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AVAILABILITY AND INTAKE OF FOODS WITH NATURALLY OCCURING OR ADDED VITAMIN D IN A SETTING OF HIGH VITAMIN D DEFICIENCY

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ABSTRACT

Vitamin D deficiency is common during pregnancy in Bangladesh. We aimed to examine availability and intake of foods with naturally occurring or added vitamin D in pregnant women in an urban, low-income setting and analyze their estimated contributions to total vitamin D intake. We examined baseline data from an ongoing, 5-arm, randomized controlled trial of vitamin D supplementation enrolling pregnant women at 17 to 24 weeks gestation in in Dhaka, Bangladesh (n=561; "MDIG" Trial goal n=1300, ClinicalTrials.gov: NCT01924013). A focused, semi-quantitative food frequency questionnaire was used to estimate dietary intake of foods containing vitamin D and potentially fortified with vitamin D in the past month. Further, local food markets were visited to document the availability of vitamin D fortified foods. Median (IQR) fish intake was 3 (2, 3) times per week, with only 5% of women reporting no fish intake. Fresh milk was commonly consumed but powdered milk was not. Total vitamin D intake was 86 IU to 123 IU per week. Naturally occurring sources of vitamin D, specifically fish and eggs, contributed the majority of dietary vitamin D intake. In market analysis, the only locally available, packaged foods labeled as vitamin D fortified were powdered milk and ice cream. Fresh milk, juice, yogurt, breakfast cereals, and crackers were not vitamin D fortified. Powdered milk was widely available, and further, we identified 12 different powdered milk brands in the markets. All were vitamin D fortified, yet only 3 products indicated 100 IU or more per serving (approximate amount in one serving of milk in the US). Vitamin D intake in this population was extremely low. Promoting use of powdered milk and fortifying fresh milk should be explored as practical ways to improve vitamin D intake in pregnant women in Bangladesh.

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LIST OF ABBREVIATIONS

$1,25(OH)_2D$=1,25-dihydroxyvitamin D=Calcitriol

$24,25(OH)_2D$ =24,25-dihydroxyvitamin D

25(OH)D=25-hydroxyvitamin D =Calcidiol

AI=Adequate Intake

Cholecalciferol=Vitamin D_3

CHW=Community health worker

DBP=α-2 globulin vitamin D-binding protein

EAR=Estimated Average Requirement

Ergocalciferol=Vitamin D_2

FFQ=Food frequency questionnaire

FRA=Field research assistant

icddr,b=The International Centre for Diarrhoeal Disease Research, Bangladesh

IOM=Institute of Medicine

IQR=Interquartile range

IU=International Unit

MCHTI=Maternal and Child Health Training Institute

MDIG=Maternal Vitamin D for Infant Growth Trial

ORT=Oral rehydration therapy

PTH=Parathyroid hormone

RDA=Recommended Daily Allowance

RXR=Retinoid X receptor

SGA=Small for gestational age

sPTB=Spontaneous preterm birth

VDR=Vitamin D receptor

VDRE=Vitamin D response elements

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Chapter 1

Introduction and Literature Review

Purpose and Specific Aims

Vitamin D is a unique micronutrient in the category of essential vitamins because there are dual opportunities for contribution to status – from diet and from synthesis in the skin. Vitamin D is best known for its role in bone health and calcium homeostasis, yet deficiency during pregnancy is also associated with non-calcemic poor health outcomes for a mother and her offspring. The best measure of status is serum 25-hydroxyvitamin D [25(OH)D], and 25(OH)D concentrations in pregnant women are inversely associated with risk of adverse outcomes such as fetal growth restriction, preeclampsia, elevated blood glucose, bacterial vaginosis, and postpartum depression (1).

Vitamin D is produced in the skin after exposure to ultraviolet B (UVB) radiation, thus vitamin D deficiency is unexpected in tropical climates. Bangladesh is one such sunny region that experiences a high prevalence of vitamin D deficiency, particularly in women of reproductive age. Nearly 90% of the population is Muslim; traditional Islam clothing practices are common (2). It is estimated that over one-third of both veiled and non-veiled women in Bangladesh are vitamin D deficient (3). Additionally, a study conducted with pregnant women in Northern India found that 42.5% of participants were considered to have hypovitaminosis D, or serum 25(OH)D, concentration below 10 ng/mL (4).

The reasons for maternal vitamin D deficiency and contribution of diet to status are largely unknown in Bangladesh; the contribution that diet may play is under appreciated. The goal of this research was to examine the local availability and intake of foods with either naturally-occurring or added vitamin D in pregnant women in an urban, low income setting in Dhaka, Bangladesh. This work was conducted within an ongoing randomized controlled trial of vitamin D supplementation. We hope to shed light on locally accepted and available foods that may contribute to vitamin D status and prevent vitamin D deficiency during the critically important time of gestation. The results could contribute to future intervention efforts to improve the health of pregnant women in Bangladesh and similar regions.

Sources and Recommendations

Vitamin D can be synthesized in the skin or consumed in the diet. 7-dehydrocholesterol is a precursor that sits in the skin and is converted to pre-vitamin D in the presence of UVB rays at 290-320 nm (5). The amount of pre-vitamin D produced is dependent on the amount of 7-dehydrocholesterol in the skin and the amount of UVB that reaches the dermis. At the point when the dermis absorbs UVB rays, pre-vitamin D may be converted to vitamin D_3 for further activation, or may converted to any number of metabolites, which protects against vitamin D toxicity (5, 6). The necessary amount of time exposed to UVB to synthesize a given amount of vitamin D varies, depending on time of day and angle of the sun, skin pigmentation, and amount of skin exposed, among other factors. Asian populations seem to require approximately nineteen minutes of sun exposure at mid-day in springtime conditions to produce 1000 IU vitamin D (7).

In foods, vitamin D may be found either as vitamin D_2, ergocalciferol, or vitamin D_3, cholecalciferol. These compounds are molecularly the same, but are different in terms of structure and source. However, vitamin D, in either form, is not abundantly found in nature. Vitamin D_2 is naturally occurring in plants and fungi, such as wild or sun-dried mushrooms, or those enriched through UV light exposure (8, 9). Animal sources contribute vitamin D_3, which can be found naturally in egg yolks, cod liver and oil, and some fish, often in high levels (10). Lichens also produce vitamin D_3; although humans do not commonly eat these, they subsequently influence human dietary intake through the animal food chain. **Table 1**, below, describes some of the highest naturally occurring food sources for vitamin D in the human diet. Fortified sources of vitamin D exist, but availability varies depending on the country. For example, Canada requires the fortification of milk and margarine, such that a reasonable amount of liquid milk consumed over the day must contain between 300 and 400 IU of vitamin D (5, 8). Per serving, this ends up being about 100 IU per cup, which is comparable to the level found in milk in the US (11, 12). The United States also has regulatory measures to govern vitamin D fortification in the food industry. Vitamin D fortification is optional, although many types of foods are eligible. Still, milk is the only routinely vitamin D fortified product, added at 400 IU per quart of milk (5); many dairy or milk-derived products, such as yogurt, are often perceived to contain added vitamin D, but do not (8). Next to milk, ready-to-eat-cereals account for the highest source of fortified vitamin D in the US (8). Calcium containing foods, like milk or calcium fortified fruit juices, are targeted for vitamin D fortification, given their interdependent functions.

