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Vitamin D Status among Bangladeshi Women of Reproductive Age

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VITAMIN D STATUS AMONG BANGLADESHI WOMEN OF REPRODUCTIVE AGE

A Thesis Presented

by

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ABSTRACT

VITAMIN D STATUS AMONG BANGLADESHI WOMEN OF REPRODUCTIVE AGE

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Vitamin D deficiency is of particular concern among women in many south Asian countries due to low availability of vitamin D-rich foods, dark skin pigmentation, and cultural and religious practices that promote the wearing of concealing clothing. However, information regarding the vitamin D status of many subpopulations in south Asian countries is limited. The current study was conducted to assess the vitamin D status of 147 Bangladeshi women of reproductive age and determine whether vitamin D status influences susceptibility to arsenic-associated skin lesions (75 cases, 72 controls). Serum 25(OH)D_3 levels were measured using a radioimmunoassay. The mean serum vitamin D level among the women in the current study was 60.1 nmol/L, which is well below the cut-off value of 75 nmol/L defining optimal vitamin D status. Over 81% of the women were below this cut-off value. Vitamin D status was not influenced by the presence of arsenic-associated skin lesions. Sun exposure and very low egg consumption were factors identified as

significant predictors of vitamin D status ($p<0.05$, $p<0.04$, respectively). Every additional hour of sun exposure per week during work was associated with a 0.32 nmol/L, on average, increase in serum vitamin D levels. Very low egg consumption corresponded to a 10.85 nmol/L lower serum vitamin D level compared to frequent egg consumption. Public health efforts in Bangladesh should promote increased consumption of food sources rich in vitamin D. Vitamin D fortification or supplementation may also be viable options to improve the vitamin D status of the population.

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CHAPTER 1

INTRODUCTION

In global public health, there are a few micronutrients that are considered to be of great importance. Vitamin A, iodine, and iron are all considered to be crucial micronutrients for the overall health of a population (WHO and FAO, 2004). Vitamin A is needed for proper immune function, cellular growth, and for the prevention of night blindness in children, while iodine is critical for proper growth and neurological development. Iron is required to prevent anemia, low birthweight, and premature birth (WHO and FAO, 2004). Although all of these nutrients are clearly essential for good health, they often overshadow the importance of other micronutrients. Of increasing interest is the role of vitamin D in global public health. Vitamin D deficiency, which has long been recognized as a contributing factor to poor bone health, has recently been associated with many public health issues that plague many countries world-wide, including cardiovascular disease (Kendrick et al., 2008), hypertension (Wang et al., 2008), autoimmune diseases (Cantorna, 2006), and cancer (Garland et al., 2006).

Vitamin D is a unique vitamin because, unlike other vitamins which must be obtained from the diet, vitamin D can be synthesized in sufficient amounts when skin is exposed to sunlight (Holick, 2004). Although humans have the ability to produce the required amount of vitamin D, certain conditions can interfere with its production, and vitamin D deficiency can develop. This is thought to be the case in many countries, as vitamin D deficiency has recently been considered a public health problem of epidemic proportions (Holick and Chen, 2008).

Vitamin D deficiency among women of reproductive age is of particular concern because it can have negative consequences for mother, fetus, infant, and child (Dawodu and Wagner, 2007). Recent research has suggested that vitamin D deficiency may put women of reproductive age at greater risk for preeclampsia during pregnancy, breast cancer, and premenstrual syndrome (Hypp□nen, 2005; Bodnar, 2007b; Evans et al., 2004; John et al., 1999; Bertone-Johnson 2005).

The body of evidence that has identified vitamin D as a potentially important factor in preventing many diseases and disorders continues to grow. If further research continues to support the positive effects that vitamin D has on preventing prevalent public health problems, vitamin D may one day be recognized as a micronutrient that is just as important as vitamin A, iodine, and iron within a global public health perspective.

CHAPTER 2

VITAMIN D

Vitamins are a category of nutrients that are essential for human health and development and generally must be obtained from the diet. Vitamins are required by the body in very small quantities and are, therefore, considered to be "micronutrients." Vitamins include niacin, riboflavin, thiamin, folic acid, vitamin B_{12}, vitamin B_6, pantothenic acid, biotin, vitamin C, vitamin K, vitamin E, vitamin A, and vitamin D. Vitamin D may be considered unique among vitamins because it is not, by definition, a true vitamin. Although the body can obtain vitamin D through a few natural food sources (including oily fish, eggs, and beef liver) and fortified foods in some developed countries (including commonly-consumed items such as milk and orange juice), humans actually have the ability to synthesize vitamin D under certain conditions. Endogenous synthesis of vitamin D is a major contributor to an individual's vitamin D status (Holick, 2004a).

2.1 Endogenous Synthesis

2.1.1 Mechanism

The exposure of skin to sunlight triggers a cascade of events leading to active vitamin D production (Figure 1). Endogenous synthesis begins in the epidermal keratinocytes and dermal fibroblasts. These cutaneous cells contain 7-dehydrocholesterol, a 4-ringed structure, which is secreted onto the skin's surface. The double bonds in ring B of 7-dehydrocholesterol absorb ultraviolet B (UVB) radiation at wavelengths of 290-315 nm from sunlight. UVB radiation causes a rearrangement of double bonds and the opening of the B ring of the 7-dehydocholesterol, converting the structure to cholecalciferol, or vitamin D3. Cholecalciferol is absorbed into the skin, diffuses into the blood, and is transported to the liver by a transport α-2 globulin vitamin D-binding protein (DBP). In the liver, cholecalciferol gains a hydroxyl group on carbon 25 by the P450 enzyme CYP27A1, or vitamin D 25-hydroxylase, producing 25-hydroxyvitamin D3. After this hydroxylation, 25-hydroxyvitamin D binds again to DBP and enters the blood. 25-hydroxyvitamin D3 is the main circulating form of vitamin D, and it serves as the best biomarker for vitamin D status. Normal levels of 25-hydroxyvitamin D are above 80 nmol/L or 32 ng/mL. The kidney takes up 25-hydroxyvitamin D3 from the blood where, in response to hormonal triggers, it undergoes another hydroxylation at carbon 1 by CYP27B1, or 25-hydroxyvitamin D 1α-hydroxylase. This enzymatic action produces 1α,25-dihydroxyvitamin D3, which is also referred to as calcitriol. Calcitriol is the active form of vitamin D, which is responsible for the vitamin's physiologic effects (Lips, 2006).

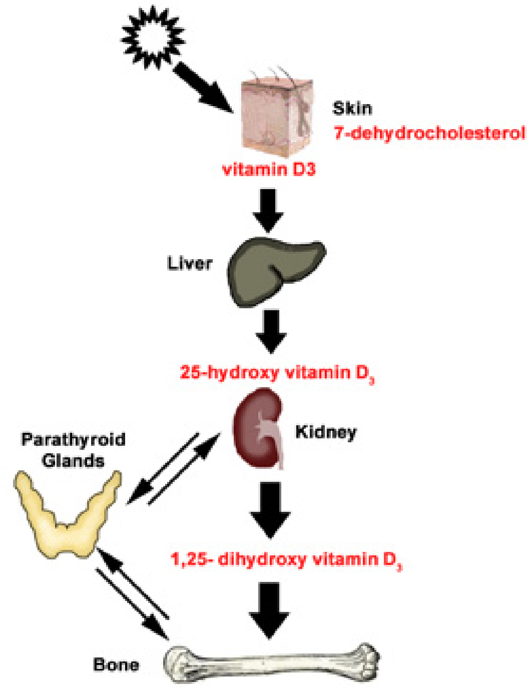

Figure 1.1. Endogenous production of vitamin D.

2.1.2 Factors Affecting Cutaneous Endogenous Synthesis

Since the body's synthesis of vitamin D relies on exposure to UVB radiation of a particular wavelength, endogenous production of vitamin D can be inhibited by factors that interfere with the skin's exposure to sunlight. Cloud cover of the sun, atmospheric pollution, geographic latitude, and season are all environmental factors that can limit skin's exposure to UVB radiation. Skin pigmentation, use of sunscreen with a skin protection factor (SPF) of eight or greater, and clothing are additional factors that can influence an individual's ability to synthesize vitamin D from available UVB radiation.

2.1.2.1. Cloud Cover and Pollution

Cloud cover and pollution can completely absorb the sun's UVB rays in the atmosphere before reaching the earth's surface, making it impossible for the skin to synthesize vitamin D (Holick, 1995). Agarwal et al. (2002) conducted a study in Delhi, India, to compare the vitamin D status of a group of children living in a highly polluted area to a group of children who lived in a less polluted area. Pollution levels were determined by a haze score, which can indicate the amount of solar UVB radiation reaching the earth's surface. The study found a significant difference in haze scores between the two areas and a significant difference in the vitamin D status of the children living in the two areas. The children living in the highly polluted section of Delhi had a mean 25-hydroxyvitamin D serum concentration of 12.4 ng/mL, and the children living in the less polluted area had a mean 25-hydroxyvitamin D serum concentration of 27.1 ng/mL. These results suggest that atmospheric pollution can have a significant impact on vitamin D status (Agarwal et al, 2002).

2.1.2.2. Geographical Latitude and Season

Geographical latitude and season can impact the cutaneous production of vitamin D by altering the angle at which the sun's rays hit the earth. When the sun's rays hit the earth more obliquely, more of the rays are absorbed by the ozone layer and fewer rays are transmitted to the earth's surface. This especially occurs above 37ºN and below 37ºS latitude between November and March, when 80-100% of the UVB rays are absorbed before reaching the earth's surface. During this time of year, very little to virtually no vitamin D can be synthesized above 37ºN or below 37ºS latitude. However, individuals residing at geographical latitudes closer to the equator (0º latitude) have fewer fluctuations in vitamin D synthesis because the angle of the sun's rays are less oblique, resulting in more constant vitamin D production year-round (Webb et al, 1988).

2.1.2.3. Skin Pigmentation and Sunscreen

Skin pigmentation, which is determined by the concentration of melanin, also affects the body's production of vitamin D. Melanin absorbs UVB radiation, as does the precursor to vitamin D (7-dehydrocholesterol). This results in competition for UVB radiation between melanin and 7-dehydrocholesterol in the skin. Individuals with more melanin (darker skin) require a longer amount of time in the sunlight than those with less melanin (lighter skin) to produce the same amount of vitamin D (Dawson-

Hughes, 2004). However, many individuals with light skin apply sunscreens before going outdoors to prevent sunburn. In essence, the sunscreen acts as an artificial melanin by absorbing the UVB radiation before it burns the skin. Therefore, individuals who consistently apply sunscreen to their skin impair their ability to synthesize vitamin D from sunlight (Matsuoka et al, 1987). Even sunscreens with a low sun protection factor (SPF) can affect vitamin D production. Sunscreens with a SPF of 8 reduces the ability of the skin to produce vitamin D by over 95% (Holick, 2004a).

2.1.2.4. Clothing

Clothing can also impact an individual's ability to synthesize vitamin D, as most fabrics absorb UVB radiation. (Matsuoka et al., 1992) The impact of clothing on vitamin D production is so significant that it has been linked to serious bone diseases, a hallmark indication of severe vitamin D deficiency. A study conducted by Allali et al. (2006) studied the impact of veiling (a traditional clothing style that completely covers the arms, legs, and head) on bone mineral density among post menopausal women in Morocco. The case-control study included 178 post menopausal osteoporotic women and 178 matched controls. The researchers found that women who wore concealing clothing had nearly twice the risk of developing osteoporosis than those who did not wear concealing clothing (ORs:1.87; 95% CI, 1.05-3.49) (Allali et al., 2006). This is of great concern in the Middle East and southeast Asia. In these regions, prominent religions (including Hinduism and Islam) often require women to wear concealing clothing which reduces the skin's exposure to sunlight. In addition, women residing in these areas tend to spend very little time in the sunlight due to cultural and social reasons (Dawson-Hughes, 2004).

2.2 Mechanisms of Action

The active form of vitamin D, calcitriol, is thought to produce biologic effects in a wide variety of tissues. Calcitriol exerts these effects through two main mechanisms: nongenomic and genomic. The nongenomic mechanisms refer to the actions of calcitriol at a cellular membrane level via interaction with a membrane-bound vitamin D receptor (VDR). The binding of calcitriol to a plasma membrance VDR can trigger a signal transduction pathway that results in the rapid opening of gated calcium ion channels located in the plasma membrane. It can also lead to increased calcium absorption in the intestines and bones (Norman, 2006).

