in which they answered questions about their social and economic status, education level, daily exposure to sunlight, smoking status, and consumption of supplements. They also completed a 24-hour dietary recall form.

**Biochemical Analysis**

All participants provided a fasting (8-10 hours) blood sample. Serum was collected and samples were then taken daily in a coolpack to the laboratory at the Al-Noor Specialist Hospital and stored at -30°C until all could be analyzed. Samples were analyzed for 25(OH)D and PTH (Appendix D) using an Elecsys 2010 Rack analysis system.

**Survey Instrument**

The primary investigator met all of the participants and informed them about the importance of vitamin D in regard to their health and why their participation in the current study was helpful. The investigator read questions from a pre-prepared questionnaire (Appendix E) to each participant and answered all questions about the study. Demographic information collected included each subject's age, height, weight, pregnancy status, number of previous pregnancies, education level, occupation, economic status, skin color, and smoking status.

**Sunlight Exposure Calculation**

Amount of exposure to sunlight was recorded for each participant based on the latitude of residence (Latitude 21.4500 degrees North and Longitude 39.8167 degrees East) (40), length of exposure to sunlight, time of the day exposed to sunlight, parts of the body exposed to the sunlight, and the use of sunscreen.
24-Hour Dietary Recall

Each participant was asked about her dietary intake during the past 24 hours. Specific information was gathered in order to determine the types of food eaten, method of preparation, and the amount of food consumed [(Appendix F- English, Appendix G- Arabic].

Food Analysis Software

Food analysis software (NutriBase 7 Clinical Edition) was used to analyze the 24-Hour Dietary Recall data for each participant. Some information related to the content of Saudi food was added to the software data pool based on the information found on local food labels. The amount of vitamin D and Ca consumed was determined for each subject.

Statistical Analysis

Statistical analysis was performed using SPSS 6.5 software. Participants were stratified for statistical analysis on the basis of age, BMI, pregnancy status, number of previous pregnancies, education level, occupation, economic status, skin color, smoking status, time exposed to sunlight, length of exposure to sunlight, body parts exposed to sunlight, supplement intake of vitamin D and Ca, and use of sunscreen. An analysis of data was performed to determine the means, standard deviation, correlations between the characteristics, significance (2-tailed), T-test, and one-way ANOVA analysis. Differences between groups were determined to be significantly different at (P ≤ 0.05).
Chapter 4: Results

25-hydroxyvitamin D status

The majority of the participants in the present study had low concentrations of serum 25(OH)D. The serum concentration of 25(OH) D was defined in the present study as being deficient when it was ≤ 11 ng/ mL; inadequate was 11.1 - 15 ng/ mL, adequate was 15.1 - 20 ng/ mL, and normal was ≥ 20.1 ng/ mL. Fifty percent of the participants [35% of the P and 65% of the NP women] were deficient; 28.9% had inadequate concentrations (32.3% of the P and 67.6% of the NP women); 17.8% had adequate concentrations (66.6% of the P and 33.3% of the NP women); and only 2.5% of the population studied had normal concentrations of 25(OH) D (66.6% of the P and 33.3% of the NP women) (Figure 1). The mean concentration of serum 25(OH)D for P women was 13 ng/ mL, and for NP women it was 10.6 ng/ mL. Non-pregnant women had significantly lower vitamin D concentrations than P women (P ≤ 0.05).
Figure 1: Mean Serum 25(OH)D Concentration of Pregnant and Non-Pregnant Women as Compared to the Recommended Concentration

Parathyroid Hormone

The majority of the women in the study had normal concentrations of PTH. A normal concentration of PTH was defined as being 15-65 pg/mL. Any values below that range were considered to be low, and values above that range were considered to be high. Two-and-a-half percent of the subjects had low concentrations of PTH, 60.2% had normal concentrations, and 37% had high concentrations. The mean PTH concentration for P women was 66.4 pg/mL, and for NP women it was 67.5 pg/mL (Figure 2). A significant negative correlation ($r = -0.254$; $P < 0.005$) was shown to occur between the PTH concentrations and serum 25(OH)D concentrations.