Table 1. Selected food sources of vitamin D[1]

Food	IUs per serving[2]	Percent DV[3]
Cod liver oil, 1 tablespoon	1,360	340
Swordfish, cooked 3 ounces	566	142
Salmon (sockeye), cooked, 3 ounces	447	112
Tuna fish, canned in water, drained, 3 ounces	154	39
Sardines, canned in oiled, drained, 2 sardines	46	12
Liver, beef, cooked, 3 ounces	42	11
Egg, 1 large (vitamin D is found in yolk)	41	10

[1]Modified from Vitamin D: Health Professional Fact Sheet (11)
[2]IUs=International Units
[3]DV=Daily Value. DVs were developed by the US Food and Drug Administration to help consumers compare the nutrient contents among products within the context of a total daily diet. The DV for vitamin D is currently set at 400 IU for adults and children age 4 and older. Food labels, however, are not required to list vitamin D content unless a food has been fortified with this nutrient. Foods providing 20% or more of the DV are considered to be high sources of a nutrient, but foods providing a low percentage of the DV also contribute to a healthful diet.

Dietary intake of vitamin D has not received much attention in Bangladesh due to the assumed high source of cutaneous production from the year-round UVB availability. The recently released Food Composition Table for Bangladesh identifies 20 key foods for the Bangladeshi diet [Table 4 (10)]. Of these, only the following are sources of any vitamin D: tilapia (raw without bones); chicken breast or leg (raw without skin); eggs; and milk. Tilapia contains only trace amounts of vitamin D; chicken contains 0.1 mcg of vitamin D; egg yolks contain anywhere between 1.8-5.4 mcg (up to 0.9 mcg per egg); and fluid milk contains only trace amounts, all for 100g of each given food. The retention rate of vitamin D when foods are cooked is excellent: 0.9 for fish and 0.95 for eggs (10). Although fish is a key food for the local diet, the FAO Bangladesh Nutrition Profile states that fish consumption is highly dependent on the season, such that it is consumed with much higher frequency during times of high availability and low prices, as is the case following monsoon season, than during other seasons (13). Of

note, the Food Composition Table for Bangladesh does not include packaged foods that may be vitamin D fortified, thus there is limited information on local fortified foods.

The Institute of Medicine (IOM) recently updated the Dietary Reference Intakes for vitamin D for each life cycle stage (5). For infants, the adequate intake (AI) is 400 IU per day (10 mcg). The recommended dietary allowance (RDA) for males and females aged 1 to 70 years, including women who are pregnant or lactating, is 600 IU per day (15 mcg) (5). For men and women older than 70 years of age, the RDA increases to 800 IU per day (20 mcg). Tolerable upper limits (UL) have been established for vitamin D across life cycle stages; they are the same between genders within a given stage. The ULs are set as follows: 1,000 IU (25 mcg) for infants 0 to 6 months; 1,500 IU (38 mcg) for infants 7 to 12 months; 2,500 IU (63 mcg) for children 1-3 years; 3,000 IU (75 mcg) for children 4-8 years; and 4,000 IU (100 mcg) for children and adults 9 years or older, including pregnant and lactating women. Though the IOM recognizes that vitamin D may be obtained through sun exposure, it also recognizes the public health concerns related to cancer and thus set these recommendations to assume minimal sun exposure. Vitamin D supplements have become popular in the US and abroad due to the increased attention given to the importance of vitamin D and the recognition of few food sources and tendencies for sun avoidance. Currently, vitamin D supplements are recommended for breastfed infants for the first six months of life, since breast milk typically has low amounts of vitamin D and sun and sunscreen avoidance is recommended for newborns.

However, the question of how sun exposure and diet each contributes to vitamin D status has garnered the attention of researchers. There is growing concern over the fact that the recommendations do not account for individual risk for deficiency. Altogether, dietary vitamin D, sun exposure, and skin reflectance contribute 55% of status and are considered significant

predictors (14). Sun exposure significantly varies between both modifiable and constant factors such as seasons, skin pigmentation (14), latitude, time of day (15), clothing, and sun screen use (5). Researchers found that dose of sun exposure was significantly less in students with South Asian ancestry than in any other studied ancestry group (14). These students also had significantly higher skin pigmentation (i.e. darker skin), which interferes with vitamin D synthesis (14-16). This combination of low sun exposure and darker skin puts the South Asian population, and those with similar dress and sun practices, at risk for deficiency (14, 17). Quite notably, the contribution of dietary vitamin D is largely unknown and understudied in these settings.

Metabolism

Vitamin D that is either consumed in the diet or synthesized in the skin is not yet biochemically active. Before the body can utilize it, it must be transported to the liver and then the kidney to undergo hydroxylation reactions completed by cytochrome P_{450} hydroxylases, which are oxidases dependent on NADPH (18, 19).

Vitamin D_3 synthesized in the skin is transported in the blood attached to α-2 globulin vitamin D-binding protein (DBP), which is produced in the liver (18). Because other metabolites of vitamin D synthesized at the skin do not have high affinity for this binding protein, they are often lost with dying skin cells (6, 18).

Dietary vitamin D needs similar assistance to reach the blood and be transported. A fat soluble molecule, dietary vitamin D requires the presence of lipid to be absorbed at the intestine because lipid stimulates the release of bile. Bile emulsifies lipid material and fat-soluble

vitamins, packaging them into micelles with hydrophilic exteriors that are capable of being passively absorbed at the enterocyte brush border (18). Approximately 50% of dietary vitamin D is absorbed, mostly at the duodenum with smaller amounts entering the gut at the distal small intestine (18). Once inside the cell, the vitamin is again repackaged into chylomicrons; these travel through the lymphatic system, reaching blood circulation at the thoracic duct. Once in the blood stream, they can be transported to the liver. This system accounts for about 40% of vitamin D_3 transport, with the remaining 60% traveling from the skin attached DBP (18). Although the liver is the primary site, protein bound vitamin D may be used by muscle and adipose tissue (18).

Upon arrival at the liver, vitamin D is hydroxylated. This first hydroxylation reaction occurs in the microsome and mitochondria, and acts on the C-25 position (6, 19). It is catalyzed by a 25-hydroxylase enzyme, and yields 25(OH)D, also known as calciferol (5). This hydroxylase is primarily expressed at the liver but has also been found in the kidney and intestine (19). The action of the 25-hydroxylase enzyme is dependent on the concentration of vitamin D and its metabolites, being more efficient in times of vitamin D deprivation (18). After enzymatic action is complete, most 25(OH)D is released to the blood, tightly attached to DBP (18).

Subsequently, 25(OH)D can then be taken up by the kidney, where it may undergo another hydroxylation reaction at either the C-1 or the C-24 position, depending on endocrine needs (6). The 1- α-hydroxylase acts on 25(OH)D to generate 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], also known as calcitriol (18). This is the physiologically functional form of the vitamin (5) and may be loosely bound to DBP for transport in the blood to exhibit action (18). The gene for this enzyme is present in extra-renal tissues, but expression is induced at much higher rates in the kidney than anywhere else (19). During pregnancy, the placenta can also produce this enzyme to create the biologically active form. 25(OH)D crosses the placenta from

mother to fetus, but 1,25$(OH)_2$D cannot and concentrations remain low in the fetus until birth (20). Notably, the 1- α-hydroxylase enzyme may also act on, and even prefer, 24,25-drihydroxyvitamin D to yield 1,24,25-trihydroxyvitamin D, but still synthesizes 1,25$(OH)_2$D at a higher rate due to its higher concentration (19). This enzyme is regulated by a variety of factors. For example, high parathyroid hormone (PTH), low plasma calcium, and low phosphorus consumption induce its activity, while inhibition occurs as a result of high dietary phosphorus consumption (18). Additionally, high concentrations of its product, 1,25$(OH)_2$D, function in a feedback loop to inhibit 1- α-hydroxylase action (6, 18).