The genomic mechanisms refer to the actions of calcitriol at the nuclear level, where it influences gene transcription. Nuclear VDRs have been discovered in cells from many different organs throughout the body. In these cells, calcitriol can enter the cell and bind with the nuclear VDR. The calcitriol-VDR complex becomes phosphorylated and binds with a retinoid X receptor (RXR), forming a heterodimeric complex that can be translocated to the nucleus. The VDR portion of the heterodimeric complex contains two zinc fingers that can interact with the vitamin D response element (VDRE) in the promoter region of DNA. This changes the transcription of certain genes, ultimately altering protein synthesis (Lips, 2006).

2.3 Physiologic Roles

The physiologic functions of calcitriol (1α,25-dihydroxyvitamin D3) can be broken down into two general categories: calcemic and noncalcemic. Calcemic functions refer to the role that calcitriol plays in calcium homeostasis and bone mineralization. Noncalcemic functions refer to the host of other physiologic actions that have been linked to vitamin D, which affect the cardiovascular system, immune system, and cell proliferation, differentiation, and apoptosis.

2.3.1 Calcemic Roles

One of calcitriol's main functions is to act along with parathyroid hormone (PTH) to strictly regulate plasma calcium concentrations. Plasma calcium concentrations are usually tightly maintained between 9.0-10.5 mg/dL. When there is a drop in plasma calcium concentration, a calcium-sensor protein in the parathyroid glands detects the drop and triggers increased secretion of PTH from the parathyroid gland. An increase in PTH stimulates the activity of the renal 1α-hydroxylase, subsequently increasing the production of calcitriol from 25-hydroxyvitamin D. Calcitriol increases serum calcium concentration by increasing calcium absorption in the intestine, increasing bone resorption, and increasing calcium reabsorption in the kidney (Deluca, 2004).

2.3.1.1. Calcium Absorption in the Intestine

At a basal level, 10-15% of the dietary calcium present in the intestines is absorbed. However, in the presence of calcitriol, intestinal calcium absorption becomes much more efficient, with about 30-80% of the dietary calcium present in the intestines being absorbed. The facilitation of calcium absorption in the intestine by calcitriol is accomplished through both calcitriol's genomic and nongenomic mechanisms of action. Through genomic mechanisms, calcitriol increases the synthesis of certain proteins that enhance intestinal absorption of calcium, including calbindin D9k and a low-affinity calcium-dependent ATPase. Calbindin D9k transports the calcium through the cytosol to the basolateral membrane of the enterocyte to be pumped out of the cell by ATP- dependent calcium pumps (Pérez, 2008). Calcitriol can also increase the expression of TRPV6, an epithelial calcium channel protein located in enterocytes. The increased expression of these proteins enhances the transport of calcium from the apical membrane to the basolateral side of the enterocyte, thereby increasing the calcium concentration in the circulation (Taparia et al.2006). Through its nongenomic actions, calcitriol can increase intestinal transcaltachia. Transcaltachia is the very rapid intestinal absorption of calcium via endocytosis at the brush-border membrane, followed by a release of calcium by the lysosome into the cytosol, which is then released across the basolateral membrane through exocytosis. The influx of calcium caused by transcaltachia triggers many critical intracellular processes, including contraction, secretion, neurotransmission, and gene expression (Norman, 2006).

2.3.1.2. Bone Resorption

When the body is unable to receive sufficient amounts of calcium from intestinal absorption, calcium stores in the bone can be mobilized into the circulation through vitamin D action. Calcitriol can induce the production of osteoclasts by enhancing the expression of the receptor activator nuclear factor-kappa beta ligand (RANKL) in osteoblast cells. RANKL interacts with its receptor located on preosteoclasts, which promotes osteoclast formation (Nagpal et al., 2005). The subsequent increase in osteoclasts mediated by RANKL increases bone resorption, thereby increasing serum calcium concentrations (DeLuca 2004).

2.3.1.3 Calcium Reabsorption in the Kidney

In an adult, about 9.7 grams of calcium are filtered by the kidneys every day. Some of this calcium can be reabsorbed by the distal convoluted tubule and the distal proximal tubule in the kidneys to increase serum calcium concentrations during hypocalcemia. Renal calcium reabsorption is increased by calbindin D28k, a protein synthesized through the genomic actions of calcitriol (Bolt, 2007).

2.3.2 Noncalcemic Roles

Upon discovery of the VDR, a host of new possibilities emerged as potential roles of vitamin D in human health. VDRs were identified not only in cells from organs involved in calcium homeostasis (intestine, bone, and kidney), but also in ovarian, lymph, skin, mammary, prostate, and other cells. Since this discovery, over 30 different types of tissues have been identified as having cells with a VDR (Norman, 2006), and numerous studies have investigated the potential role of vitamin D in diseases affecting these tissues.

2.3.2.1. Cardiovascular System

Epidemiologic, clinical, and laboratory studies have all provided evidence that poor vitamin D status may negatively impact cardiovascular health. A recent prospective study involving 1739 participants in the Framingham Offspring Study investigated the relationship between the risk of experiencing a cardiovascular event and 25-hydroxyvitamin D status. A “cardiovascular event” encompassed a variety of diagnoses relating to the cardiovascular system, including myocardial infarction, coronary insufficiency, angina, stroke, transient ischemic attack, peripheral claduication, and heart failure. Analysis of the data indicated that the risk of experiencing a cardiovascular event increased as vitamin D concentration decreased (Wang et al., 2008). Although an exact mechanism explaining the relationship between cardiovascular health and vitamin D is not readily available, Li et al. (2002) suggested that vitamin D may suppress the expression of the gene that codes for renin, an enzyme that triggers a cascade of events that results in the production of angiotensin II, which increases blood pressure. In theory, if an individual were vitamin D deficient, the gene coding for renin may be overexpressed, causing increased production of angiotensin II, and, therefore, high blood pressure.

2.3.2.2. Immune System

The immune system is the body’s intricate network of lymphocytes, macrophages, and specific molecules that interact to defend the body from foreign organisms or substances. B lymphocytes are responsible for forming antibodies that bind to foreign organisms and facilitate destruction of the organism by phagocytes. The T lymphocytes orchestrate the immune response and lyse cells in the body that are infected with a pathogen. Macrophages are phagocytic cells that are responsible for engulfing foreign particles and activating T cells through secretion of cytokines (Parkin and Cohen, 2001).

The VDR is expressed in active B cells, T cells, and macrophages, which suggests that vitamin D is involved in immune system regulation on some level (van Etten and Mathieu, 2005). Epidemiological evidence gathered over the past two decades supports this theory, as it has been observed that individuals with autoimmune diseases (such as multiple sclerosis, diabetes, Crohn's disease, inflammatory bowel disease, and ulcerative colitis) and certain infectious diseases, such as tuberculosis, tend to be deficient in vitamin D (Holick, 2004; Sentongo, 2001; Chan, 2000; Sita-Lumsdem et al, 2007).

Vitamin D affects autoimmunity by influencing the transcription of several T lymphocyte cytokines. Vitamin D inhibits the production of the T helper (Th)1 cell cytokines interferon-γ and interleukin-2, which promote the activation and proliferation of Th1 cells (van Etten and Mathieu, 2005). Th1 cells are considered to be key mediators in the development of autoimmune diseases. Therefore, reduced production may decrease the likelihood of developing an autoimmune disease. In addition, vitamin D increases the production of Th2 cell cytokines, including interleukin-4 and interleukin-10 (van Etten and Mathieu, 2005), Interkeukin-4 promotes Th2 cell activity, while interleukin-10 down-regulates Th1 cells (Parkin and Cohen, 2001). Due to the modulating effect that vitamin D has on Th1 cell and Th2 cell cytokines, it may be considered as a protective factor in autoimmunity.

Vitamin D also affects the body's ability to defend itself against invading organisms by stimulating the innate immune system. Vitamin D promotes the maturation of normal and neoplastic myelomonocytic cells to more differentiated monocytes and macrophages, leading to enhanced granuloma formation and increased phagocytosis of the infecting organism (Sita-Lumsdem et al, 2007). This effect could decrease susceptibility to infections such as tuberculosis (van Etten and Mathieu, 2005).

2.3.2.3. Cancer

It was first suggested that there might be a link between cancer and vitamin D status by F. Apperly in 1941 (Holick, 2004a). Apperly found that Americans residing in Massachusetts, Vermont, and New Hampshire were more likely to die of cancer than those Americans residing in states farther south, such as Texas, Georgia, and Alabama. Apperly attributed the variation in cancer risk to the different amounts of sunlight that the states received. Since then, it has been established that there is an association between residence and cancer risk: the further from the equator, the greater the risk (Garland et al., 2006, Holick, 2004a). Further epidemiologic research has indicated that reduced exposure to UVB radiation is linked to increased risk of cancers of the breast, rectum, ovary, prostate, stomach, bladder, esophagus, kidney, lung, pancreas, and uterus, in addition to non-Hodgkin lymphoma and multiple myeloma (Holick, 2006b). In an intervention study, Lappe et al. (2007) analyzed the effect of fours years of supplemental calcium, calcium plus vitamin D or placebo on cancer risk among 1179 randomly-selected healthy women. At the end of four years, participants who were given the calcium supplement with vitamin D had a lower incidence of all types of cancer than those given the placebo or just the calcium supplement (Lappe et al., 2007). Although the exact mechanisms by which poor vitamin D status increases cancer risk is unknown as of yet, it is thought that the vitamin D response elements located on over 200 human genes may regulate cell proliferation, differentiation, and apoptosis, making vitamin D a key protective factor against cancer (Lappe et al., 2007)

2.4 Vitamin D Status

2.4.1 Attaining Adequate Vitamin D Status

Adequate vitamin D status is clearly an important aspect of human health. Not only is vitamin D crucial to maintaining appropriate blood calcium levels, but it may also help prevent a wide range of chronic diseases. In order to aid in the prevention of many potential adverse health effects caused by inadequate vitamin D, circulating 25-hydroxyvitamin D levels should be at least 75-80 nmol/L (Calvo and Whiting, 2006). To achieve this level of serum vitamin D, a recommended adequate intake (AI) of 5 µg (or 200 IUs) of vitamin D per day for adults and 10 µg (or 400 IUs) for pregnant or lactating women has been established (IOM, 1997). Recent studies have suggested, however, that these recommendations for adults are exceedingly insufficient, especially for women of reproductive age (Vieth R et al., 2001; Datta et al., 2002). A much higher AI for vitamin D should be established to help the population achieve ideal serum levels of vitamin D. It is thought that for adults, an AI of 50 µg (2,000 IUs) would be more appropriate than the current recommendation (Hollis, 2005). An AI of 150 µg (or 6,000 IUs) for pregnant and lactating women has also been suggested (Yetley et al. 2009). It should be noted that adequate levels of vitamin D can also be obtained via endogenous synthesis, triggered by casual sun exposure (lasting only about a quarter of the amount of time that it takes for the skin to become pink) on the face, hands, and arms two to three times each week (Holick, 2004b).

2.4.2 Vitamin D Insufficiency and Deficiency

If vitamin D sufficiency cannot be attained through sun exposure, vitamin D must be obtained through the diet to avoid vitamin D insufficiency or deficiency (Heaney, 2007). Serum concentrations of 25-hydroxyvitamin D between 75 nmol/L and 37.5 nmol/L indicate vitamin D insufficiency, and levels below 37.5 nmol/L indicate deficiency (Holick 2004a; Hollis 2005). Without sun exposure, a daily intake of 25 µg (1000 IU) of dietary vitamin D is recommended (Tangpricha et al., 2003). In countries where foods are not fortified, the only viable option is to increase the intake of fatty fish, which provides 5-13 µg of vitamin D per three ounces of fish (USDA/ARS, 2005). Although there are other natural sources of vitamin D, including egg yolk and beef liver, it would be difficult to obtain the necessary 25µg of vitamin D through these sources alone, as only 0.5 µg of vitamin D is available in one medium egg yolk and only 0.3 µg of vitamin D is available in three ounces of beef liver (USDA/ARS, 2005). Therefore, when fish or fortified foods are not available and exposure to sunlight is limited, supplementation is the only recourse for attaining adequate vitamin D status (Calvo et al., 2005).

Unfortunately, due to the limited number of dietary sources of vitamin D and a plethora of environment, social, and cultural factors that limit sun exposure for many persons, vitamin D deficiency and insufficiency appear to be quite prevalent in many countries, such as India, Lebanon, Turkey (Calvo et al. 2005; Guzel, 2001; Gannagé-Yared, 2000). Vitamin D deficiency results in decreased calcium absorption and secondary hyperparathyroidism, which causes bone abnormalities in both children and adults (Holick and Chen, 2008; Lips, 2001). Vitamin D deficiency produces rickets in children and osteomalacia in adults.