*Note: Bars with different superscripts were significantly different at ($P \leq 0.05$)
Vitamin D Intake

The dietary vitamin D intake was ≤ 400 IU daily (the DRI recommendation) for 74.6% of the subjects. Vitamin D intake for P women was 422.4 IU/day, whereas, it was 462.4 IU/day for NP women (Figure 3). Key dietary products that contained vitamin D and were consumed in Saudi Arabia were dairy products (milk, buttermilk, and yogurt). Each of these dairy products contained approximately 400 IU of vitamin D/cup. Fish intake, especially fatty fish, was very low among Saudi Arabian women. Types of fish that were mostly consumed in Mecca were Najel, hareed, tuna, and shrimp. Consumption of sardines and salmon was rarely found to occur. A positive correlation (r = 0.269; P < 0.003) was shown to exist between dietary vitamin D Intake and serum concentrations of 25(OH)D.
Dietary Ca intake of Saudi Arabian woman was very low. Ninety-seven and a half percent of the participants did not reach the DRI recommendations for Ca intake, which were established to be 1000 mg/day for NP women and 1200 mg/day for P women. The mean intake of dietary Ca for P women was 226.9 mg/day, and for NP women it was 261.2 mg/day (Figure 4). A significant correlation was shown to exist between Ca intake and vitamin D intake ($r = 0.605; P < 0.000$) and also between Ca Intake and serum 25(OH)D concentrations ($r = 0.192; P < 0.038$).
Supplemental Vitamin D Intake

Consumption of dietary supplements is uncommon among Saudi Arabian women; however, during pregnancy the majority of women generally do consume needed amounts of supplements. Approximately 70% of the subjects in the present study did not use any supplements (31.3% of the P women and 68.7% of the NP women). Only 16.1% of the subjects used vitamin D supplements during the sampling period; 53% of those were P women. In general most vitamin D supplements were consumed along with Ca (Figure 5). Participants who consumed supplements had significantly higher serum 25-(OH) D concentrations than those who did not (P = 0.05).
Figure 5: Consumption of Vitamin D and Calcium Supplements by Saudi Arabian Women

Sun Exposure and Vitamin D Status

Results of the current study showed a lack of exposure to sunlight among Saudi Arabian woman. This is partially due to the fact that most of the time that the participants spent outdoors was time that they were covered (completely, or with only hands or face showing) (Figure 6). Participants who had a swimming pool or a backyard behind their houses were exposed to the sun more often and had significantly higher serum 25(OH)D concentrations ($P \leq 0.05$) (Figure 7). A positive correlation was shown to exist between the length of exposure to sunlight and serum of 25(OH)D concentrations ($r = 0.293; P < 0.001$) (Figure 8).
Figure 6: Amount of Sunlight Exposure Amongst Saudi Arabian Women

*Note: Bars with different superscripts were significantly different at (P ≤ 0.05)

Figure 7: Relationship Between Body Parts Exposed to Sunlight and Serum 25(OH)D Concentrations of Saudi Arabian Women

*Note: Bars with different superscripts were significantly different at (P ≤ 0.05)
Figure 8: Relationship Between Length of Exposure to Sunlight and Serum 25(OH)D Concentrations of Saudi Arabian Women

Extraneous Factors That Influence Vitamin D Status

Only nine participants who were in the study used sunscreen. Two of the subjects used it daily and had serum 25(OH)D concentrations that were less than participants who did not use sunscreen (7.9 ng/mL vs. 11.8 ng/mL). Significant correlations were not shown between skin color and vitamin D status. All participants were living in the same region where year-round skin production of vitamin D is possible. Participants who were students or teachers generally had access to backyards, which allowed sunlight exposure and, consequently, they had higher concentrations of serum 25(OH)D (11.6 ng/mL), whereas participants who worked in hospitals did not have access and had lower concentrations (8.2 ng/mL). Participants who smoked and second-hand smokers had lower vitamin D concentrations (8.6 ng/mL) than participants who did not smoke (12.4 ng/mL) (Figure 9).
Figure 9: Relationship Between Smoking and Serum 25(OH)D Concentrations of Saudi Arabian Women

*Note: Bars with different superscripts were significantly different at (P ≤ 0.05)
Chapter 5: Discussion