Moreover, 1,25$(OH)_2$D stimulates action of the 24-hydroxylase and production of 24,25-dihydroxyvitamin D [24,25$(OH)_2$D] (6). This enzyme is present in nearly all tissue cells, but is most prominent in the kidney (19). Although 25(OH)D may be a substrate of this enzyme, it has preference for 1,25$(OH)_2$D (19). Still, 24,25$(OH)_2$D is formed when 25(OH)D status is adequate and when calcium is in homeostasis (18). The 24,25$(OH)_2$D form of vitamin D may also attach to DBP for transport to other tissues. During pregnancy, 25(OH)D concentrations remain fairly constant but 1,25 concentrations increase 2- to 3-fold (20).

Function

Calcitriol reaches target tissues and mediates its action through the vitamin D receptor (VDR) (21). Mediation of action may occur in a genomic fashion, by interacting with the nuclear VDR to influence genetic expression, or through a nongenomic mechanism in which the vitamin participates in an intracellular signal transduction pathway involving VDRs on the cell membrane (18). With regards to calcium and phosphorus homeostasis, cell membrane VDRs

mediate action at the bone and intestine and nuclear VDRs are found in the bone, intestine and kidney (18). Both nuclear and cell membrane receptors are found in cells of tissues other than those involved in the calcium and phosphorus regulation systems, including the placenta (5, 22-24).

Nongenomic action occurs rapidly due to an intracellular mechanism. The signal transductions are proposed to act through the phosphorylation or dephosphorylation of various enzymes and second messengers. These act to increased absorption of calcium at the intestine (transcaltachia); opening of gated calcium channels; and increased uptake of calcium by osteoblastic and skeletal muscle cells. (18)

Genomic reception, on the other hand, triggers conformational change of the ligand-receptor complex between $1,25(OH)_2D_3$ and VDR, which is phosphorylated, initiating a pathway influencing transcription of genes (22, 24, 25). This complex then forms a heterodimer with the retinoid X receptor (RXR) at the VDR; the VDR-RXR portion of the complex is physiologically functional in that it binds with high affinity to vitamin D response elements (VDREs), which are promoter gene sequences (25). The subsequent mechanisms to up-regulate transcription are not clear (25). On the other hand, calcitriol may down-regulate gene expression. These mechanisms, too, are not yet completely understood. In some cases, the VDR may bind to a down-regulated gene and interfere with the binding to up-regulators (24). In other circumstances, the VDR may bind to inhibitory VDREs that interact with repressor, rather than promoter, proteins to decrease gene transcription (22, 24).

Vitamin D, in its bioactive form $1,25(OH)_2D$, functions through these mechanisms for various purposes, depending on location. At the bone, intestine, and kidney, $1,25(OH)_2D$ is well

known for its role in regulating serum calcium and phosphorus levels, which in turn effect bone mineralization. Calcitriol fulfills these roles as a component of the endocrine system.

Its synthesis is triggered in response to hypocalcemia and increased in parathyroid hormone (PTH). Hypocalcemia first stimulates the parathyroid gland to secrete PTH. PTH targets the kidney and stimulates the conversion of 25(OH)D to $1,25(OH)_2D$. At the intestine, calcitriol functions through both genomic and nongenomic mechanisms. Calcitriol has high affinity for enterocyte receptors and is introduced in the nucleus. Here, it promotes the synthesis of mRNA that translates proteins involved in calcium transport. The brush border calcium-binding protein Calbindin D_{9k} is synthesized in response to calcitriol. A nongenomic response causes transcaltachia, which involves endocytosis of calcium, exocytosis at the basolateral membrane, release of calcium within the cytosol, and opening of voltage-gated calcium channels. At the same time, calcitriol also increases absorption of phosphorus by increasing the activity of the alkaline phosphatase enzyme at the brush border (18). Notably, calcium absorption is independent of vitamin D during pregnancy.

Still, the kidney does not only function in activing 25(OH)D to physiological form during hypocalcemia. Under this condition, calcitriol stimulates production of Calbindin D_{28k} to increase calcium and phosphorus reabsorption at the distal tubule. This action is thought to be mediated through genomic mechanisms (18).

At the kidney and intestine, calcitriol functions as a result of stimulation by PTH. At the bone, though, function is achieved through PTH and calcitriol, or PTH alone. Increased levels of these hormones stimulate resorption, or release, of calcium and phosphorus from osteoblasts. Calcitriol influences the differentiation of bone cells through the direction of osteoclasts. (18)

Until this point, only conditions of hypocalcemia have been discussed. However, calcitriol is active in bone mineralization, in addition to resorption. Calcitonin is secreted by the thyroid in response to higher-than-normal levels of calcium to trigger mineralization. Calcitriol and 24,25$(OH)_2$D may also participate in mineral deposition (18).

When calcitriol is elevated and serum calcium levels are high, a negative feedback loop decreases PTH, thus decreasing conversion of 25(OH)D and abating the actions of calcitriol. However, increased calcitriol, itself, regulates production. Through genomic action at the nuclear VDR, calcitriol decreases PTH synthesis (18).

However, vitamin D's functional activity stretches beyond the scope of bone health, as evidenced by the presence of vitamin D receptors in the nuclei of cells of various tissues and cell throughout the body (5). Evidence is growing to support the role of calcitriol in other functions, such as muscle strength (26), cell differentiation, immunity, and disease prevention (21). According to one review, target sites for calcitriol include islet cells of the pancreas, skins, ovaries, mammary tissue, macrophages and T lymphocytes, and the parathyroid gland (24).

1,25$(OH)_2$D is implicated in cell development and proliferation. For example, calcitriol is proposed to have a role in regulating growth of mammary glands as demonstrated in VDR knockout mice. One study showed that these knockout mice expressed increased glandular growth in response to exogenous progesterone and estrogen, as compared to controls (27). These and other findings on calcitriol's role in growth inhibition and differentiation may lend evidence towards its efficacy in breast cancer therapies (25). Another important system affected by calcitriol is immunity, both autoimmunity and infection. For example, when calcitriol was administered in mice induced with a model disease for multiple sclerosis, the disease was completely prevented; moreover, disease progression was inhibited when calcitriol was

administered with appearance of symptoms (28). Additionally, mice infected at the ear expressed impaired recovery response when vitamin D deficient (29). The immune response was improved with 8 weeks administration of vitamin D sufficient diet (29). The function of calcitriol extends to chronic conditions as well. Macrophages from vitamin D deficient, obese, diabetic, hypertensive patients were compared to various controls submerged in calcitriol rich or deficient media; all were exposed to low-density lipoproteins. In cells of diabetic patients, calcitriol suppressed foam cell formation by reducing acetylation or oxidation of LDL; foam cell formation was enhanced with VDR deletion in diabetic cells. Additionally, calcitriol regulated enzymatic action to reduce oxidized LDL uptake, lending evidence to its role in progression or prevention of cardiovascular disease in diabetics (30).

Evidence suggests that calcitriol influences reproductive health and maternal and infant outcomes. This line of research will be discussed in a later section.

Status and Deficiency

Circulating 25(OH)D is widely accepted as the best indicator of e vitamin D status. This intermediate metabolite of vitamin D is used because it has a half-life of about three weeks, which means that it can accurately represent concentrations accumulated from *both* diet and cutaneous production over a long course of time (26). 1,25(OH)D_2, on the other hand, has a half-life of between 4 and 6 hours, so although it is the form we are most interested in biologically, it is not a stable indicator of status (23).

Serum 25(OH)D levels have not been defined in terms of vitamin D toxicity due to a lack of well-controlled studies (5). However, the IOM sites research that concludes that vitamin D toxicity-induced hypercalcemia occurs at vitamin D intakes of 25,000 IU per day, which results in serum 25(OH)D concentration of approximately 500 nmol/L. Usually signs of toxicity are not visible until serum 25(OH)D is at least 500-600 nmol/L and frank toxicity is reported at 750 nmol/L. 50 nmol/L of circulating 25(OH)D is considered sufficient for 97% of the population; insufficiency is considered below this point. Circulating 25(OH)D below 30 nmol/L indicates risk for deficiency (5). Despite the IOM definition of sufficient 25(OH)D levels, some researchers and specialized organizations suggest that the desirable level should be higher. For example, the National Osteoporosis Foundation considers 25(OH)D insufficiency to be below 75 nmol/L (31). Another researcher argues for this to be the minimum desirable concentration, due to evolutionary development and epidemiological evidence, and assessment of IOM data regarding bone density and osteomalacia (32).