Rickets is a bone disorder characterized by insufficient mineralization of bone, which may be manifested as bowed legs or knocked knees. When a child is deficient in vitamin D, sufficient amounts of calcium are not absorbed by the intestines to support the calcium demand required by the epiphyseal plates for mineralization and growth (Holick, 2006a). Rickets continues to be a problem worldwide, with at least 59 countries, including the U.S., reporting cases of rickets over the past two decades (Thacher et al., 2006).

Osteomalacia, a term that literally means “soft bones,” refers to disorders in which bones are not mineralized properly, causing a weakened bone structure that is prone to fractures. The inadequate mineralization that occurs in osteomalacia can be caused by secondary hyperparathyroidism due to vitamin D deficiency. Secondary hyperparathyroidism develops when 25-hydroxyvitamin D levels are low, causing decreased production of 1,25-dihydroxyvitamin D and, therefore, decreased intestinal calcium absorption. The decreased calcium absorption causes a low serum calcium concentration, which triggers the release and subsequent increase in serum concentrations of PTH (secondary hyperparathyroidism) (Lips, 2001). Increased PTH concentration increases bone turnover in order to maintain calcium levels, eventually leading to mineral depletion and bone loss if sustained for a long period of time (Lips, 2001). High PTH levels also promote urinary loss of phosphorus, a mineral that is required for bone mineralization (Holick, 2004b). The consequent bone loss and impaired mineralization due to vitamin D deficiency in adults result in the distinct weak bone structure of osteomalacia.

CHAPTER 3

VITAMIN D AND REPRODUCTIVE HEALTH

Studies indicate that vitamin D insufficiency and deficiency during a woman's reproductive years have profoundly negative consequences for her offspring, impairing growth, contributing to skeletal abnormalities (Dawodu et al., 2005), and increasing the risk diseases such as type 1 diabetes (Dawodu and Wagner, 2007). These offspring-focused concerns surrounding maternal vitamin D status often overshadow the importance of vitamin D sufficiency for the woman herself, not just for her offspring. Recent research has suggested that the vitamin D status of women of reproductive age may have a direct effect on the risk of preeclampsia, female cancers, and premenstrual syndrome (PMS), all of which profoundly impact women's health and well being (Hypponen, 2005; Bodnar, 2007b; Evans et al., 2004; John et al., 1999; Bertone-Johnson 2005). Therefore, improved understanding of the role of vitamin D among women of reproductive age can potentially influence the health of women as well as their offspring.

3.1 Preeclampsia

Preeclampsia is a pregnancy-specific syndrome that is estimated to affect 3-7% of women during their first pregnancy (Bodnar, 2007). This disorder usually develops after the twentieth week of pregnancy and exists until delivery. It is characterized by pregnancy-induced hypertension, proteinuria, and renal impairment (Hladunewich et al., 2007). Preeclampsia increases the risk for placental abruption, cerebrovascular and cardiovascular complications, renal failure, and maternal and offspring death (Mackay et al., 2001). In developed countries, nearly 25-45% of preterm births can be attributed to medically indicated deliveries due to preeclampsia (Ananth and Vintzileos, 2006). In developing countries, where prenatal care is less accessible than in developed countries, maternal mortality due to preeclampsia is common. It is estimated that over 50,000 maternal deaths in developing countries can be attributed to preeclampsia annually (Roberts and Gammill, 2005).

The pathophysiology of preeclampsia is somewhat elusive. Many factors are thought to contribute to the development of the disorder, including environmental, behavioral, immunological, and hereditary factors. Additional risk factors for preeclampsia are nulliparity, advanced maternal age, and dark skin (Hypp□nen, 2005). It is currently thought that preeclampsia progresses in two stages. The first stage arises when a woman's uterine spiral arteries do not remodel normally during early pregnancy. The remodeling of these arteries usually occurs when the trophoblast invades the spiral arteries and dilates the vessels, which helps supply sufficient amounts of blood to the placenta and fetus (Roberts and Gammill, 2005). When uterine spiral arteries are not remodeled, there is decreased perfusion of the placenta and fetus and increased secretion of placental materials into the maternal circulation (Walker, 2000; Hladunewich et al., 2007). This can trigger a maternal systemic response in some women, which is considered to be stage two, or the symptomatic stage, of preeclampsia. The clinical manifestations of this stage (hypertension, proteinuria, and renal impairment) can be attributed to glomerular endotheliosis, increased vascular permeability, and systemic inflammatory responses (Hladunewich et al., 2007).

Recent epidemiologic evidence suggests that there may be a link between the risk of developing preeclampsia and vitamin D status (Hypp□nen, 2005). In the United States, the prevalence of preeclampsia among white women 14-44 years of age was found to be lowest during July and August and is highest in January (Bodnar et al., 2007a). Magnu and Eskild (2001) also found similar results in Norway; in a retrospective cohort study of nearly 2 million births, women who delivered in the month of August had the lowest risk of preeclampsia. The risk of preeclampsia gradually increased during the fall, and peaked during the month of December (Magnu and Eskild, 2001). Interestingly, the vitamin D status of women varies throughout the seasons as well, typically reaching the highest concentrations in the summer months and lowest concentrations during the late winter months (Bolland 2007).

Racial disparities have also been observed between vitamin D status and preeclampsia. Preeclampsia is more common and fatal among black women than white women; black women of reproductive age also tend to have lower vitamin D status than white women (MacKay et al. 2001; Harris and Hughes 1998;). This evidence further reinforces the potential link between preeclampsia risk and vitamin D status.

A study by Bodnar et al. (2007b) was one of the first to examine the potential link between maternal early-pregnancy vitamin D status and preeclampsia risk. In this nested case-control study, 25-hydroxyvitamin D was measured in banked serum obtained from women 14-44 years of age who were less than 16 weeks pregnant. Non-hispanic whites represented 68.5% of the population, and non-hispanic blacks represented 31.5% of the population. Analysis included 55 women who developed preeclampsia later in pregnancy and 219 women who did not. The 25-hydroxyvitamin D concentrations in early pregnancy were significantly lower in the group of women who developed preeclampsia than in those who did not develop preeclampsia (Bodnar 2007b). These results suggest that maternal vitamin D deficiency may be an independent risk factor for preeclampsia.

Many possible mechanisms have been proposed to explain why vitamin D status may impact preeclampsia risk. Because vitamin D is thought to influence gene transcription, immune function, and blood pressure, some have suggested that 1,25-dihydroxyvitamin D may play a role in the regulation of genes responsible for placental invasion and blood vessel formation (Evans et al., 2004). In addition, since preeclampsia may be due to an immunological intolerance to pregnancy, the role of vitamin D in modulating immune function may affect the immunological response to fetus (Hypp□nen 2005). Lastly, vitamin D deficiency may exacerbate the hypertension that is clinically associated with preeclampsia through its potential effects on the renin-angiotensin system (Bodnar et al., 2007b).

3.2 Breast Cancer

Breast cancer is a major public health problem across the globe. It is the most commonly diagnosed cancer in women in both developed and developing countries; worldwide, nearly one-fifth of the 4.7 million cancer cases diagnosed in women are breast cancer. It is also a major cause of cancer death among women; in the year 2000 alone, about 375,000 deaths were attributed to breast cancer (Bray et al., 2004). A growing body of evidence supports the theory that vitamin D metabolites, such as 25,hydroxyvitamin D and 1,25-dihydroxyvitamin D, may significantly reduce the risk of many types of cancers, including breast cancer (Holick, 2006b; Pérez-López, 2008).

Risk factors for breast cancer involve genetic, hormonal, behavioral, and lifestyle factors. Certain genes, such as BRCA1 and BRCA2, put women at greater risk for the disease (Dunning et al., 1999). Hormonal risk factors are related to a women's exposure to estrogen and progesterone. These risk factors include early age of menarche, never giving birth, giving birth at a late age, short duration of breast feeding, low parity, late menopause, oral contraceptive use and hormone replacement therapy. Behavioral and lifestyle risk factors refer to dietary and physical activity patterns that have been linked to increased breast cancer risk, including low physical activity, high caloric intake, and excessive alcohol intake (Bray et al., 2004; Key et al., 2003; Hamajima, 2002).

Due to the high prevalence and multitude of risk factors for breast cancer, there is a great effort to identify new targets to help prevent and treat the disease. Vitamin D is one such target, as certain geographical patterns in breast cancer incidence are thought to be related to the amount of sunshine available to the region, presumably affecting the vitamin D production of its population (Pérez-López, 2008). Epidemiologic, clinical, and animal studies have added strength to this speculation, supporting the theory that vitamin D status impacts breast cancer risk (John et al., 1999; Janowsky et al. 1999; Lipkin and Newmark, 1999; Welsh, 2004).

The first epidemiological study that explored the relation between breast cancer and an individual's exposure to sunlight was conducted by John et al. (1999). Using data from the National Health and Nutrition Examination Survey (NHANES) I Epidemiologic Follow-up Study, the authors assessed whether sunlight exposure, dietary intake, and/or supplement intake affected breast cancer development in study participants. A cohort of 5009 white women between the age of 25-74 were included in this study, 191 of whom had a diagnosis of or death due to breast cancer. A 20-33% reduction in breast cancer risk (RR = 0.67 – 0.80) was found among women who had considerable sunlight exposure, defined by moderate to severe sun-induced skin damage, frequent recreational and occupational sunlight exposure determined by self-report, and physician assessment. Women who resided in southern locations (high solar radiation) also had reduced risk compared to those who resided in regions farther north (low solar radiation). In addition to sunlight exposure, women with low dietary vitamin D intake (<100 IU/day, as assessed by a

24-hour food recall questionnaire) were slightly more likely to have breast cancer, but the results were not statistically significant (John et al., 1999).

During the same year as the study by John et al. (1999), Janowsky et al. (1999) reported the results of a clinical study designed to determine if blood levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D differed between women with and without a breast cancer diagnosis. Women with a first diagnosis of breast cancer (n = 156) were matched to controls (n=184), by race, age, and month of blood draw. Low levels of the active form of vitamin D, 1,25-dihydroxyvitamin D, were associated with a higher risk of breast cancer (Janowsky et al., 1999). Bertone-Johnson et al. (2005a) conducted a study with similar objectives, but utilized a much larger study population. Using blood samples from a cohort of the Nurse's Health Study, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels were examined in 701 women who had a new diagnosis of breast cancer and 724 matched controls who did not have a breast cancer diagnosis. In this study, high levels of plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, were associated with a decreased risk of breast cancer in white women, although the association was not statistically significant (Bertone-Johnson, 2005a).

Many animal studies have also demonstrated a connection between vitamin D and breast cancer (Lipkin and Newmark, 1999). In one experiment, a population of rats was treated with a known carcinogen, but one group was fed a diet adequate in vitamin D and calcium, and a second group was fed a diet low in vitamin D and calcium. The rats that were given sufficient amounts of vitamin D and calcium developed fewer mammary tumors than the rats fed a diet low in vitamin D and calcium (Welsh, 2004).

These studies suggest that vitamin D status may play an important role in breast cancer prevention. Vitamin D is thought to exert anticarcinogenic effects through both its genomic and nongenomic actions within the breast tissue. Some evidence indicates that breast tissue has its own enzymatic system of CYP271B1 (the 1α hydroxylase) that converts 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D locally(Pérez-López, 2008). Once activated to its 1,25-dihydroxy form, vitamin D can bind to the nuclear VDRs in mammary cells, helping to regulate cell proliferation, differentiation, and apoptosis of the breast cells (Holick, 2006).

3.3 Premenstrual Syndrome

Premenstrual syndrome (PMS) is a collection of physical and mental symptoms experienced by 8-20% of women during the luteal phase of their menstrual cycle (Johnson, 1987). These symptoms can include breast tenderness, abdominal bloating, headache, swelling of the arms and feet, depression, anxiety, and irritability (Andrzej and Diana, 2006). Very severe symptoms can impair a women's ability to perform normal activities of daily living. Some researchers believe that the severity of symptoms and lowered quality of life experienced by many women with PMS is comparable to dysthymic disorder, a recognized mood disorder classified within the depression spectrum (Halbreich, 2003). Although the exact cause or causes of

PMS are still rather obscure, sex steroids produced in the ovaries are thought to trigger the symptoms, which are further aggravated by the serotonin and central nervous systems (Andrzei and Diana, 2006).