Numerous studies have shown that people from various parts of the world including Saudi Arabia have low serum concentrations of vitamin D (1, 5, 8). Data reported in the present study indicate that Saudi Arabian women who live in the western region of their country have serum concentrations of 25(OH)D that are even lower than previously suggested in other regions of Saudi Arabia (1-7). Previously reported data from the eastern province of Saudi Arabia showed that 30% of the population between the ages of 25-35 years and 55% of the population between the ages 36-50 years had serum 25(OH)D concentrations that were ≤ 20 ng/mL (7). In the central province of Saudi Arabia, Al Faraj et al. (4) showed that 83% of the population had 25(OH)D concentrations that were ≤ 20 ng/mL. Vitamin D status studies of women in the western region of Saudi Arabia (Jeddah) showed that 74% of the population had serum 25(OH)D concentrations that were ≤ 12 ng/mL (5). In the present study it was shown that 97.5% of Mecca women had 25(OH)D concentrations ≤ 20 ng/mL. These unexpected results may be a result of the fact that the consumption of fish is more common in the eastern and western provinces of Saudi Arabia because they are coastal regions. Also the eastern region is located on the Arabian Gulf, which contains fatty fish like kanad, shaour, and sardines, whereas the Red Sea, where Jeddah city is located on its coast, contains sardines and shaour, which are fatty fish. Also, dairy products are more commonly consumed in the central region of Saudi Arabia than in the western region. Saudis who live in the central region drink significantly large amounts of buttermilk daily within the main food dishes. They also consume large quantities of milk and yogurt. In addition, women who live in the eastern and western costal regions expose more parts of their bodies to the sunlight than women in the central and western regions. Finally, women in the central and eastern provinces spend more time outdoors than women who reside in the western provinces. Fatty fish like kanad, shaour, and sardines are also more commonly eaten in the eastern region.
Results from the present study indicate that women from Mecca (Western province) have very low concentrations of serum 25(OH)D. Most researchers define vitamin D deficiency as a serum 25(OH)D concentration that is \( \leq 20 \text{ ng/ mL} \). Ninety-seven percent of the women in the present study were classified as vitamin D deficient. These low vitamin D concentrations have historically been associated with clinical disorders like osteoporosis, osteomalacia, and fractures in adults and children (14). Furthermore, recent studies have shown a connection between low concentrations of vitamin D and immune deficiency, cardiovascular disease, type 1 and type 2 diabetes, PCOS, and many types of cancer (breast, colon, and prostate cancers) (11, 12).

There are many factors that are responsible for the extremely low vitamin D concentrations that have been shown to occur in Saudi women. A primary cause seems to be a lack of exposure to the sunlight. The level of latitude in Saudi Arabia does not affect the efficiency of the sun throughout the year since Saudi Arabia is located at latitude of 21° (1, 10, 41). Unfortunately, most Saudi women in Mecca (80%) spend less than 15 minutes in the sun. Fifteen to 20 minutes a day is the minimal recommended amount (10, 17). Due to the sun's intensity and high temperatures found in Saudi Arabia, Saudis usually limit their time outdoors to the nighttime hours. Because of the intense heat, most daytime public activities are done in indoor places. Also the outdoor traditional dress for women consists of a dark veil that covers the body. Seventy-two percent of the participants indicated that they covered their entire body except for their hands when they were outdoors. Only 6.8% of the participants said that they had private places like a backyard or a swimming pool where they could expose their bodies to the sun. These women had significantly higher vitamin D concentrations (mean=12.98) than other participants. Students and workers who had to go out during the daylight hours also had higher vitamin D concentrations (11.59 ng/ mL) than women who worked in closed buildings (8.15 ng/ mL).
Many studies have shown that the daily use of sunscreen decreased the dermal production of vitamin D (9, 19). Sunscreen has been shown to block the UVB radiation that is required for the skin to produce vitamin D. The present study showed that using sunscreen is not common among Saudi women who live in Mecca. Those women who did use sunscreen had significantly lower 25(OH)D concentrations (7.88 ng/mL) than women how did not use sunscreen.

No relationship was shown between women with different skin colors and status of serum vitamin D concentrations. This was unexpected since most studies have shown that vitamin D concentrations vary according to the concentration of the melanin in the skin (22). The present data probably reflect a lower level of exposure to sunlight, which occurs for almost all women who lived in Mecca because of outdoor veil.

Age has been shown to affect vitamin D status due to the fact that skin production of vitamin D declines between the ages of 20-70 years (18, 21). However, the present study did not show this relationship for Mecca women. This trend could be due to the low levels of sunlight Mecca women are exposed to since temperatures are extremely hot in the daytime and, therefore, most Saudis prefer to stay indoors.

Normal concentrations of PTH are important to maintain since irregular concentrations can negatively affect the human body in many different ways (41). High concentrations of PTH can be a sign of low 25(OH)D concentrations (16). Sixty percent of the participants in the present study had normal PTH concentrations, while 37% had high concentrations of PTH. This may be due to the prevalence of vitamin D deficiency that was shown to occur in the present study.