Consequences of Deficiency

Given the important roles of vitamin D, insufficient amounts can have serious consequences. Deficiency can cause nutritional rickets when it occurs in children and osteomalacia in cases of adults. Rickets can be caused by vitamin D, calcium, or phosphorus deficiency, and results in the inability of cartilage to ossify; thus bone cannot mineralize normally at the growth plate (5, 33). This excess of cartilage causes bone to be soft and malleable, resulting in characteristics such as widening of the long bone, rachitic rosary, and

deformations of the skeleton, such as bowed legs and knees (5). Signs and symptoms of rickets are initially expressed due to deossified bone, but may progress to secondary hyperparathyroidism, hypocalcemia, and hypophosphatemia (5). The parathyroid gland becomes overactive, causing activity of osteoblasts, in a compensatory attempt to prevent hypocalcemia (5). Hypocalcemia and hypophosphatemia also occur because a lack of calcitriol means that bone is not signaled to mobilize these minerals to increase plasma concentrations. At progressive stages of rickets, hypocalcemia may cause tetany, and poorly ossified bone may cause stunting and bowing (5). Nutritional rickets may be treated with vitamin D therapy if discovered in the early stages; however, it cannot be reversed once progressive stages or puberty have been reached (5).

Like rickets, osteomalacia occurs when bone fails to mineralize new matrix materials, resulting in a softened and weak skeleton (5). Similarly, osteomalacia may cause secondary hyperparathyroidism and increased levels of parathyroid hormone (5). Unlike rickets, though, plasma calcium and phosphate levels are normal; because of physiological priority to maintain homeostasis, they may be mobilized from bone in an effort to maintain circulating levels. Because of high osteoblastic activity, osteomalacia is associated with increased markers of bone turnover (5). Symptoms include muscle weakness, and bone pain and fractures, yet, a bone biopsy is the only way to diagnose osteomalacia (5).

Although biomarkers are not used diagnostically, serum 25(OH)D concentrations lower than 20 nmol/L are associated with nutritional rickets and osteomalacia (33). A review found that bone mineral density increases as 25(OH)D rises from 22.5 nmol/L to 94 nmol/L (26).

Importance to Public Health

Since the discovery of vitamin D's role in preventing nutritional rickets, the deficiency disease has re-emerged (33, 34). While children are primarily effected by rickets, elderly people are prone to falls and bone fractures. Every 25 nmol/L reduction in serum 25(OH)D was associated with an odds ratio of 1.33 for hip fracture risk in a nested case-control study (35). A meta-analysis of randomized control trials found that vitamin D supplementation of 700-800 IU/day reduced the risk of hip fracture by 26% in ambulatory and institutionalized elderly people (36).

Considering the widespread presence of VDRs in the body, discussed previously, vitamin D is important for disease states outside of the skeletal system and these have vast public health implications. For example, type 2 diabetes mellitus is prevalent and is characterized by an inability to use circulating glucose, resulting in hyperglycemia. Novel evidence shows that vitamin D supplementation may enhance glucose utilization by increasing expression and translocation of GLUT4 receptors at adipocytes treated with high glucose (37). A vitamin D supplementation trial in type 2 diabetic patients found that, compared to the control, the supplemented group had significantly lower fasting plasma glucose and insulin levels, and glycated hemoglobin at the end of the intervention period (38). Zhou et al. also found other metabolic benefits in the supplemented group, such as significantly reduced body mass index and waist circumference (38).

Vitamin D has also been shown to benefit patients of chronic kidney disease. A systematic review and meta-analysis concluded that mortality in chronic kidney disease patients decreases with vitamin D supplementation or analogue (39). Cardiovascular mortality is also

associated with vitamin D status. A cross-sectional study observed that subjects in the lowest two quartiles for 25(OH)D status had cardiovascular mortality hazard ratios of 2.22 and 1.82, respectively (40). A similar relationship was observed with all-cause mortality (40).

Concern over vitamin D is not limited to the effects of the vitamin on disease states; there is also concern for the reverse relationship. In one study, obese and lean subjects were exposed to whole body irradiation; 24 hours post-exposure, obese participants experienced a 57% lower increase in circulating 25(OH)D than did lean subjects (41). Worstman and colleagues attribute this reduced increase in status to deposition in body fat (41).

Reduced 25(OH)D status has gained attention, not only because of its effects, but also because of its prevalence. Vitamin D insufficiency is well documented in northern latitudes. For example, NHANES III data from the United States showed that 42.4% of African American women of reproductive age had hypovitaminosis D [25(OH)D $\leq$37.5 nmol/L]; the prevalence was 4.2% among white women or reproductive age (42).

Still, vitamin D deficiency is not restricted by geography, as it is a concern in Bangladesh. Among infants between 1 and 6 months in rural Bangladesh, vitamin D deficiency had a prevalence rate of just about one third, as determined by 25(OH)D concentration < 25 nmol/L (43). Moreover, premenopausal women, aged 16 to 40 years, were evaluated for vitamin D across two socioeconomic groups in rural and urban Bangladesh. Seventeen percent of women in the lower socioeconomic group and twelve in the higher has 25(OH)D below 25 nmol/L; 50% of lower socioeconomic participants and 38% of higher socioeconomic participants had 25(OH)D concentration at or below 37.5 nmol/L and were considered to have hypovitaminosis D (44). Lactating women in both groups were at even higher risk for hypovitaminosis (44).

Relation to Maternal and Infant Health Outcomes

Although serum 25(OH)D remains largely unchanged during pregnancy, without the presence of confounding behaviors and environments, it is nonetheless important (1). A review conducted by Christesen et al. reports that for every 50 nmol/L decrease in 25(OH)D at 16 weeks gestation, the odds of preeclampsia more than doubles; pregnant women who have preeclampsia had a 15% lower 25(OH)D concentration than those without the condition (1). A case control study at mid-gestation found that women whose 25(OH)D was lower than 50 nmol/L were nearly 4 times as likely to experience preeclampsia than women with a concentration of greater than 75 nmol/L (45). However, a Cochrane review of vitamin D supplementation on pregnancy reported only one trial relating to preeclampsia, and it found that women who received 1200 IU of vitamin D plus 375 mg of calcium per day has a preeclampsia risk ratio of 0.67 (95% confidence interval 0.33 to 1.35) (46).

Among mothers in the US, the association between spontaneous preterm birth (sPTB) and vitamin D was dependent on race in a large, multi-site study. An inverse relationship was found between 25(OH)D collected at ≤26 weeks gestation and sPTB at 35 weeks, but only among nonwhite mothers (47). Serum levels between 30 and 75 nmol/L were associated with a 1-1.6 reduction in sPTB per 100 live births; among nonwhite mothers, there was a 20-30% reduction in sPTB risk at 25(OH)D levels above 30 nmol/L. No associations were found in white mothers (47). Bodnar et al. also examined histological data for placenta samples. Reduction of inflammation-associated sPTB was observed in nonwhite women with 25(OH)D levels above 30 nmol/L. These findings support other evidence for the role of vitamin D in the immune response, such that vitamin D deficiency impairs the function of toll-like receptors, thus increasing risk for

infection, an established determinant of sPTB (47). The infection and inflammation response may predispose preeclampsia, placental insufficiency, and preterm birth (20). Moreover, a strong association was found for sPTB without placental pathology. This possibly implicates an interaction between vitamin D and other pathways in preterm birth; the authors suggest examination of the myometrium response to labor stimuli in relation to vitamin D (47).