Although research efforts are in their early stages, recent studies have proposed a potential role for vitamin D in the prevention of premenstrual syndrome. Bertone-Johnson et al. (2005b) conducted a study among 116,678 women from the Nurse's Health Study II to assess the relationship between calcium and/or vitamin D intake and the risk of PMS. High intake of vitamin D and calcium was linked with a decreased risk of PMS. Women who had the highest intake of vitamin D had a relative risk of premenstrual syndrome of 0.59 (95% confidence interval, 0.40-0.86) compared to those with the lowest intake of vitamin D. These results suggest that high vitamin D intake may help prevent premenstrual syndrome (Bertone-Johnson, 2005b). Further investigation of the relationship between vitamin D and PMS appears warranted.

CHAPTER 4

BANGLADESH

Bangladesh is a predominantly Muslim country located in southeastern Asia, surrounded by India, Myanmar, and the Bay of Bengal. It is situated latitudinally between 20º34' and 26º38' north and longitudinally between 88º01' and 92º41' east. Bangladesh, which is comprised of 144,000 square kilometers, is inhabited by 156 million people – a population that is expected to grow by 1.8 percent annually (World Bank, 2008). This large and growing population of Bangladesh currently faces many public health challenges.

One of the most pressing public health concerns involves wide-spread arsenic contamination of water sources throughout the country. Nearly 97% of Bangladesh's population depends on shallow tube wells for drinking water (Smith et al., 2000). The tube wells were installed to provide clean and healthy water to much of the population in order to reduce mortality caused by gastrointestinal diseases from unhealthy water sources. However, the water from the millions of installed tube wells was not tested for arsenic. It is thought that nearly 35% of the tube wells that were installed have arsenic concentrations above the safe level of 50 µg/L (Smith et al., 2000; Chowdhury et al., 2000). These high levels of arsenic may be negatively affecting the health of an estimated 30-35 million people in Bangladesh (Smith et al., 2000). Long-term exposure to high arsenic levels in water can lead to arsenic poisoning, or arsenicosis. Arsenicosis causes painful skin lesions including keratosis and pigment changes. Keratosis often occurs in the palms of the hand and soles of the feet, and pigment changes are typically observed in the trunk or extremities in a freckled raindrop pattern (Mazumder DNG, 2003). These skin lesions are distinct indicators of arsenicosis (Tchounwou et al., 2003; Tondel et al., 1999), and usually appear five to ten years after the initial exposure to arsenic (Mazumder et al., 1998).

Arsenic-related skin lesions are associated with other major health problems, including cancers of the skin, lungs, bladder, and kidneys, hypertension, cardiovascular issues, diabetes, reproductive problems, respiratory disease, and neurological disorders among adults (Chen et al., 1995; Mazumder et al., 2007; Rahman et al. 2007; Ratnaike, 2003; Tchounwou et al., 2003). In younger generations, clinical manifestations of arsenic exposure are not always obvious and can be misdiagnosed. The effects of arsenic exposure early in life are not well understood compared with the effects of adult exposure (Watanabe et al, 2003). In recent years, there has been an effort to determine whether nutritional status influences the susceptibility to arsenicosis (Milton et al., 2004; Kile & Ronnenberg, 2008).

In addition to arsenic contamination of water sources, human welfare in Bangladesh is also challenged by high rates of poverty. Poverty maintains a vicious cycle of malnutrition, ill health, impaired physical and mental development, and low productivity (Nawani, 1994). Poverty is rampant throughout the country, as over one third of the population lives below the absolute poverty line, defined as subsisting on an income of less than one U.S. dollar per day (UNDP, 2006). In addition, nearly 24 million people in Bangladesh have a dietary intake of less than 1,805 kilocalories per day, an indicator of extreme poverty (Hong, 2006).

Women in impoverished nations tend to suffer more than other members of their family, as men and children often receive a disproportionate allocation of available resources (Santow, 1995). This holds true in Bangladesh, as studies have indicated that females are more likely to be undernourished and suffer a far higher mortality rate than their male counterparts (Ahmed, 1998). Chronic undernourishment leads to multiple micronutrient deficiencies, potentially including vitamin D, which may be exacerbated by additional cultural and social practices, including purdah (a common practice that involves wearing concealing clothing) and the avoidance of sunshine to maintain a light skin tone, which may impede the endogenous production of vitamin D in Bangladeshi women (Islam et al, 2002; Goswami et al., 2000).

4.1 Female Nutrition in Bangladesh

Over 50 percent of Bangladeshi women experience chronic energy deficiency. The prevalence of women with a Body Mass Index (BMI) less than 18.5 kg/m^2 ranges from 47.6% to 59.6%, depending on the country region. (FAO, 2009). In a study conducted within slum households in Dhaka, Bangladesh, almost 11 percent of the women in the sample had a BMI less than 16 kg/m^2, indicating severe malnutrition (Pryer, 2006). The vast prevalence and severity of malnutrition is of particular concern within this population because not only are women at greater health risk due to malnutrition, but their offspring are at increased risk of morbidity and mortality due to low maternal BMI (Ronnenberg et al., 2003). Low maternal BMI is associated with adverse pregnancy outcomes, including preterm birth (Schieve et al., 2000), low birth weight, and small-for-gestational age (Ronnenberg,et al., 2003). If the child survives, these adverse pregnancy outcomes have been shown to increase the child's risk of certain health problems later in life, including asthma (Gessner and Chimonas, 2007) and neurodevelopmental delays (Allin et al., 2006).

One of the main causes of malnutrition in Bangladesh is persistent food insecurity due to an inability of households to either grow or purchase enough food. Combs et al. (2005) studied the local food system in southeastern Bangladesh, and found among a sample of 480 households, the dominate foods produced included rice and starchy vegetables, such as potatoes and gourds. Very few micronutrient-dense foods were consumed on a regular basis. Only one third of households used pulses (bean, peas, lentils), and one quarter used leafy vegetables. Households very rarely used meat, eggs, dairy products, or fruits (Combs et al., 2005). However, fish was consumed in 73 households that lived close to water. Fish is generally a good source of dietary vitamin D, and it may represent one of the few food sources of vitamin D that may be accessible to a substantial portion of the population in Bangladesh. However, the vitamin D content of fish depends on the type of fish consumed, with generally lower amounts of vitamin D available in fresh water fish versus oceanic species; therefore, the contribution that fish intake makes to vitamin

D status will likely depend on the species consumed (Lu et al., 2007). Bangladesh currently does not fortify any foods with vitamin D (MOST, 2004).

4.2 Vitamin D Deficiency in Bangladeshi Women

Suboptimal vitamin D status is common among women in many Southeast Asian countries, including India and Pakistan (Goswami, 2000; Atiq, 1998). The extent of vitamin D insufficiency in these countries is rather surprising given their geographic latitude, which supports cutaneous vitamin D synthesis year-round. Researchers speculate that certain social and cultural practices, such as avoiding sunshine to maintain a light skin tone and wearing concealing clothing, may prevent vitamin D synthesis despite available sunlight (El-Sonbaty and Abdul-Ghaffar, 1998; Guzel et al., 2001). This hypothesis was validated by Guzel et al. (2001), who compared serum 25-hydroxyvitamin D levels in Turkish women who did and did not wear traditional Islamic dressing (veils). Although dietary habits were similar between the two groups, mean 25-hydroxyvitamin D level was significantly lower among veiled compared to unveiled women, suggesting that attire can greatly impact endogenous vitamin D synthesis in this region (Guzel et al., 2001). There is potential that concealing clothing may negatively affect the vitamin D status of many Bangladeshi women as well, since the vast majority of Bangladesh's population is Muslim and a covered style of dress (*Sari*) is common among Muslim Bangladeshi women (Islam et al., 2002). In addition to skin-concealing clothing, many Bangladeshi women avoid sun exposure in order to maintain a lighter skin color, as a dark skin color is often considered undesirable (Islam et al., 2002).

Few studies have investigated the vitamin D status of women in Bangladesh (Islam et al., 2002; Islam et al., 2008). One of the first studies documented that suboptimal vitamin D status and vitamin D deficiency existed among Bangladeshi women, regardless of socioeconomic status, and that breastfeeding women of low socioeconomic status had the lowest serum 25-hydroxyvitamin concentrations (Islam et al., 2002). Another study examined the vitamin D status of 200 Bangladeshi women who worked in a garment factory (Islam et al, 2008). All women wore concealing clothing, such as the sari or salwar, leaving only the hands and face exposed. Nearly all of the women (>99%) had serum 25-hydroxyvitamin D concentrations below the defined optimal range of 75-125 nmol/L, 86% had serum levels below 50 nmol/L, indicating hypovitaminosis D, and 16% had levels below 25 nmol/L, indicating vitamin D deficiency. Interestingly, about 90% of the women studied applied sunscreen to their face and hands, which covered the only skin that was exposed to sunlight (Islam, 2008). Although extensive research has not been completed, many dietary and cultural factors appear to negatively affect the vitamin D status of women in Bangladesh.

4.3 Importance of Vitamin D Sufficiency in Bangladeshi Women

Vitamin D sufficiency may be an important aspect of reproductive health for women in Bangladesh. If vitamin D insufficiency is determined to be a wide-spread problem in Bangladesh, improving the vitamin D status of the population may help reduce the prevalence of reproductive health concerns within the country, including preeclampsia, breast cancer, and premenstrual syndrome. Preeclampsia is one of the most commonly reported complications during pregnancy in Bangladesh; in one study, nearly 40% of pregnant women reported having more than one symptom of preeclampsia (Koenig, 2007). The exact prevalence of breast cancer in Bangladesh is unknown, as many cases are presumed to go undetected due to a lack of access to or utilization of healthcare, but the disease is thought to be the third leading cause of cancer death among Bangladeshi women (WHO, 2005). The prevalence of PMS in Bangladesh is also unknown, but if it is similar to that in the United States, extrapolations would suggest that PMS could potential affect nearly 7.1 million women, or 15% percent of women of reproductive age in Bangladesh.

There is great potential for high vitamin D insufficiency within Bangladesh due to dietary patterns and cultural habits present within the country, as illustrated by Guzel et al. (2001) and Islam et al. (2002). Further research regarding the vitamin D status of Bangladeshi women is of particular interest, as vitamin D may impact an array of reproductive health concerns.

CHAPTER 5

PURPOSE OF THE STUDY

Vitamin D is an especially important micronutrient for women of reproductive age. Not only does vitamin D insufficiency lead to osteomalacia, but it has also been associated with many female-specific health concerns, including preeclampsia, breast cancer, and PMS (Hypp□nen, 2005; Bodnar, 2007b; Evans et al., 2004; John et al., 1999; Bertone-Johnson, 2005). Since very few foods contain significant amounts of vitamin D naturally, women living in countries that do not fortify foods with vitamin D need to rely on sunlight exposure to attain adequate vitamin D status.

Despite abundant sunshine year-round in southern Asia, studies in this region have documented a high prevalence of vitamin D deficiency among women (Goswami, 2000; Atiq, 1998). Vitamin D deficiency is thought to be common in southern Asia due to limited dietary sources of vitamin D, dark skin pigmentation, and religious or cultural practices that limit skin exposure to sunlight and prevent endogenous production of vitamin D (El-Sonbaty and Abdul-Ghaffar, 1998; Guzel et al., 2001).

The main objective of the current study was to examine the vitamin D status of a cohort of reproductive-age women from Bangladesh and to identify dietary, cultural and sociodemographic factors that might influence vitamin D status in this population. Few studies have examined the vitamin D status of women from Bangladesh, although many Bangladeshi women are at high risk for vitamin D deficiency due to limited dietary sources and cultural and religious customs practiced throughout the country. The results from this study will lead to a greater understanding of the determinants of vitamin D status in Bangladeshi women of reproductive age and will help to inform future interventions aimed at improving the vitamin D status of this population.

A secondary objective of this study was to determine whether vitamin D status influences susceptibility to arsenic-associated skin lesions. To date, no studies have examined the potential relationship between vitamin D status and arsenic-associated skin lesions. The results from this study will help to determine whether vitamin D status interacts with arsenic exposure in the development of arsenic-associated skin lesions and will help inform nutritional interventions aimed at preventing this condition.

5.1 Hypotheses and Specific Aims

Hypotheses:

- Substantial proportions of Bangladeshi women in this cohort will have suboptimal vitamin D status [37.5 nmol/L ≤ 25(OH)D < 75 nmol/L] and biochemical evidence of vitamin D deficiency [25(OH)D < 37.5 nmol/L].
- Suboptimal vitamin D status will be associated with dietary factors, such as fish or eggs, and factors associated with length of time spent outdoors.
- Suboptimal vitamin D status will be more common among women with arsenic-associated skin lesions than in women without these lesions.