The dietary intake of vitamin D unexpectedly reached the DRI recommendation (400 IU/day) in 74.6% of the participants. The mean dietary vitamin D intake was 446.12 IU/day and a significant correlation was shown between dietary vitamin D intake and serum
25(OH)D concentrations. This correlation was probably due to the high amount (400 IU/cup) of vitamin D found in fortified dairy products in Saudi Arabia. The intake of vitamin D from foods occurred primarily via the consumption of eggs (21 IU/egg) and tuna fish (200 – 350 IU/100 g) consumption. Unfortunately, these results demonstrate that when vitamin D is consumed at a level of 400 IU/day, serum concentrations of 25(OH)D are still pitifully low.

Calcium intake was extremely low in Mecca women. The average Ca intake was 247.2 mg/day, while the recommended amount is 1000 mg/day for NP women and 1200 mg/day for P women. Ninety-seven percent of the participants consumed less than 1000 mg/day of Ca. This can have a major affect on serum vitamin D status. Moreover, low concentrations of Ca and P in the blood stimulate the secretion of PTH. The increase of PTH concentrations then stimulates the kidney to produce 1,25(OH)2D, which can cause changes in gene expression. This change in gene expression then normalizes serum Ca concentrations by increasing intestinal Ca absorption, kidney reabsorption, and bone resorption (9, 16).

Supplement consumption is not common among Saudi Arabian women. It is mostly prescribed for people who have health conditions that require vitamin treatment. During pregnancy, women consume the vitamins that are necessary for maintaining their health and their baby's health. In most public hospitals in Saudi Arabia, gynecologists prescribe folic acid for the mother for the first three months of pregnancy and recommend additional supplements (i.e. Ca and Iron) if the blood tests show they are necessary. In the present study only 29.7% of the Mecca women used supplements. Thirty-one percent of the participants who used supplement were P. Of the participants who used supplements, only 16.1% used vitamin D supplements. These results may partially explain the vitamin D deficiency noted among Mecca women. Participants who consumed vitamin D supplements had significantly higher serum 25(OH)D concentrations. Usually vitamin D supplements were consumed along with Ca, but some times they were consumed separately.
It was not anticipated that P women would have higher serum vitamin D concentrations than NP woman. The serum concentration of 25(OH)D for P women was 13.01 ng/ mL, and for NP women it was 10.64 ng/ mL. Since dietary intake and sunlight exposure were not significantly different between the two groups, a possible explanation is that the higher consumption of vitamin D supplements by the PW led to increased serum concentrations of their vitamin D.
Chapter 6: Conclusions

In conclusion, more attention should be paid to vitamin D intake in the kingdom of Saudi Arabia because it is an important essential vitamin that is sadly lacking in P and NP Saudi women who live in Mecca. These women need to become involved in more outdoor activity during the daytime in order to increase their exposure to sunlight. Fatty fish consumption should also be increased since these fish are a good source of vitamin D. No additional fortification of vitamin D is needed; however, women should consume more vitamin D containing dairy products and eggs. People in Mecca should consume additional amounts of vitamin D in order to avoid health-related deficiencies. More research is needed in the area of vitamin D and disease; however, more education is especially needed to make the public more aware of this serious problem.
REFERENCES


24. Lamberg-Allardt CJ, Outla TA, Karkkainen MU, Rita HJ, Valsta LM. Vitamin D Deficiency and Bone Health in Healthy Adults in Finland: Could This Be a Concern in Other Parts of Europe? J Bone Miner Res 2001; 16:2066-2073.


APPENDICES
Appendix A: Informed Consent Form

You have been invited to participate in a research study that is designed to determine the vitamin D status and Intake in pregnant and non-pregnant Saudi women. Vitamin D helps strengthen your bones and also helps protect against cancer, therefore, it is important for women in general, as well as mothers who are pregnant to insure that they have adequate levels of Vitamin D in their bodies.

The study you are being asked to participate in will require that you allow that we study the vitamin D status in your body by analyzing a small portion of the blood that will be taken from you as part of your medical examination. There is minimal risk involved in blood draws. This minimal risk involves slight pain due to a needle stick and the possibility of bruising. If bruising is excessive you can contact the clinic for advice on how to take care of it. If you provide your consent your vitamin D test results will be provided to our research group from your medical record by your attending physician.