Using data from the same project, the Collaborative Perinatal Project, Gernand et al. examined the newborn and placenta weight, in addition to infant head circumference, for association with 25(OH)D level. Small for gestational age (SGA) was also tested for association using multivariate logistic regression because of its ability to indicate pathological or developmental growth (48). Birth weight and head circumference were found to be positively associated with serum 25(OH)D; no association was found with ponderal index (a ratio of birth weight to length), placenta weight, or placenta to fetal weight ratio (48). This is consistent with findings from three trials in a Cochrane review that suggested women who received vitamin D supplementation during pregnancy less frequently bore babies weighing less than 2500 grams than did controls (46).

During the first trimester, maternal 25(OH)D has also been negatively associated with risk for SGA (48). Further, Eckhardt et al. observed the persistence of these effects. Infants of mothers with 25(OH)D concentration above 30 nmol/L had higher length for age and head circumference throughout the first year of life (49). Shin et al. report that the placenta produces large amounts of vitamin D through the action of 1- α -hydroxylase, and that this increase may allow higher maternal gut absorption of calcium to meet fetal skeletal needs (50). However, Gernand et al. suggest that the positive association with fetal growth outcomes may be due to

vitamin D and its receptors' ability to regulate pregnancy hormones, thus effecting glucose and fatty acid transport and anabolic metabolism (48).

Findings from a third trimester supplementation in Bangladesh support these effects on infant length (51). Unlike the findings from the observational study conducted by Gernand et al., the trial observed similar length for age z-scores at birth between supplemented and control groups; on average, though, length for age z-score increased more in infants of supplemented mothers by one month (51). Although divergence did not continue after, the early postnatal growth is important because it represents a near halving of stunting prevalence (51).

Chapter 2

Methods

This thesis was a sub-study of the Maternal Vitamin D for Infant Growth (MDIG) Trial, a 5-arm, placebo controlled trial of vitamin D supplementation during pregnancy and lactation. Offspring are followed to 2 years of age. Data was collected at the Maternal and Child Health and Training Institute (MCHTI), also known as the Azimpur Maternity Clinic. This is a public facility in Dhaka, the capital city of Bangladesh; most of the patients to this clinic are from nearby, low-income areas. MDIG received ethical approval from ethical review boards at the Hospital for Sick Children in Toronto, Canada and The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) in Dhaka, Bangladesh. The study is funded by The Bill and Melinda Gates Foundation.

The MDIG trial has two primary aims: to determine the effect of maternal prenatal vitamin D_3 supplementation versus placebo on infant length at 1 year; and to determine the effect of postpartum maternal vitamin D_3 supplementation versus placebo on infant length at 1 year. Other trial objectives include examining inflammatory and hormonal determinants of infant growth, epigenetic interactions with vitamin D metabolism, and diarrheal and respiratory morbidity in infants.

During pregnancy, vitamin D_3 supplemented subjects are given 4,200 IU, 16,800 IU, or 28,000 IU, as a weekly dose. Post-partum, the vitamin D_3 dose was 28,000 IU weekly and it was only administered to half the women given 28,000 IU/week prenatally. All dosing assignments were random and occurred at the time of enrollment. This intervention continues for 6 months

post-partum. MDIG will follow infant growth for 2 years to document the persistence of any observed effects at 1 year of age.

The MDIG trial is ongoing and intends to enroll 1,300 pregnant women in the study. This thesis reflects data for 561 women. Our sample size is limited due to the time constraints on this project and the need to use data before the enrollment period is complete. Eligibility requirements are 18 years of age and above, gestational age of 17 to 24 completed weeks (as determined by last menstrual period and/or ultrasound), and intended permanent residency within the determined geographical area for at least 18 months. Women may not participate if they have a medical history of conditions that may make them prone to vitamin D sensitivity or altered metabolism, hypercalcemia, or renal calculi. High-risk pregnancies, including those with severe anemia, moderate-severe proteinuria, and hypertension, are excluded from the trial. If maternal history and/or ultrasound reveals multiple gestation, major congenital abnormalities, or severe oligohyrdamnios, women are ineligible. Exclusion criteria also include current physician-prescribed vitamin D supplementation for deficiency, or a woman's unwillingness to stop taking non-study administered supplements containing vitamin D or calcium.

Eligibility is initially determined through screening conducted by a community health worker on all women waiting in the lobby of MCHTI. If eligible, women are enrolled and baseline data is collected regarding anthropometry, diet (by food frequency questionnaire), demographic characteristics, and medical history. A blood draw occurs prior to the beginning supplementation.

Data were collected from all participants at baseline. The trial used a food frequency questionnaire (FFQ) to capture intake of calcium, phosphorus, phytate, and vitamin D. Forty foods in the Bangladeshi diet were identified for inclusion in terms of frequency of consumption

in the past month; portion size was not asked. We analyzed consumption of all 40 foods, and created new variables in terms of intake scenarios per week. For the current project, we created a supplementary questionnaire to the FFQ specifically to capture information regarding potentially fortified vitamin D foods. The foods asked were identified as being available in fortified forms during a market survey. We asked about frequency of consumption of these foods, ways these products were used or consumed, brand names purchased, and package labels or pictures that many influence their purchases. These data were collected for a subset of 51 women. This form can be found in **Appendix A**. A reference page for use during the supplement to the FFQ was created with pictures of product brands we identified on the market to prompt women's responses. This can be found in **Appendix B**.

Fieldwork

As part of the research, there was a one-week field visit to Dhaka. The purposes for this visit were dual: 1) to observe and learn about logistics and quality control measures of a research study, and 2) to visit local markets for a market analysis of fortified vitamin D items. This in-person visit framed participant food habits and firmly established the context of the trial for the thesis author. Of particular importance was an informal focus group conducted with study staff, including community health workers (CHWs), field research assistants (FRAs), and one research physician. This discussion included information on perceptions and purchasing practices of vitamin D foods. See **Appendix C** for more detail on the questions and findings of this focus

group. Also during the fieldwork was the pilot administration of the supplementary FFQ form on 2 participants. These data were not included in the final analysis.

Appendix D contains additional information regarding other trial observations made during the fieldwork.

Market Analysis

To evaluate the cost and availability of vitamin D fortified products, we visited markets within and around the neighborhoods (called "catchment areas") of the trial. Research physicians and a data manager accompanied us to facilitate translation from Bangla and purchasing of products at the dukahs, or small market stalls. At each, we asked to see all of the products that we predetermined might be fortified with vitamin D, including dry and malted milk, biscuits, hot and cold cereals, yogurt, and juice. We documented information about price, package weight, and vitamin D content for each product, both in writing and in photographs of each product. Photographs from the market visit may be seen in Figure 1.

Samples were taken of 12 dry milk brands, including malted milk, with the intention to explore a vitamin D extraction method using high performance liquid chromatography to measure actual vitamin D concentration. Due to time constraints and lack of collaborative support, this path was not realized.

Figure 1. Milk products available in the markets

Statistical Analysis

Data were analyzed using Stata 13 (StataCorp, College Station, Texas). The sample of participants was characterized by analyzing the socio-demographic survey administered at enrollment for mean values. Variable were categorized into groups, as needed, and treated as discrete. For example, age of participant is a continuous variable but was analyzed as under 21 years, 21 to 25 years, or older than 25 years.

Intake of vitamin D foods, either naturally occurring or fortified, was analyzed using data from the food frequency questionnaire (n=561) and the supplemental form (n=51). Items were recorded in terms of instances of consumption per month, week, and day, and we recoded each item to describe instances of consumption in terms of times per week. We calculated frequency of consumption of each food and median and interquartile range (IQR). Within the subsample of

women asked additionally about vitamin D fortified foods, we analyzed the frequency of responses to methods of using items and to specifications about products.