Specific Aims:

1. Measure serum concentrations of 25(OH)D and identify predictors of vitamin D among women of reproductive age from the Pabna district of Bangladesh.
    - Possible predictors include body mass index (BMI), dietary intake of vitamin D, and sun exposure.
2. Determine the prevalence of suboptimal vitamin D status and vitamin D deficiency among women of reproductive age from the Pabna district of Bangladesh.
3. Assess the potential relationship between vitamin D status and risk of arsenic-associated skin lesions.

CHAPTER 6

MATERIALS AND METHODS

6.1 Subjects

Dr. David Christiani from the Department of Environmental Health at the Harvard School of Public Health in collaboration with the Dhaka Community Hospital conducted a study from 2001-2003 to examine the relationship between arsenic exposure and skin lesions in Bangladesh (Breton et al., 2006). The study enrolled 1800 men and women (900 case-control pairs) recruited through community meetings held by the Dhaka Community Hospital. Case selection was based on a physician's diagnosis of skin lesions, including keratosis of the extremities, spotted melanosis, Bowen's disease, or squamous cell carcinoma. Controls were participants without any visible skin lesions and were matched to cases based on gender, age (within 3 years), and area of residence. Recruitment meetings took place in 23 villages throughout the Pabna district of Bangladesh. The Pabna district is located north of Dhaka on the Jamuna River in central Bangldesh. This region is considered to be moderately affected by arsenic contamination in the drinking water (Breton et al., 2006). The current study included 147 women (72 cases, 75 controls) selected among women subjects 18 to 33 years of age who had serum archives of ≥ 1.3 mL. All participants provided interviewer-administered informed consent, and the study protocol was approved by Human Subjects Committees of the University of Massachusetts Amherst and the Harvard School of Public Health.

6.2 Data Collection

6.2.1 Survey Data

At the time of recruitment, all participants visited a local clinic where a questionnaire was administered by a study-associated community health worker. Anthropometric, behavioral and demographic data was collected through the questionnaire. We had a data sharing agreement with the primary researchers from the Department of Environmental Health at the Harvard School of Public Health, which included limited access to collected survey data. Available anthropometric data included height (cm), weight (kg), BMI (kg/m^2), age (years), systolic blood pressure (mm/Hg), and diastolic blood pressure (mm/Hg), Behavioral data included whether or not the participants chewed betel nuts, chewed tobacco leaves, or had smokers in their daily environment. Behavioral data also included sun exposure (hours per week during work) and food habits. The food habits were assessed by the frequency of consumption of the following food categories: bean products, beef, bread, canned food, egg, fish, poultry, fruit or juice, milk, noodles, pickled food, rice, salty snacks, soda, sweet snacks, and vegetables. Food frequency categories were classified as almost none, 1-3 times monthly, 1-6 times weekly, 1-2 times daily, or more than 3 times daily. Demographic data included education level (illiterate, able to write, primary education, secondary education, higher secondary education, college, or postgraduate education), marital status (unmarried, married, divorced, or widowed), and whether or not the participant had chronic bronchitis or respiratory problems.

6.2.2 Blood Sample Collection and Laboratory Measurements

Between 2001 and 2003, researchers from the Department of Environmental Health at the Harvard School of Public Health collected blood samples from all study subjects during the initial clinic visit. Serum was isolated by centrifugation, and aliquots were reserved and frozen. Frozen serum samples from 147 women 18 to 33 years of age were transported to the Department of Nutrition, University of Massachusetts Amherst for biochemical analysis. Serum samples were transferred on dry ice and stored at -80^{o}C until biomarker assessment.

Serum 25(OH) vitamin D concentrations were determined using commercially available radioimmunoassay kits from DiaSorin (MN, USA). This assay procedure has been validated (Hollis, 2000). Gamma irradiation of I^{125} (in counts per minute) was quantified using a Beckman "Gamma 4000" gamma counter (Beckman Coulter, California, USA). Radioimmunoassay analysis involved extraction and preparation of the sample in duplicate followed by measurement of gamma counts per time unit for each sample, which were plotted against a standard curve to determine biomarker concentrations. Every assay included 42 subjects, six calibrators ranging from 0-100 ng/mL, two controls (one control represented a low-normal range and the other control represented a high-normal range), and an additional control sample from the lab at UMass to establish internal validity. Samples whose replicate gamma counts varied by more than 10% (N=13) were rerun. Biomarker concentrations were calculated using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California, USA). Suboptimal vitamin D status was defined as serum

25(OH)D ≥ 37.5 nmol/L but < 75 nmol/L, and vitamin D deficiency was defined as serum 25(OH)D < 37.5 nmol/L (Dawson-Hughes et al., 2005).

6.3 Statistical Analysis

The statistical analysis was completed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Characteristics of the study sample were assessed using means +/- standard deviations, ranges, and percentiles. The normality of the distribution of all variables was evaluated using the Shapiro-Wilks statistic, which indicated that all variables were normally distributed.

A *t* test was used to compare mean vitamin D levels between cases and controls. To investigate whether mean vitamin D levels differed between exposure variables, *t* tests were used for dichotomous variables and ANOVA was used for categorical variables with more than two groups.

A chi-squared analysis was used to determine whether the proportions of women with suboptimal vitamin D status and vitamin D deficiency differed between the case and control groups. A chi-squared test was also used to compare other potential differences in vitamin D status (insufficient or sufficient) between categorical variables, including chewing betel nut, chewing tobacco leaves, experiencing chronic bronchitis or respiratory problems, having smokers in their daily environment, education level, and fish and egg consumption.

Because serum $25(OH)D_3$ concentration and vitamin D status were not significantly different between cases and controls, the data were collapsed to create one cohort for the remaining analyses.

Predictors of vitamin D concentratons were identified using univariable linear regression. Variables that were significant at the level of $p \leq 0.25$ (height, sun exposure, education, and egg consumption) were included the multivariable linear regression model. Multivariable logistic regression was used to estimate the risk of vitamin D insufficiency associated with the selected variables.

CHAPTER 7

RESULTS

Characteristics of the study sample are shown in Table 7.1. The women in this study ranged in age from 18-33 years; most (73%) women were married. The mean BMI of the women was 20.1 kg/m^2 (n=147). About one third (30.6%) of participants had a BMI less than 18.5 kg/m^2, and almost all of the remaining participants (62.6%) had a BMI between 18.5 kg/m^2 and 25 kg/m^2. About one-third (36%) of study participants had no formal education. On average, participants were exposed to the sun for 20.4 hours per week. One fifth of the participants (21.1%) reported respiratory problems (including cough, shortness of breath, or asthma) and 8.8% experienced chronic bronchitis. Eleven participants indicated that they chew tobacco leaves, and fifteen reported chewing betel nut on a regular basis. Mean serum 25(OH)D_3 was 60.1 ± 17.4 nmol/L, with concentrations ranging from 18.6 to 110.0 nmol/L. Vitamin D levels below 75 nmol/L, indicative of suboptimal vitamin D status, were observed in 81% of participants. Of those, five subjects had vitamin D levels below 37.5 nmol/L, suggesting vitamin D deficiency. Just 28 subjects (19% of the study sample) had vitamin D levels above 75 nmol/L, the cut-off value used to describe optimal status.

Table 7.1. Characteristics of Bangladeshi Women in the Study Sample

Characteristic	n	Mean ± SD
Age (years)	147	25.4 ± 5.0
Weight (kg)	146	46.6 ± 7.3
Height (cm)	147	152.0 ± 5.7
Body Mass Index, BMI (kg/m^2)	146	20.2 ± 3.0
Blood Pressure – Diastolic (mm/Hg)	147	75.1 ± 9.8
Blood Pressure – Systolic (mm/Hg)	147	113.8 ± 14.3
Sun Exposure (hrs/week during work)	147	20.4 ± 9.3
Vitamin D Status		
Deficient ($25(OH)D_3$ < 37.5 nmol/L)	5	27.7 ± 7.7
Suboptimal ($25(OH)D_3$ 37.5 – 75 nmol/L)	114	55.0 ± 10.0
Optimal ($25(OH)D_3$ > 75 nmol/L)	28	88.1 ± 10.2
		%
Chews betel nut regularly	15	10.2
Chews tobacco leaves	11	7.5
Chronic bronchitis	13	8.8
Respiratory problems	31	21.1
Smokers in daily environment	93	63.7
Martial Status		
Unmarried	37	25.2
Married	107	72.8
Divorced	1	0.7
Widowed	2	1.4
Education completed		
No formal education	53	36.0
Primary education	17	11.6
Secondary education	52	35.4
Higher secondary education	17	11.6
College	8	5.4

Estimated intake frequencies of foods containing vitamin D (eggs and fish) are summarized in Table 7.2. All women consumed some fish, with the majority (88.3%) reporting that they ate fish one to six times a week. Egg consumption was less frequent, as roughly one half (53.1%) ate eggs one to six times per week and more than one third (34.3%) ate eggs just one to three times per month.

Table 7.2. Estimated Intake Frequency of Selected Foods Containing Vitamin D

Food Item	n	Percent
Fish		
None/Almost none	0	0.0
1 to 3 times monthly	2	1.4
1 to 6 times weekly	128	88.3
1 to 2 times daily	14	9.7
3 or mores times per day	1	0.7
Eggs		
None/Almost none	12	8.6
1 to 3 times monthly	48	34.3
1 to 6 times weekly	73	52.1
1 to 2 times daily	7	5.0
3 or mores times per day	0	0.0

Nearly all characteristics describing the study subjects were similar between skin lesion cases and controls (Table 7.3). The proportion of women experiencing chronic bronchitis as well as the proportion experiencing respiratory problems were significantly higher among women with arsenic-associated skin lesions than in controls ($p=0.01$), but no other subject characteristics differed significantly by case-control status. Because serum 25(OH)D_3 concentration and vitamin D status were not significantly different between cases and controls, the data were collapsed to create one cohort for the remaining analyses.

Table 7.3. Characteristics of Study Subjects, Stratified by Case/Control Status

Characteristic	Controls	Cases	p-value[1]
	Mean ± SD		
Age (years)	25.7 ± 5.3	25.4 ± 4.7	0.66
Weight (kg)	47.3 ± 7.5	46.0 ± 7.1	0.27
Height (cm)	151.9 ± 5.7	152.1 ± 5.7	0.83
Body Mass Index, BMI (kg/m^2)	20.5 ± 3.0	19.9 ± 2.9	0.22
Blood Pressure – Diastolic (mm/Hg)	74.2 ± 8.5	76.1 ± 10.8	0.24
Blood Pressure – Systolic (mm/Hg)	115.6 ± 11.3	112.1 ± 16.5	0.13
Sun Exposure (hrs/week during work)	20.7 ± 9.5	20.1 ± 9.0	0.70
25(OH)D_3 (nmol/L)	61.1 ± 16.7	59.7 ± 18.2	0.64
	n (%)		
Vitamin D Status			
Deficient (<37.5 nmol/L)	2 (40.0)	3 (60.0)	0.87
Suboptimal (37.5 – 75 nmol/L)	57 (50.0)	57 (50.0)	
Optimal (>75 nmol/L)	13 (46.4)	15 (53.6)	
Chews betel nut regularly			
Yes (15)	8 (11.1)	7 (9.5)	0.74
No (131)	64 (88.9)	67 (90.5)	
Chews tobacco leaves			
Yes (11)	7 (9.9)	4 (5.3)	0.30
No (135)	64 (90.1)	71 (94.7)	
Chronic bronchitis			
Yes (13)	2 (2.8)	11 (14.7)	0.01[2]
No (134)	70 (97.2)	64 (85.3)	
Respiratory problems			
Yes (31)	8 (11.3)	23 (30.7)	0.01[2]
No (115)	63 (88.7)	52 (69.3)	
Smokers in daily environment			
Yes (93)	42 (58.3)	51 (68.9)	0.18
No (53)	30 (41.7)	23 (31.1)	
Education completed			
No formal education	23 (31.9)	30 (40.0)	0.15
Primary education	5 (6.9)	12 (16.0)	
Secondary education	31 (43.1)	21 (28.0)	
Higher secondary education	10 (13.9)	7 (9.3)	
College	3 (4.2)	5 (6.7)	
Egg Consumption			
None/Almost none	7 (10.0)	5 (7.1)	
1 to 3 times monthly	27 (38.6)	21 (30.0)	0.26
1 to 6 times weekly	31 (44.3)	42 (60.0)	
1 to 2 times daily	5 (7.1)	2 (2.9)	
Fish consumption			
Less than 3 times monthly	0 (0.0)	2 (2.7)	
1 to 6 times weekly	61 (85.9)	67 (90.5)	0.23
1 to 2 times daily	9 (12.7)	5 (6.8)	
3 or mores times per day	1 (1.4)	0 (0.0)	

[1] Statistical significance was assessed using t-tests for continuous variables and chi-square analysis for categorical variables.
[2]Statistically significant at p<0.05

Although mean serum vitamin D levels did not vary significantly across variable categories (Table 7.4), it tended to be higher in women with more sun exposure and more frequent egg consumption (ANOVA $p = 0.07$ and 0.10, respectively), and this dose-response tendency was significant via linear trend tests ($p = 0.02$ and 0.03, respectively). Characteristics of the study sample did not vary significantly by vitamin D status (insufficient vs. sufficient; Table 7.5).