After your blood is analyzed you will be notified by your physician for follow-up care if your vitamin D levels are lower then normal. You will also be asked some questions from a survey form by the dietitian that deals with you: amount of exposure to sunlight, age, socioeconomic status, and consumption of vitamin and mineral supplements. The present study will also require that you complete a 3 day dietary recall during which time you describe the foods you ate on three different days. The first dietary recall will be obtained from you at the present time; the other two day dietary recalls will be obtained by phone on two other days of the week. It will take approximately 15-30 minutes/interview-phone call for you to provide feedback regarding your daily diet.

All Information that we obtain about your vitamin D status during this study will be maintained confidentially in a locked cabinet that is maintained in a locked office that only
the investigator will have access too. Your name will not be associated with this research and all data will be maintained using confidential code numbers. Results of this study will be presented at a national forum in Saudi Arabia/United States and will be submitted for publication in an internationally recognized, peer reviewed health publication. All published results will present only group data. Subject names will not be used in any presentations/publications.

Your participation in this study is voluntary and you may withdraw at any time without negative consequences regarding your health care.

Thank you…

Wedad Azhar, RD
Eastern Michigan University, Masters of Science Graduate Students

I _______________ understand the terms of this study. I also understand that my participation is voluntary and that I may withdraw from the study at any time without negative consequences regarding my health care.

Participant signature: ___________________ Date: ________________
نموذج الموافقة على المشاركة في البحث

لقد تم دعوتكم للمشاركة في دراسة بحثية لتحديد حالة فيتامين (D) والكمية المتناولة منه لدى النساء السعوديةن والإناث غير الحوامل. إن فيتامين (D) يساعد على تقوية عظامك والوقاية من الأصابة بالسرطان، وبالتالي فإنهم من المهم بالنسبة للنساء بصفة عامة وأمهات الحوامل بصفة خاصة التأكد من أن لديهم المستوي الكافي من فيتامين (D) في أجسامهم.

للمشاركة في هذا البحث سوف نطلب منك أن تستحلي لنا بدارسة حالة فيتامين (D) لديك وذلك بإخراج عينة دم بسيطة منك كجزء من الفحص الطبي. إن هناك حد أدنى من المخاطرة عند أخذ عينة الدم، هذه المخاطرة تكون في الإحساس بالمкусة بسيطة بنتائج عرض الإبرة، وكذلك إمكانية الإصابة بكبدة طفيفة في منطقة أخذ العينة. يرجى في حال إزدياد حدة الكبدة سرعة الإتصال بالعناية المختصة لأخذ الاستشارة اللازمة بكيفية العناية بها. في حال موافقتكم على المشاركة في هذا البحث، فإن نتيجة التحليل سوف تقدم إلى مجموعتنا البحثية من خلال سجللك الطبي من قبل طبيبك المعالج.

بعد ظهور نتيجة التحليل سوف يتم إعلامكم من قبل طبيبكم المعالج لغرض المتابعة الصحية في حال أن نسبة فيتامين (D) لديك أقل من العلامة الطبيعي. أيضا سوف يتم طرح بعض الأسئلة عليك من قبل أخصائية التغذية لتحديد الجزء المتعلق بذلك في استمارة البحث. هذه الأسئلة بخصوص: مقدار التعرض للشمس، العمر، الحالة الاجتماعية والاقتصادية، وكذلك الكمية المستهلكة من الفيتامينات والمعادن. إن هذه الدراسة تتطلب منك أيضًا إعطاء وصف دقيق للغذاء المستهلك لثلاثة أيام مختلفة ومقدار الكمية المستهلكة، حيث أن الإغاد المعطى لليوم الأول سوف يسجل منك في الوقت الحالي، أما بالنسبة لليومين الأخرين فانه سوف يتم أخذ بياناتهما من خلال الإتصال الهاتفي بك في يومين آخرين من أيام الأسبوع. إن المكالمات الهاتفية سوف تستغرق من 15 إلى 30 دقيقة لاستكمال معلومات الاستهلاك الغذائي.