For select items (or groups of items) from the FFQ and FFQ supplement (fresh milk; powdered and condensed milk; yogurt; ice cream; cheese; eggs; poultry; beef/mutton/pork; organ meats; fish, small, medium, large and dried; milk, dry; breakfast cereals, hot; and breakfast cereals, cold), we estimated a plausible range of vitamin D that women are consuming from these foods. This considered portion size, vitamin D concentration in micrograms (as determined using the Food Composition Table for Bangladesh) (10), and frequency of consumption. Given that the FFQ did not inquire about portion size and amount consumed each time is not uniform across the sample, we used a low and high portion size for each food to represent the low and high ends of usual intake. These portion sizes were identified using best available knowledge among the investigative team (in particular, from Dr. Aditi Roy). Thus, for each food item, two variables were created: one to estimate the amount of vitamin D consumed from a smaller portion size and one from a larger portion size. The low end was calculated by multiplying consumption per week by concentration of vitamin D (in micrograms) per gram of item by estimated low intake of item in grams. This was repeated for the high estimate of intake in grams; the calculations were applied to each of the 13 foods. For each participant, this yielded an estimation of the range of vitamin D consumed from each food over one week. The low end of each food was totaled, as was the high, to give an estimate of the range of the total vitamin D consumed over one week from all sources. The range of vitamin D for each item and the total range were subsequently converted to IU. We then analyzed the median (IQR) range of vitamin D consumed from each food by running median (IQR) analysis on the low and high ends of each

food; we repeated this analysis for the range of total vitamin D consumption. Finally, we calculated contribution in percent of each food to total vitamin D for each end of the range.

A cost analysis of 13 vitamin D fortified milk packages (of 12 different brands) was conducted using information collected during the fieldwork visit. One serving was considered to be 25g of powder, unmixed. For each package, cost per serving was calculated in US dollars using package weight and price; a conversion rate of 77.3 Bangladeshi Taka to 1 USD was used. Vitamin D content per serving was calculated from vitamin D concentration per 100 g powder identified on nutrition labels. Given that a usual glass of milk in the US contains 100 IU of vitamin D, we calculated the cost of using enough milk powder to reach 100 IU of vitamin D. We accomplished this by dividing 100 IU by the amount of vitamin D per serving, to indicate the number of 25g servings needed. This was then multiplied by the cost per serving.

My role in this thesis process was to conduct the statistical analysis described and to create the supplemental form to the FFQ. More information on the context collected to create this form can be found in **Appendix C**. Additionally, I was involved in the collaborative process of forming the research objective and in collecting market information from the field.

Chapter 3

Results

Characteristics can be found in **Table 2**. Over one third of the sampled women were under 21 years of age. The median age was 22.5 (20.0, 26.0). The majority of participants were educated in school, with a small minority educated in madrasha (a religious education). One quarter of participants were educated to the point of receiving the equivalent of a high school degree or higher. Almost all women keep their head covered when in public; yet, the majority of women expose their face. Most women reported usually covering their arms and spending limited time outdoors or exposed to the sun. Most women remained covered during the interview, with the exceptions of the face and back of hands, as observed by the CHWs. Over one third of women are primigravida, but of women who have been pregnant before, over 40% have experienced one or more spontaneous abortions or miscarriages. Still, most participants who have previously been pregnant have at least one living child; nearly 95% of living children are between the ages of 1 and 3 years.

Table 2. Sample Characteristics (n=561)

Category	Frequency	Percent
Current Age (years)		
Under 21	191	34.1
21-25	220	39.3
Over 25	149	26.6
Education (type)		
School	487	86.8
Madrasha	40	7.1
School & Madrasha	6	1.1
Neither	28	5.0
Highest Class Completed		
Grades 1-5	151	30.6
Grades 6-9	219	44.5
High school equivalent or higher	123	25.0
Outdoor/Public Habits		
Top of head		
Usually covered	531	94.7
Usually uncovered	30	5.3
Unknown	0	0
Face (except eyes)		
Usually covered	205	36.5
Usually uncovered	356	63.5
Unknown	0	0
Arms		
Usually covered	536	95.5
Usually uncovered	25	4.5
Unknown	0	0
Burqa		
No	73	13.0
Yes	488	87.0
Hours spent outside per day		
0	5	0.9
1	495	88.6
2 or more	59	10.55
During daytime job		
Usually covered	559	99.6
Usually uncovered	2	0.4
Sunscreen use		
Always	21	3.8
Most of the times	2	0.3
Sometimes	10	1.8
Rarely/never	527	94.1
Clothing as observed by CHW during interview		
Top/back of head		

Not covered	173	30.8
Covered	388	69.2
Face (entire)		
Not covered	560	99.8
Covered	1	0.2
Abdomen		
Not covered	7	1.3
Covered	554	98.7
Back of hands		
Both covered	69	12.3
One covered	1	0.2
None covered	491	87.5
Arms below elbow		
Both covered	369	65.8
One covered	4	0.7
None covered	188	33.5
Arms above elbow		
Both covered	513	91.4
One covered	5	0.9
None covered	43	7.7
Legs below knee		
Both covered	550	98.0
One covered	0	0
None covered	11	2.0
Top of feet		
Both covered	29	5.2
One covered	2	0.3
None covered	530	64.7
Total pregnancies		
1	208	37.1
2	178	31.7
3	124	22.1
4	35	6.2
5	10	1.8
6 or more	6	1.1
Total abortions/miscarriages		
0	202	57.6
1	127	36.2
2	17	4.9
3	3	0.9
4	2	0.6
Total live births		
0	45	12.8
1	217	61.5
2	74	21.0

3	12	3.4
4+	5	1.4
Total # living children		
0	4	1.3
1	224	72.7
2	68	22.1
3	10	3.3
4 or more	2	0.6
Age of youngest child, years		
<1	4	1.3
1 to <3	292	94.8
3 to <6	12	3.9

With regards to food consumption, fresh milk was consumed at least once a week by 50% of participants **(Table 3)**. Eighteen percent of women consumed powdered milk at least once a week, but nearly 75% reported never consuming it. Fifty percent ate eggs between 1 and 6 times per week; over half ate poultry with the same frequency. The median fish intake was 3 (2,3) times per week; less than 5% of women reported never consuming fish. Forty-five percent of participants ate organ meats occasionally. Results for all 40 foods in the main FFQ are in **Appendix E**.

Table 3. Frequency of food intake for pregnant women over the past month for foods with for foods with naturally occurring vitamin D, Dhaka, Bangladesh, 2014-2015 (n=561)

Food	Never	Less than once per week	Once per week	2-6 times per week	Once per day	More than once per day
			n (%)			
Milk, fresh[1]	127 (22.6)	156 (27.8)	26 (4.6)	145 (25.9)	105 (18.7)	2 (0.4)
Milk, powdered or condensed[1]	416 (74.2)	41 (7.3)	12 (2.1)	43 (7.7)	39 (7.0)	10 (1.8)
Yogurt[1]	314 (56.0)	210 (37.4)	21 (3.7)	14 (2.5)	2 (0.4)	0 (0)
Ice cream[1]	212 (37.8)	233 (41.5)	46 (8.2)	63 (11.2)	6 (1.1)	1 (0.2)
Cheese1	522 (93.1)	35 (6.2)	2 (0.4)	2 (0.4)	0 (0)	0 (0)
Egg	51 (9.1)	95 (17.0)	52 (9.3)	229 (40.8)	131 (23.4)	3 (0.5)
Poultry	76 (13.6)	176 (31.8)	129 (23.0)	176 (31.4)	3 (0.5)	1 (0.2)
Beef/Mutton/Pork	92 (16.4)	202 (36.0)	108 (19.3)	150 (26.7)	9 (1.6)	0 (0)
Organ Meats	309 (55.1)	198 (35.3)	27 (4.8)	21 (3.7)	6 (1.1)	0 (0)
Fish (fresh or dried)	23 (4.1)	74 (13.2)	105 (18.7)	239 (42.6)	54 (9.6)	66 (11.8)

[1] Trace amount of vitamin D naturally occurring per the Food Composition Table for Bangladesh, 1st Edition, University of Dhaka, June 2013.