Univariable linear regression did not reveal any significant associations between the covariates and serum 25(OH)D_3 (Table 7.6). However, the p values for height, sun exposure, education, and egg consumption were <0.25 in univariable linear regression analyses, and these were entered in a multiple linear regression model with vitamin D as the outcome variable (Table 7.7). Age also had a p-value <0.25, but it was not included in the multivariable model because it was significantly correlated with sun exposure ($r=0.24$, $p=<0.01$; data not shown). Multivariable linear regression, using the forced-entry procedure, revealed two significant predictors of vitamin D levels: almost no egg consumption ($p=0.05$) and sun exposure ($p=0.04$). The regression coefficient for almost no egg consumption was -10.85 (SE=5.43), and the regression coefficient for sun exposure was 0.32 (SE=0.16).

Univariable logistic regression did not reveal any significant associations between the covariates and optimal vitamin D status (Table 7.8).

Table 7.4. Mean Vitamin D Status by Various Demographic and Health Characteristics

Variable (n)	Mean ± SD	p-value[1]
Age		0.44
18-22 (50)	59.9 ± 19.7	
23-26 (30)	59.0 ± 21.7	
27-30 (35)	60.5 ± 15.9	
30-33 (32)	64.4 ± 12.7	
BMI		0.40
<18.5 (44)	58.8 ± 18.7	
18.5 – 24.9 (92)	61.9 ± 17.5	
25 – 29.9 (10)	57.4 ± 8.3	
Sun Exposure		0.07[2]
<10 hours per week (20)	53.7 ± 14.7	
10 – 30.0 hours per week (113)	60.7 ± 18.2	
>30 hours per week (14)	67.6 ± 10.5	
Education		0.56
No formal education (53)	59.5 ± 15.6	
Primary education (17)	66.0 ± 18.4	
Secondary education (52)	61.2 ± 17.9	
Higher secondary education (17)	57.9 ± 19.1	
College (8)	55.3 ± 21.6	
Chews Betel Nut		0.31
Yes (15)	64.9 ± 11.3	
No (131)	60.5 ± 18.6	
Chews tobacco leaves		0.28
Yes (11)	66.0 ± 12.3	
No (135)	60.0 ± 17.8	
Smokers in daily environment		0.66
Yes (93)	60.0 ± 17.6	
No (53)	61.3 ± 17.4	
Respiratory problems		0.39
Yes (31)	62.7 ± 18.3	
No (115)	59.7 ± 17.2	
Chronic bronchitis		0.41
Yes (13)	64.2 ± 21.7	
No (134)	60.0 ± 17.0	
Fish consumption		0.71
1 to 3 times monthly (2)	71.5 ± 1.0	
1 to 6 times weekly (128)	60.4 ± 17.5	
1 to 2 times daily (14)	60.1 ± 18.0	
3 or mores times per day (1)	74.2 ± 0.0	
Egg consumption		0.10[3]
Almost none (12)	49.1 ± 11.2	
1 to 3 times monthly (48)	61.1 ± 15.2	
1 to 6 times weekly (73)	60.7 ± 18.9	
1 to 2 times daily (7)	67.3 ± 21.1	

[1]Statistical significance was assessed using t-tests for dichotomous variables and ANOVA for categorical variables with >2 groups.
[2] p-value for linear trend analysis = 0.02 [3]p-value for linear trend analysis = 0.03

Table 7.5. Characteristics of Study Subjects, Stratified by Vitamin D Status

Characteristic	Vitamin D Status (nmol/L)		p-value[1]
	Insufficient $25(OH)D_3 \leq 75$ N = 119	Sufficient $25(OH)D_3 > 75$ N = 28	
Mean (± SD)			
$25(OH)D_3$ (nmol/L)	53.9 ± 11.3	88.1 ± 10.2	<0.01
Age (years)	25.4 ± 4.0	25.9 ± 5.0	0.67
Weight (kg)	46.9 ± 7.8	45.3 ± 4.8	0.28
Height (cm)	152.3 ± 5.9	151.1 ± 4.8	0.34
Body Mass Index, BMI (kg/m^2)	20.3 ± 3.2	19.8 ± 1.8	0.50
Blood Pressure – Diastolic	75.0 ± 10.0	75.4 ± 8.4	0.90
Blood Pressure – Systolic	113.8 ± 14.6	13.75 ± 12.8	0.98
Sun Exposure (hrs/week during work)	20.3 ± 9.6	21.1 ± 7.7	0.66
Percent			
Chews betel nut regularly			
Yes (15)	73.3	26.7	0.44
No (131)	81.7	18.3	
Chews tobacco leaves			
Yes (11)	63.6	36.4	0.13
No (135)	82.2	17.8	
Chronic bronchitis			
Yes (13)	84.6	154	0.73
No (134)	80.6	19.4	
Respiratory problems			
Yes (31)	77.4	22.6	0.77
No (115)	81.7	18.3	
Smokers in daily environment			
Yes (93)	80.6	19.4	0.94
No (53)	81.1	18.9	
Education			
No formal education (53)	81.1	18.9	0.98
Primary education (17)	76.5	23.5	
Secondary education (52)	80.8	19.2	
Higher secondary education (17)	82.4	17.6	
College (8)	87.5	12.5	
Egg consumption			
Almost none (12)	100.0	0.0	0.33
1 to 3 times monthly (48)	77.1	22.9	
1 to 6 times weekly (73)	84.9	15.1	
1 to 2 times daily (7)	85.7	14.3	
Fish consumption			
1 to 3 times monthly (2)	100.0	0.0	0.51
1 to 6 times weekly (128)	78.9	21.1	
1 to 2 times daily (14)	92.9	7.1	
3 or mores times per day (1)	100.0	0.0	

[1] Statistical significance was assessed using t-tests for continuous variables and chi-square analysis for categorical variables.

Table 7.6. Univariable Linear Regression of 25(OH)D_3 (nmol/L) by Covariates

Variable	Regression Coefficient	Standard Error	p-value
Age (years)	0.50	0.287	0.08[1]
Weight (kg)	-0.02	0.200	0.90
Height (cm)	-0.32	0.254	0.21[1]
BMI (kg/m^2)	0.24	0.490	0.62
Blood Pressure – Diastolic (mm/Hg)	-0.01	0.148	0.97
Blood Pressure – Systolic (mm/Hg)	-0.05	0.101	0.65
Sun Exposure (hrs/week during work)	0.30	0.154	0.06[1]
Education			
No formal education	ref		
Primary education	6.52	4.88	0.18[1]
Secondary education	1.73	3.42	0.61
Higher secondary education	-1.57	4.88	0.75
College	-4.11	6.64	0.54
Chews betel nut regularly			
No	ref		
Yes	4.84	4.76	0.31
Chews tobacco leaves			
No	ref		
Yes	5.96	5.48	0.28
Chronic bronchitis			
No	ref		
Yes	4.20	5.07	0.41
Respiratory problems			
No	ref		
Yes	2.15	1.89	0.26
Smokers in daily environment			
No	ref		
Yes	-1.34	3.02	0.66
Fish consumption			
1 to 3 times monthly	11.2	12.42	0.37
1 to 6 times weekly	ref		
1 to 2 times daily	-.05	4.93	0.99
3 or mores times per day	14.01	17.60	0.43
Egg consumption			
Almost none	-12.00	5.34	0.03[1]
1 to 3 times monthly	0.01	3.15	0.99
1 to 6 times weekly	ref		
1 to 2 times daily	6.17	6.79	0.37

[1] Variable will be included in multiple linear regression model (p<0.25)

Table 7.7. Multivariable Linear Regression of 25(OH)D_3 (nmol/L) by Selected Covariates

Variable	Regression Coefficient	Standard Error	p-value
Constant	102.15	40.24	
Height (cm)	-0.32	0.27	0.23
Sun Exposure (hrs/week during work)	0.32	0.16	0.04[1]
Education			
No formal education	ref		
Primary education	5.90	4.91	0.23
Secondary education	1.70	3.40	0.62
Higher secondary education	-1.57	4.83	0.75
College	-2.04	7.00	0.77
Egg consumption			
Almost none	-10.85	5.43	0.05[1]
1 to 3 times monthly	0.71	3.27	0.83
1 to 6 times weekly	ref		
1 to 2 times daily	5.09	6.83	0.46

[1]Statistically significant at p<0.05

Table 7.8. Univariable Logistic Regression of Optimal Vitamin D Status[1] by Covariates

Variable	Odds Ratio	95% CI	p-value
Age (years)	1.02	(0.94, 1.11)	0.66
Weight (kg)	0.97	(0.91, 1.03)	0.28
Height (cm)	0.97	(0.90, 1.04)	0.34
BMI (kg/m^2)	0.95	(0.82, 1.10)	0.50
Blood Pressure – Diastolic (mm/Hg)	1.00	(0.96, 1.05)	0.89
Blood Pressure – Systolic (mm/Hg)	1.00	(0.97, 1.03)	0.98
Sun Exposure (hrs/week during work)	1.01	(0.97, 1.06)	0.66
Education			
No formal education	ref		
Primary education	1.32	(0.36, 4.93)	0.68
Secondary education	1.02	(0.39, 2.71)	0.96
Higher secondary education	0.92	(0.22, 3.83)	0.91
College	0.61	(0.07, 5.57)	0.67
Chews betel nut regularly			
No	ref		
Yes	1.62	(0.48, 5.53)	0.44
Chews tobacco leaves			
No	ref		
Yes	2.64	(0.72, 9.75)	0.14
Chronic bronchitis			
No	ref		
Yes	0.76	(0.16, 3.62)	0.73
Respiratory problems			
No	ref		
Yes	0.97	(0.54, 1.72)	0.91
Smokers in daily environment			
No	ref		
Yes	1.03	(0.44, 2.44)	0.94
Fish consumption			
Less than once per day	ref		
1 or more times per day	1.43	(0.53, 33.05)	0.173
Egg consumption			
Less than 3 times monthly	-0.108	(0.38, 2.11)	0.81
1 to 6 times weekly	ref		
1 to 2 times daily	-0.41	(0.08, 5.94)	0.72

[1]Vitamin D status was dichotomized at 75 nmol/L

CHAPTER 8

DISCUSSION

A significant proportion of the Bangladeshi women involved in this study had suboptimal vitamin D status, regardless of the presence of arsenic-associated skin lesions. The mean serum vitamin D level among the women in the current study was 60.1 nmol/L, which is well below the cut-off value of 75 nmol/L defining optimal vitamin D status. Over 81% of the women were below this cut-off value, indicating that vitamin D insufficiency is a substantial problem in this population. These findings are consistent with results from other studies conducted among women of reproductive age in Bangladesh (Islam et al., 2002; Islam et al, 2008). Even though women in this region live at a latitude that supports cutaneous vitamin D synthesis year-round, our results suggest that vitamin D insufficiency may be a common public health problem in Bangladeshi women that needs to be addressed.

Results from this study indicate that both sun exposure and egg consumption are influential factors that may affect the vitamin D status of Bangladeshi women within the population. Although mean duration of sun exposure per week did not vary significantly by vitamin D status (insufficient vs. sufficient; p=0.07), mean vitamin D levels showed a significant positive trend as hours of sun exposure per week during work increased (p=0.02). In univariable linear regression with serum 25(OH)D_3 in nmol/L as the outcome variable, sun exposure tended to be associated with vitamin D status, but this association did not reach statisitical significance (p=0.06) In multiple linear regression with serum 25(OH)D_3 as the outcome variable and controlling for height, education level, and egg consumption, sun exposure was identified as a significant predictor of vitamin D status. Every additional hour of sun exposure per week during work was associated with a 0.32 nmol/L, on average, increase in serum vitamin D levels, A larger sample size may have produced more significant results, revealing an even stronger relationship between weekly hours of sun exposure at work and vitamin D status.