إن كافة المعلومات التي سوف نحصل عليها خلال هذا البحث حول حالة فيتامين (D) لديك سيتم الاحتفاظ بها سراً في خزانة مغلقة داخل مكتب مغلق بحيث لا يستطيع الوصول إليها إلا من قبل الباحث. كما أن إمكنتن لن تكون موجودة في البحث، حيث أن جميع البيانات سوف تحفظ باستخدام أرقام شفرة سرية. كذلك فإن نتائج هذه الدراسة سوف تُقدِّم في ندوات علمية في المملكة العربية السعودية وفي الولايات المتحدة الأمريكية، وأيضا سوف تُنشر في مجلة صحية عالمية. إن كل النتائج التي سوف تُنشر ستكون عبارة عن بيانات حول المجموعة، أما فيما يخص أسماء المشاركين في البحث فلن تُستخدم في أي عرض أو نشر.
إن مشاركتك في هذا البحث مشاركة تطوعية حيث يمكنك الانسحاب من المشاركة في أي وقت من دون أي عواقب سلبية
على صحتك.

شكرًا جزيلا لتعاونك...

وداد أزهر، أخصائية تغذية
طالبة دراسات عليا، ماجستير علوم تغذية الإنسان، جامعة شرق ميشيغان

أقر أنا __________________________ بفهم كافة المصطلحات الواردة في هذه الدراسة. كذلك أنا على
علم تمام بأن مشاركتي تطوعية وأنه بإمكاني الانسحاب من الدراسة في أي وقت من دون أي عواقب سلبية على صحتي.

توقيع المشاركة __________________________
التاريخ __________________________
Appendix C: Approval from The College of Health and Human Services Human Subject Review Committee at Eastern Michigan University

EASTERN MICHIGAN UNIVERSITY

May 14, 2008

Dr. George Liepa
313 Marshall
School of Health Sciences

Dear Dr. George Liepa:

The Human Subjects Institutional Review Board (IRB) of Eastern Michigan University has granted approval to your proposal, “Determination of Vitamin D Intake and Vitamin D Status of Pregnant and Non-pregnant Women in Saudi Arabia.”

After careful review of your completion application, the IRB determined that the rights and welfare of the individual subjects involved in this research are carefully guarded. Additionally, the methods used to obtain informed consent are appropriate, and the individuals participating in your study are not at risk.

You are reminded of your obligation to advise the IRB of any change in the protocol that might alter your research in any manner that differs from that upon which this approval is based. Approval of this project applies for one year from the date of this letter. If your data collection continues beyond the one-year period, you must apply for a renewal.

On behalf of the Human Subjects Committee, I wish you success in conducting your research.

Sincerely,

Deb de Laski-Smith, Ph.D.
Interim Dean
Graduate School
Administrative Co-Chair
University Human Subjects Review Committee

Note: If project continues beyond the length of one year, please submit a continuation request form by 5/12/09.

Reference # 080413
# Appendix D: Example of Blood Tests Results

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Flags: 49 = Result below expected value range  
.49 = Result above expected value range
Appendix C: Questionnaire

File Number:_______________________
Name_______________________

1. □ Married □ Unmarried

2. □ Non pregnant □ Pregnant: if yes in which trimester:__________

3. How many times have you been pregnant? ____________

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

5. Age(Years): It should be between (20 – 60) ____________

6. Education level:
   □ Illiterate □: what is your education level? ____________

7. Socioeconomic status (According to family income):
   □ Below 5000 RS □ 5000-10000 □ above 10000

8. Number of family members(Person):__________
9. Skin color:
   □ White □ Tan □ Brown □ Dark

10. Region: ________________

11. Smoking:
   □ Yes □ No
   If yes: □ light □ Heavy

12. Occupation:
   □ Student □ House wife □ Employee
   □ Teacher
   □ Hospital worker
   □ Other ________________

13. How often do you expose to the sun:
   □ Daily □ Weekly □ Monthly

14. At what time do you get exposed to the sun?
   □ 7-11 □ 11-4 □ 4-6

15. How much time do you spend outdoors in the day time:
   □ 0-15 min □ 15-30 min □ 30 - 60 min □ 1-4 hours
16. Which parts of your body get exposed to the sun:

☐ None ☐ Face ☐ Hands ☐ Other parts of the body

17. Do you use sunscreen whenever you are outside?

☐ Yes ☐ No

If Yes: ☐ Daily ☐ Weekly ☐ Monthly

18. Do you consume any vitamins or minerals supplement?

☐ Yes ☐ No ☐ Sometimes

If Yes: What type: _______________________

Phone # _____________
### Appendix F: 24 Hour Dietary Recall

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## Appendix G: 24 Hour Dietary Recall (Arabic)

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