Food frequency results for the supplement to the FFQ are found in **Table 4**. Regular use of powdered milk was not reported in our sample. Additionally, breakfast cereals (either hot or cold) were reported to never be consumed by every participants surveyed; in fact, one participant commented on the unfamiliarity with cereals. Of women consuming powdered milk (n=18), most participants reported using it to prepare tea or coffee (n=15, 83%) but 11 reported mixing dry milk with water for drinking purposes (61%). Only 2 women (11%) reported cooking it with rice or porridge or other grains, and no one used dry milk in their preparation of vegetables. When asked if they buy milk products specifically labeled for pregnant or lactating women, only 2 responded yes (11%), and no one purchased milk products marketed specifically towards infants or children. Dano was reported to be the most frequently purchased dry milk brand, with 5 respondents. Diploma, Fresh, and Mark's each had 2 responders, and Arong, Mother's Horlicks,

Red Cow, and Nido were reported once each. Three participants stated that they bought a dry milk powder brand other than those pictured on the reference sheet.

Table 4. Frequency of food intake for pregnant women over the past month for foods potentially fortified with vitamin D, Dhaka, Bangladesh, 2014 (n=51)

Food	Never	Less than once per week	Once per week	2-6 times per week	Once per day	More than once per day
	n (%)					
Milk, dry[1]	33 (64.7)	7 (13.7)	0 (0)	0 (0)	9 (17.6)	2 (3.9)
Hot Cereal[2]	51 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cold Cereal[2]	51 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[1] Includes malted milk products.
[2] One participant additionally commented "cereals not familiar in this society".

The estimated contributions to total vitamin D intake for each food containing vitamin D can be found in **Table 5**. Large and medium fish, together, contributed over 50% of dietary vitamin D intake by percent, regardless of the portion size (**Table 5**). On the low end of the estimation, eggs contributed nearly 14%; considering a larger serving, they contributed almost 18%. Small fish contributed around 8% of vitamin D intake for both lower and higher estimations. The median estimated intake from liquid milk was 0.114 IU and 0.285 IU from low and high estimates, respective, while the estimated median for that of powdered milk was 0.0 IU for both. The estimated range of total vitamin D intake was between 86.1 and 123.1 IU per week (which is 12 to 18 IU per day).

Table 5. Estimated contribution of foods to vitamin D intake[1]

	Small serving sizes		Large serving sizes	
Food	Estimate of vitamin D intake (IU) per week[2]	% of total vitamin D intake	Estimate of vitamin D intake (IU) per week[2]	% of total vitamin D intake
Milk, liquid	0.114 (0.043, 0.952)	1.08	0.285 (0.108, 2.38)	1.58
Milk, powdered or condensed	<0.001 (0.000, 0.016)	0.10	<0.001 (0.000, 0.033)	0.14
Yogurt	<0.001 (0.000, 0.046)	0.08	<0.001 (0.000, 0.092)	0.09
Ice Cream	0.025 (0.00, 0.066)	0.35	0.050 (0.000, 0.133)	0.41
Cheese	<0.001 (0.000, 0.000)	0.31	<0.001 (0.000, 0.000)	0.40
Eggs	7.89 (1.73, 14.5)	13.9	23.7 (5.20, 43.4)	17.6
Poultry	0.648 (0.428, 1.94)	1.89	0.97 (0.64, 2.92)	1.85
Beef/Mutton/Pork	1.17 (1.17, 5.31)	4.73	1.75 (1.75, 7.96)	4.63
Organ Meats	<0.001 (0.000, 5.13)	3.93	0.0 (0.000, 8.55)	4.35
Small Fish	3.77 (0.000, 5.71)	7.74	6.03 (0.000, 9.14)	8.31
Medium Fish	13.4 (0.000, 53.4)	26.2	17.7 (0.000, 70.7)	26.1
Large Fish	23.0 (8.72, 104.7)	37.5	25.7 (9.74, 116.9)	32.0
Dried Fish	0.336 (0.000, 0.89)	1.55	1.01 (0.000, 2.66)	2.71
Total	86.1 (40.5, 170.6)		123.1 (58.2, 221.1)	

[1] Vitamin D intake was calculated for each food based on an estimated smaller portion size for and a larger portion size because the food frequency questionnaire did not ask women to specify the portion size. Each total is based on all foods with the "smaller" or "larger" portion sizes to estimate a range of intake (rather than a single estimate).
[2] Data are presented as median (interquartile range).

In surveying the market, the only observed locally available, packaged foods labeled as containing vitamin D were powdered milk and ice cream. Liquid milk, juice, yogurt, breakfast cereals, and crackers were not fortified. Powdered milk was widely available; we identified 12 different fortified powdered milk product brands (**Table 6**), of which only 3 products indicated 100 IU or more per serving (the approximate amount in one serving of liquid milk in the US). Over fifty percent of the products we observed contained between 25 and 50 IU per 25g serving. One product indicated on the nutrition label that it was vitamin D enriched but did not specify the amount. **Figure 5** represents powdered milk products by cost and vitamin D content per serving. Each dot on the scatter plot represents one brand. The color of the circle darkens as

package weight increases. Overall, cost was not higher for higher amounts of vitamin D added, but was generally higher for smaller package weight.

Table 6. Vitamin D content and cost of powdered milk products in Dhaka, Bangladesh, 2014

Milk Brand Name	Package Weight	Cost per 25 g serving[1,2]	Vitamin D content per 25 g serving	Cost per 100 IU vitamin D[1]
Horlicks[3]	200 g	0.33 USD	46 IU	0.70 USD
Complan Growth[3]	200 g	0.49 USD	38 IU	1.30 USD
Fresh	250 g	0.21 USD	200 IU	0.10 USD
NIDO Fortified[3]	350 g	0.28 USD	58 IU	0.48 USD
Mark's	400 g	0.26 USD	"enriched"	-
Dano	400 g	0.24 USD	100 IU	0.25 USD
Anchor	400 g	0.23 USD	38 IU	0.61 USD
Junior Horlicks[3]	400 g	0.32 USD	42 IU	0.75 USD
Shape Up Women's Horlicks[3]	400 g	0.28 USD	100 IU	0.29 USD
Horlicks[3]	450 g	0.40 USD	46 IU	0.86 USD
Diploma	500 g	0.22 USD	50 IU	0.44 USD
Diploma	1000 g	0.22 USD	50 IU	0.44 USD

[1] Conversion rate: 77.3 Taka (currency of Bangladesh) = 1 USD (US Dollar).
[2] For comparison, fresh liquid milk (515 g package) was 16 Taka per serving and was not fortified with vitamin D.
[3] Malted milk products.