In addition to sun exposure, mean vitamin D status showed a significant positive trend as reported egg intake increased ($p=0.03$), although there was no significant difference in the proportion of vitamin D-insufficient and -sufficient women across categories of egg consumption ($p=0.33$). In univariate linear regression with serum 25(OH)D_3 in nmol/L as the outcome variable, very low egg consumption (almost none) was significantly associated with serum vitamin D levels($p=0.02$). In multiple linear regression with serum 25(OH)D_3 as the outcome variable and controlling for height, sun exposure, and education level, very low egg consumption was identified as a significant predictor of vitamin D status. Very low egg consumption corresponded to a 10.85 nmol/L lower serum vitamin D level compared to frequent egg consumption (one to six per week). One egg yolk typically contains about 20 IU of vitamin D (USDA/ARS, 2005). Although the Adequate Intake (AI) for vitamin D is currently set at 200 IU per day for women 19-50 years of age, much of the scientific community supports increasing the AI to 1000 IU per day (Yetley EA et al., 2009). Regardless of the AI level, it would be difficult to reach this recommended intake of vitamin D through eggs alone. In the current study, egg intake may be related to some other factor, such as farming, which could also influence vitamin D status. Unfortunately we were not able to assess this potential relationship due to limited survey data.

Among our study subjects, fifteen women reported chewing betel nut on a regular basis. Previous studies have suggested that chewing betel nut may aggravate vitamin D deficiency (Ogunkolade WB et al, 2006) since compounds present in betel nut may increase expression of the enzyme 24-hydroxylase, which catalyzes conversion of **1**,25 $(OH)_2$ vitamin D to the relatively inactive **24**,25 $(OH)_2$ vitamin D. However, the current study found no significant association between betel nut chewing and vitamin D status or mean serum $25(OH)D_3$ levels. Given the small sample size of individuals who chewed betel nut (n=15), it is likely that we did not have enough power to reliably assess this potential association. We also did not measure **1**,25 $(OH)_2$ vitamin D or **24**,25 $(OH)_2$ vitamin D, which would be necessary to adequately assess the relationship between betel nut chewing and overall vitamin D status. A larger sample size would have provided us with greater power and may have produced results more consistent with the current literature.

There was no relationship between vitamin D status and arsenic-associated skin lesions. This is an important finding, however, because it suggests that arsenic-associated skin lesions may not interfere with cutaneous vitamin D synthesis. The painful skin lesions on hands and feet may not impact the amount of time spent outdoors, and the skin pigment changes may not affect the absorption of UV rays.

These finding also suggest that vitamin D status does not influence the appearance of arsenic-associated lesions, although vitamin D status has been associated with other cutaneous hyperproliferative conditions, such as psoriasis (Holick et al. 1987).

Limitations of this study include a relatively small sample size (n=147) and limited demographic data. A larger sample size would have increased the statistical power to detect associations, and it is possible that the associations between sun exposure and serum 25(OH)D_3 levels may have reached statistical significance. Additional characteristics describing the study sample would have been desirable. Specifically, data regarding religion, parity, season of blood draw, occupation, income, typical attire, and duration of sun exposure during recreational time would have been appropriate variables to consider as potential predictors of vitamin D status. This study was a secondary data analysis; we were not involved in the survey development, nor did we have any control over data collection methods. It is possible that a larger sample size and the use of a questionnaire designed to collect more detailed socioeconomic, dietary and health data may have revealed other statistically significant associations with vitamin D status.

The major strength of the study was the use of a biomarker (25(OH)D_3) to assess vitamin D status. Measuring the serum concentration of 25(OH)D_3 is the most reliable method for determining vitamin D status because it provides a measurement of the amount of vitamin D available to tissues (Calvo and Whiting, 2006) and includes both dietary and cutaneously synthesized. Biomarker measurement of

25(OH) vitamin D_3 levels is particularly important because dietary intake of the vitamin is a relatively poor predictor of overall vitamin D status (Holick and Chen, 2008).

It is important to recognize the potential impact of vitamin D insufficiency among this population of reproductive-age women. A large proportion (72%) of women in our study were married and, consequently, had a heightened likelihood of becoming pregnant. Inadequate vitamin D has been associated with a greater risk of preeclampsia (Bodnar, 2007) and increased bone turnover (Prentice, 2000). Moreover, recent evidence suggests that poor maternal vitamin D status may increase the risk of bacterial vaginosis (Bodnar et al., 2009), a condition that has been linked to preterm delivery (Hillier et al., 1995), as well as gestational diabetes (Zhang et al., 2008), which increases the risk of morbidity and mortality for both the mother and fetus (Langer et al., 2005). Poor maternal vitamin D status may also contribute to poor fetal skeletal mineralization and calcium homeostasis, causing congenital rickets, craniotabes, and low bone mineral content (van der Meer et al, 2006). In addition, the child may be at increased risk for developing diseases later in life that have been linked to poor vitamin D status, such as multiple sclerosis, cancer, diabetes, and schizophrenia (Holick and Chen, 2008).

Public health efforts in Bangladesh should address the need for vitamin D sufficiency within their population. Despite abundant sunshine at this latitude, many women in our study clearly did not synthesize adequate amounts of vitamin D. Although increasing skin exposure would be a simple and beneficial behavior modification to aid in cutaneous vitamin D synthesis, it would be inappropriate to suggest such a change since many women wear concealing clothing for religious reasons (Islam et al. 2002). Therefore, encouraging the consumption of natural food sources rich in vitamin D, such as egg, may be a more effective method for improving the vitamin D status of this particular population. Vitamin D fortification or supplementation may also be viable options to improve the vitamin D status of the population. Commonly used foods, such as wheat flour or cooking oil, could be fortified with vitamin D (Misra, 2008). Bangladesh has the necessary human and institutional capacity to implement large-scale food fortification programs, and it has recently employed a variety of strategies to reduce micronutrient malnutrition (MOST, 2004). Food fortification is in pilot stages, but efforts are being made to establish sustainable programs to fortify wheat flour with vitamin A, iron, zinc, thiamin, riboflavin niacin, and folic acid. If future studies with large sample sizes continue to find widespread vitamin D deficiency among the Bangladeshi population, perhaps vitamin D should also be considered for inclusion in the country's fortification efforts.

BIBLIOGRAPHY

Agarwal KS, Mughal MZ, Upadhyay P, Berry JL, Mawer EB, Puliyel JM. The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi, India. *Arch Dis Child* 2002;87:111-113.

Ahmed, SM, Adams A, Chowdhury AMR, Bhuiya A. Chronic energy deficiency in women from rural Bangladesh: some socioeconomic determinants. *J. Biosoc Sci* 1998;30:349-358.

Allin M, Rooney M, Griffiths T, Cuddy M, Wyatt J, et al. Neurological abnormalities in young adults born preterm. *J Neurol Neurosurg Psychiatry* 2006;77:484-499.

Ananth CV and Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. *J Matern Fetal Neonatal Med* 2006;19(12):773-782.

Andrzej M and Diana J. Premenstrual syndrome: From etiology to treatment. *Maturitas* 2006;55S:S47-S54.

Atiq M, Suria A, Nizami SQ, Ahmed I. Maternal vitamin D deficiency in Pakistan. *Acta Osbtet Gynecol Scand* 1998;77:970-973.

Bertone-Johnson ER, Chen WY, Holick MF, et al. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2005a;14(8):1991-1997.

Bertone-Johnson ER, Hankinson SE, Bendich AB, et al. Calcium and vitamin D intake and risk of incident premenstrual syndrome. *Arch Intern Med* 2005b;165:1246-1252.

Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab*. 2007a;92(9):3517-3522.

Bodnar LM, Catov JM, Roberts JM. Racial/ethnic differences in the monthly variation of preeclampsia incidence. *Am J of Obstet Gynecol*. 2007b;196:324.e1-324.e5.

Bolland MJ, Grey AB, Amers RW, et al. The effects of seasonal variation of 25-hydroxyvitamin D and fat mass on a diagnosis of vitamin D sufficiency. *Am J Clin Nutr*. 2007;86:959-964.

Bolt MJG, Coa LP, Kong J, Sitrin MD, Li YC. Vitamin D receptor is required for dietary calcium-induced repression of calbindin-D9k expression in mice. *J. Nutr. Biochem* 2005;16:286-290.

Bray F, McCarron P, Parkin DM. The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Res* 2004;6:229-239.

Buchanan JR, Saten R, Cauffman S, et al. The effect of endogenous estrogen fluctuation on metabolism of 25-hydroxyvitamin D. *Calcif Tissue Int* 1986;39:139-144.

Calvo MS and Whiting SJ. Public health strategies to overcome barriers to optimal vitamin D status in populations with special needs. *J Nutr* 2006;136:1139-1139.

Calvo MS, Whiting SJ, Barton CN. Vitamin D intake: a global perspective of current status. *J Nutr* 2005;135:310-316.

Cantorna MT. Vitamin D and its role in immunology: multiple sclerosis and inflammatory bowel disease. *Prog Biophys Mol Biol* 2006;92(1):60-64.

Chan TYK. Vitamin D deficiency and susceptibility to tuberculosis. *Calcif Tissue Int* 2000;66:476-478.

Chen CJ, Hsueh YM, Lai MS, et al. Increased Prevalence of Hypertension and Long-term Arsenic Exposure. *Hypertension* 1995;25:53-60.

Chowdhury UK, Biswas BK, Chowdhury TR, et al. Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ Health Perspect* 2000;108(5):393-397.

Combs GF Jr, Hassan N. The Chakaria food system study: household-level, case-control study to identify risk factors for rickets in Bangladesh. *Euro J Clin Nutr* 2005;59:1291-1301.

Datta S, Alfaham M, Davies D, et al. Vitamin D deficiency in pregnant women from a non-European ethnic minority population – and international study. *Br J Obstet Gyneacol* 2002;109:905-908.

Dawodu A, Agarwal M, Sankarankutty M, Hardy D, Kochivil J, Badrinath P. Higher prevalence of vitamin D deficiency in mothers of rachitic than nonrachitic children. *The Journal of Pediatrics* 2005;147(1):109-111.

Dawodu A and Wagner CL. Mother-child vitamin D deficiency: an international perspective. *Arch Dis Child* 2007;92:737-740.

Dawson-Hughes B. Racial/ethnic considerations in making recommendations for vitamin D for adult and elderly men and women. *Am J Clin Nutr*. 2004;80:176S-176S.

Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. *Osteoporosis Int* 2005;16:713-716.

Deluca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004;80(suppl):1689S-1696S.

Dunning AM, Healey CS, Pharoah PD, et al. A systematic review of genetic polymorphisms and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 1999;8:843-854.

El-Sonbaty MR, Abdul-Ghaffar NU. Vitamin D deficiency in veiled Kuwaiti women. *Eur J Clin Nutr* 1996;50:315-320.

Evans KN, Bulmer JN, Kilby MD, Hewison M. Vitamin D and placental-decidual function. *J Soc Gynecol Investig* 2004;11:262-271.

The Food and Agriculture Organization of the United Nations (FAO). Nutrition country profiles: Bangladesh. FAO April 28, 2009 <http://www.fao.org/ag/agn/nutrition/bgd_en.stm>

Gannagé MH, Chemali R, Yaacoub N, Halaby G. Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. *J Bone Miner Res* 2000;15:1856-1862.

Garland CF, Garland FC, Gorham ED, Lipkin M, Newmark H, et al. The role of vitamin D in cancer prevention. *Am J Public Health* 2006;96(2):252-261.

Gessner BD and Chimonas MAR. Asthma is associated with preterm birth but not small for gestational age status among a population-based cohort of Medicaid-enrolled children <10 years of age. *Thorax* 2007;62:231-236.

Goswami R, Gupta N, Goswami D, et al. Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. *Am J Clin Nutr* 2000;72:472-475.

Guzel R, Kozanoglu E, Guler-Uysal F, Soyupack S, Sarpel T. Vitamin D status and bone mineral density of veiled and unveiled Turkish Women. *J Womens Health Gend Based Med* 2001;10(8):765-770.

Halbreich U, Borenstein J, Pearlstein T, Kahn LS. The prevalence, impairment, impact, and burden of premenstrual dysphoric disorder (PMS/PMDD). *Psychoneuroendocrinology* 3004;28:1-23.

Hamajima N, Hirose K, Tajima K, et al. Alcohol, tobacco and breast cancer – collaborative reanalysis of individual data from 53 epidemiological studies, including

58,515 women with breast cancer and 95,067 women without the disease. *Br J Cancer* 2002;87:1234-1245.

Harris SS, Dawson-Hughes B. Seasonal changes in plasma 25-hydroxyvitamin D concentration of young American black and white women. *Am J Clin Nutr* 1998;67:1232-1236.

Heaney RP. The case for improving vitamin D status. *J Steroid Biochem Mol Biol* 2007;103:635-641.

Hillier SL, Nugen RP, Eschenbach DA, Krohn MA, Gibbs RS, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. *N Engl J Med* 1995;333:1737-1742.

Holick MF. Environmental factors that influence the cutaneous production of vitamin D. *Am J Clin Nutr* 1995;61(suppl):638S-645S.

Holick MF. Resurrection of vitamin D deficiency and rickets. *J Clin Invest* 2006a;116:2062-2072.

Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune disease, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004a;80(suppl):1678S-1688S.

Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* 2004b;79:362-371.

Holick MF. Vitamin D: Its role in cancer prevention and treatment. *Prog Biophys Mol Biol* 2006b;92:49-59.

Holick MF and Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* 2008;87(suppl):1080S-1086S.

Holick MF, Smith E, Pincus S. Skin as the site of vitamin D synthesis and target tissue for 1,25-dihydroxyvitamin D3. Use of calcitriol (1,25-dihydroxyvitamin D3) for treatment of psoriasis. *Arch Dermatol* 1987;123(12):1677a-1683a.

Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D Sufficiency: Implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr* 2005;135(2):317-323.

Hong R, Banta JE, Betancourt JA. Relationship between household wealth inequality and chronic childhood under-nutrition in Bangladesh. *Int J Equity Health* 2006;5:15.

Hladunewich M, Karumanchi SA, Lafayette R. Pathophysiology of the clinical manifestations of preeclampsia. *Clin J Am Soc Nephrol* 2007;2:543-549.

Hypp□nen E. Vitamin D for the prevention of preeclampsia? A hypothesis. *Nutr Rev* 2005;63(7):225-232.

Institute of Medicine (IOM). 1997. *Dietary Reference Intakes: Calcium, Phospohorus, Magnesium, Vitamin D, Fluoride*. Washington, DC, USA.

Islam MZ, Lamberg-Allardt C, Kärkkäinen M, et al. Vitamin D deficiency: a concern in premenopausal Bangladeshi women of two socio-economic groups in rural and urban region. *Eur J Clin Nutr* 2002;56:51-56.

Islam MZ, Shamim AA, Kemi V, et al. Vitamin D deficiency and low bone status in adult female garment factory workers in Bangladesh. *Br J Nutr* 2008;99:1322-1329.

Janowsky EC, Lester GE, Weinberg CR, et al. Association between low levels of 1,25-dihydroxyvitamin D and breast cancer risk. *Public Health Nutr* 1999;2(3):283-291.

John EM, Schwartz GG, Dreon DM, Koo J. Vitamin D and breast cancer risk: The NHANES I epidemiologic follow-up study, 1971-1975 to 1992. *Cancer Epidemiol Biomarkers Prev* 1999;8:399-406.

Johnson SR. The epidemiology and social impact of premenstrual symptoms. *Clin Obstet Gynecol.* 1987;30:367-376.

Kendrick J, Targher G, Smits G, Chonchol M. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in The Third Health and Nutrition Examination Survey. *Atherosclerosis* 2008;205(1):255-60.

Key TJ, Naomi AE, Spence EA, Travis RC. Nutrition and breast cancer. *Breast* 2003;12:412-416.

Kile ML, Ronnenberg AG. Can folate intake reduce arsenic toxicity? *Nutr Rev* 2008 Jun;66(6):349-353.

Koenig MA, Kamil K, Streatfield PK, et al. Maternal health and care-seeking behavior in Bangladesh: findings from a national survey. *Int Fam Plan Perspect* 2007;33(2):75-82.

Langer O, Yogev Y, Most O, Xenakis EM. Gestational diabetes: the consequences of not treating. *Am J Obstet Gynecol* 2005;192(4):989-997.

Li YC, Kong J, Wei M, et al. Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensis system. *J Clin Invest* 2002;110:229-238.

Lipkin M and Newmark HL. Vitamin D, Calcium and Prevention of Breast Cancer: A Review. *J Am Coll Nutr* 1999;18(5):392S-397S.

Lips P. Vitamin D physiology. *Prog Biophys Mol Biol* 2006;92:4-8.

Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocr Rev* 2001;22(4):447-501.

Lu Z, Chen TC, Zhang A, Persons KS, Kohn N, et al. An evaluation of the vitamin D3 content in fish: Is the vitamin D content adequate to satisfy the dietary requirement for vitamin D? *J Steroid Biochem Mol Biol* 2007;103(3-5):642-644.

Mackay AP, Berg CJ, Atrash HK. Pregnancy-related mortality from preeclampsia and eclampsia. *Obstet Gynecol* 2001;97(4):533-538.

Magnu P, Eskild A. Seasonal variation in the occurrence of pre-eclampsia. *BJOG*. 2001;108:116-119.

Matsuoka LY, Ide L, Wortsman J, MacLaughlin JA, Holick MF. Sunscreens suppress cutaneous vitamin D3 synthesis. *J Clin Endocrinol Metab* 1987;1165-1168.

Matsuoka LY, Wortsman J, Dannenberg MJ, et al. Clothing Prevents Ultraviolent-B Radiation-Dependent Photosynthesis of Vitamin $D_{3.}$ *J Clin Endocrinol Metab* 1992;75(4):1099-1103.

Mazumder DNG, Haque R, Ghosh N, De BK, Snatra A, Chakraborty D. Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. *Int J Epidemiol* 1998;27(5):871-877.

Mazumder DNG, Haque R, Nilima G, et al. Arsenic in drinking water and the prevalence of respiratory effects in West Bengal, India. *Int J Epidemiol* 2000;29:1047-1052.

Milton AH, Hasan Z, Shahidullah SM, et al. Association between nutritional status and arsenicosis due to chronic arsenic exposure in Bangladesh. *Int J Epidemiol* 2004;14(2):99-108

Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. *Pediatrics* 2008;122:398-417.

MOST, USAID Micronutrient Program. 2004. Elements of a National Food-Fortification Program for Bangladesh. Arlington, Virginia, USA.

Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev* 2005;26(5):662-687.

Nawani NP. 1994. Indian experience on household food security and nutrition security. RAP Publication, Bangkok, Thailand.

Norman AW. Minireview: Vitamin D receptor: new assignments for an already busy receptor. *Endocrinology* 2006;147(12):5542-5548.

Ogunkolade WB, Boucher BJ, Bustin SA, et al. Vitamin D metabolism in peripheral blood mononuclear cells is influenced by chewing "betel nut" (*Areaca catechu*) and vitamin D status. *J Clin Endo & Met* 2006;91(7):2612-2617.

Parkin J, Cohen B. An overview of the immune system. *Lancet* 2001;357:1777-1789.

Pérez AV, Picotto G, Carentieri AR, et al. Minireview on regulation of intestinal calcium absorption: emphasis on molecular mechanisms of transcellular pathway. *Digestion* 2008;77:22-34.

Pérez-López FR. Sunlight, the vitamin D endocrine system, and their relationships with gynaecologic cancer. *Maturitas*. 2008;59(2):101-113.

Prentice A. Maternal calcium metabolism and bone mineral status. *Am J Clin Nutr* 2000;71(suppl):1312S-1316S.

Pryer JA, Rogers S. Epidemiology of undernutrition in adults in Dhaka slum house holds, Bangladesh. *Eur J Clin Nutr* 2006;60:815-822.

Rahman A, Vahter M, Ekstrom EC, et al. Association of Arsenic Exposure during Pregnacy with Fetal Loss and Death: A Cohort Study in Bangladesh. *Am J Epidemiol* 2007;165(12):1389-1396.

Ratnaike, RN. Acute and chronic arsenic toxicity. *Postgrad Med J* 2003;79:391-396.

Roberts JM, Gammill HS. Preeclampsia: recent insights. *Hypertension* 2005;46:1243-1249.

Ronnenberg AG, Wang X, Xing H, Chen C, Chen D, Guang W, Guang A, Wang L, Ryan L, Xu X. Low preconception body mass index is associated with birth outcome in a prospective cohort of Chinese women. *J Nutr* 2003 Nov;133(11):3449-55.

Santow G. Social roles and physical health: the case of female disadvantage in poor countries. *Soc Sci Med* 1995;40:147-161.

Schieve LA, Cogswell ME, Scanlon KS, Perry G, Ferre C, et al. Prepregnancy body mass index and pregnancy weight gain: associations with preterm delivery. *Obstet Gynecol*. 96:194-200.

Sentongo TA. Semaeo EJ, Stettler NS, et al. Vitamin D status in children, adolescents, and young adults with Crohn disease*. Am J Clin Nutr* 2002;76:1077-1081.

Sita-Lumsden A, Lapthonrn G, Swaminathan R, Milburn HJ. Reactivation of tuberculosis and vitamin D deficiency: the contribution of diet and exposure to sunlight. *Thorax* 2007;62:1003-1007.

Smith AH, Lingas EO, Rahman M. Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bull World Health Organization* 2000; 78(9):1093-1103.

Tangpricha V, Koutkia P, Rieke SM, et al. Fortification of orange juice with vitamin D: a novel approach to enhance vitamin D nutritional health. *Am J Clin Nutr* 2003;77:1478-1483.

Taparia S, Fleet JC, Peng JB, Wang XD, Wood RJ. 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D – mediated regulation of TRPV6 (a putative epithelial calcium channel) mRNA expression in Caco-2 cells. *Eur J Nutr* 2006;45:196-204.

Thatcher TD, Fischer PR, Strand MA, Pettifor JM. Nutritional rickets around the world: causes and future directions. *Ann Trop Paediatr* 2006;25:1-16.

Tchounwou PB, Patlolla AK, Centeno JA. Carcinogenic and Systemic Health Effects Associated with Arsenic Exposure – A Critical Review. *Toxicol Pathol* 2003;31:575-588.

Tondel M, Rahman M, Magnuson A, Chowdhury IR, Faruquee MH, Ahmad SA. The relationship of arsenic levels in drinking water and the prevalence rate of skin lesions in Bangladesh. *Environ Health Perspect* 1999;107(9):727-729.

United Nations Development Program (UNDP). 2006. *Human Development Report.* New York, New York, USA.

U.S. Department of Agriculture/Agricultural Research Service (USDA/ARS). 2005. USDA National Nutrient Database for Standard Reference, Release 18. USDA/ARS, Washington D.C.

van der Meer IM, Karamali NS, Boeke JP, et al. High prevalence of vitamin D deficiency in pregnant non-western women in The Hague, Netherlands. *Am J Clin Nutr* 2006;84:350-353.

van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol* 2005;97:93-101.

Vásárhelyi B, Cseh A, Kocsis I, Treszl A, Gy□rffy B, Rigó J Jr. Three mechanisms in the pathogenesis of pre-eclampsia suggested by over-represented transcription factor-binding sites detected with comparative promoter analysis. *Mol Human Repro* 2006;12(1):31-34.

Vieth R, Cole D, Hawker G, Trang H, Rubin L. Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it. *Eur J Clin Nutr* 2001;55:1091-1097.

Wang TJ, Pencina MJ, Booth SL, et al. Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008;117:503-511.

Watanabe C, Inaoka T, Matsui T, Ishigaki K, Murayama N, et al. Effects of arsenic on younger generation. *J Environ Sci Health* 2003;A38:129-139.

Walker JJ. Pre-eclampsia. *Lancet*. 2000;356:1260-1265.

Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitaminD3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab* 1988;67:373–378.

Welsh J. Vitamin D and breast cancer: insights from animal models. *Am J Clin Nutr* 2004;80:1721S-1724S.

Whelton PK. Epidemiology of hypertension. *Lancet* 1994;344:101-106.

World Health Organization (WHO). Global InfoBase. WHO. January 15, 2008. <http://www.who.int/infobase>.

WHO and FAO. Vitamin and mineral requirements in human nutrition, second edition. Geneva, Switzerland: World Health Organization, 2004.

The World Bank. Data Profile: Bangladesh The World Bank. June 9, 2008. <http://worldbank.org.bd>

Yetley EA, Brulé D, Cheney MC, et al. Dietary Reference Intakes for vitamin D: justification for a review of the 1997 values. *Am J Clin Nutr* 2009;89:719-727.