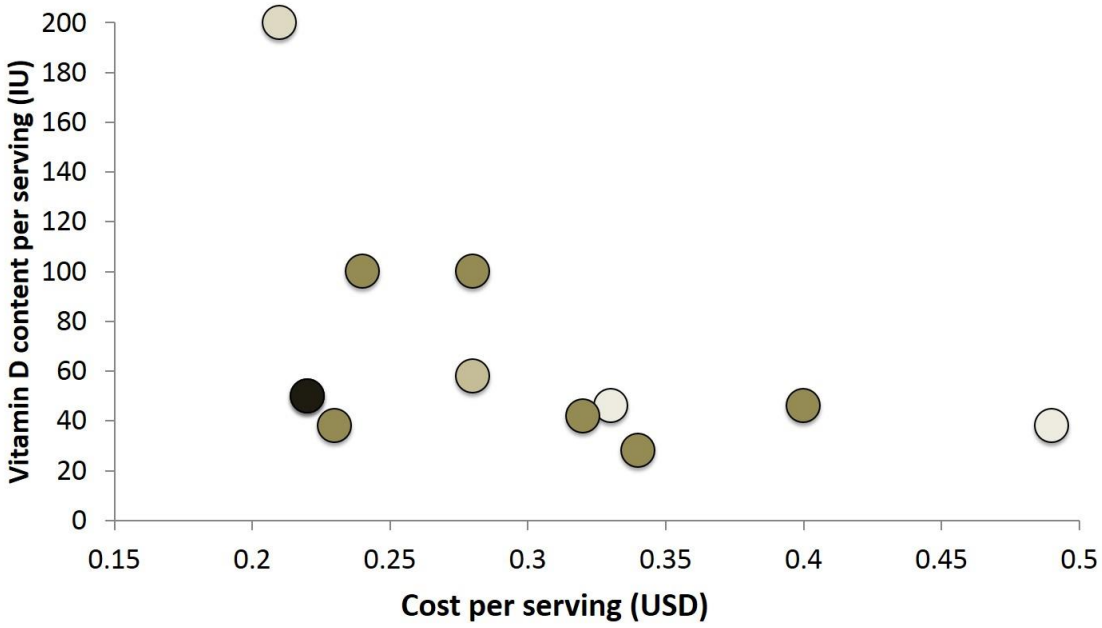


Figure 2. Cost and vitamin D content per serving of powdered milk products

Chapter 4

Discussion

Interpretation of Findings

One aim of this study was to identify dietary sources of vitamin D in the Bangladeshi diet. We found that fresh milk was commonly consumed and widely available; however, it is not high in naturally occurring vitamin D, nor is it fortified in this setting. As such, it did not contribute highly to the vitamin D consumption of our population. Higher amounts of vitamin D in liquid milk would increase its contribution to intake. In contrast, an increase in frequency, rather than in vitamin D content, would increase the contribution of powdered milk, given that it is widely available and vitamin D fortified. Naturally occurring sources of vitamin D were the largest contributors. Fish and eggs together contributed the majority of vitamin D intake for both low and high estimates due to frequent consumption and relatively high vitamin D content.

The median total vitamin D intake over a week was 86 and 123 IU per week for low and high estimates, respectively. In the United States, the estimated average requirement (EAR), or intake estimated to meet the needs of half of healthy individuals in a population, is set at 400 IU *per day* (5). Using this as a reference point, the current intake of the study population is highly insufficient. NHANES data from 2005-2006 showed that the mean dietary vitamin D intake among women 19 to 30 years of age in the US was 144 IU per day (52). When supplement use was accounted for, the mean intake in US women of the same age group was 232 IU; 25% in this age group used a vitamin D containing supplement (53). In the US population, the largest food group to contribute to vitamin D intake is dairy, and calcium-vitamin D-fortified juices provide

high amounts of vitamin D for dairy-avoiders (53). It is clear from the current study that dietary vitamin D intake is very low, and much lower than in the US.

From our market analysis, we found a wide variety of types of vitamin D fortified milk products, including those intended for pregnant or lactating women, and those marketed for use by children. Additionally, we found that the cost per serving and vitamin D content per serving varied across a wide range. However, cost did not necessarily increase with vitamin D content. We informally observed that larger package size did reduce cost per serving. During our market visits, we observed availability of a variety of breakfast cereals. Despite our speculations, cereals were not fortified with vitamin D (although they were highly fortified with other vitamins and minerals) and no one reported eating them. Similarly, biscuits and yogurt, were not fortified, but also not consumed with high frequency and may not be considered as targets for fortification. Juice was available but not identified as a fortified source of vitamin D either. In contrast, fresh milk, ready-to-eat breakfast cereals, and calcium-fortified juices are all targets of vitamin D fortification in the US (8).

Future Directions for Public Health

Public health efforts, including campaigns, programs, and policies may facilitate the improvement of dietary vitamin D intake of the study population. Given the absence of breakfast cereals in the diet, fortifying them would not be a realistic target in an attempt to increase the vitamin D intake of low-income pregnant women in this setting. We identified that fresh, liquid milk was relatively commonly consumed. Given this general acceptance, fortification could be explored as a practical way to improve vitamin D intake in pregnant women. Obstacles could

include non-acceptance by some groups, lactose intolerance, and added expense of liquid milk once it is fortified due to the longer chain of processing.

Additionally, promotion of fortified powdered milk could be considered as another intervention. In discussion with CHWs in the field, we found that cost was perceived to be a barrier in purchasing powdered milk. Social marketing campaigns may identify the cost per serving of powdered milk, and highlight that powdered milk is an economic option when evaluated per serving, especially given its additional nutritional benefit. The CHWs indicated that fresh milk consumption was encouraged by doctors in prenatal visits as a "health drink." During these visits, doctors may alternatively, or additionally, encourage use of fortified milk products, highlighting these same points. Still, social marketing would be an important tool. Many pregnant women do not visit the doctor until approximately the second trimester, so this technique could capture the attention of women at earlier stages of gestation.

Oral rehydration therapy (ORT), famously used in Bangladesh to manage widespread diarrheal diseases, has gained acceptance and can serve as a model for the success of social marketing and household education (54). Campaigns to encourage appropriate use have highlighted individual packets of ORTs, outlining that the only steps involved are to open the package, mix with a specified amount of water, and serve. This simple message was mass promoted, and paired with brief education via personal contact, print material, or other media (54). Given that ORT and powdered milk are used and prepared in analogous ways, promotion of powdered milk in Bangladesh has the potential to be accepted and used.

The CHWs reported an important barrier to accepting the use of powdered milk is the lower quality, particularly in taste. Additional uses for powdered milk could be encouraged outside of drinking it plain, mixed with water. In using powdered milk in cooking applications,

taste may be less of a deterrent. For example, mixing it into cooking rice or daal, which are commonly consumed foods, may be acceptable mediums for powered milk. Future research should evaluate the acceptance of various applications of powdered milk.

For non-dairy consumers, fish and egg consumption may be highlighted as entrances for vitamin D in the diet. From our analysis, these foods have already been identified as commonly consumed, and contribute a large percentage of vitamin D to the diet. However, due to the cost of these foods, women might not be able to increase intake enough to have a reasonable impact on achieving recommended intake of vitamin D.

Strengths and Limitations

This research has suggested possible directions for future public health research and interventions. The application of our findings is possible due to its consideration of already accepted or available sources of dietary vitamin D. We evaluated consumption of vitamin D by analyzing frequency, but also utilized an innovative method to estimate vitamin D in the absence of reported serving size. Assessing the Bangladeshi diet for specific contributors to vitamin D intake allowed foods to be understood in the context of the diet. Thus, contribution could be understood as an intersection between frequency and vitamin concentration across a range of plausible serving sizes. Additionally, the estimation of contributors to dietary vitamin D intake identified a range. This benefited our research and future directions because it acknowledges inter-individual differences in portion patterns, thus providing a range that could more accurately be applied to a population.

Although we identified a plausible range of portion sizes, our estimations may or may not accurately represent actual portion size for the women. Improvements could be possible through the inclusion of portion size in the FFQ or through 24-hour dietary recalls. Another limitation in our study is the defined concentration of vitamin D in powdered milk, according to the Food Composition for Bangladesh. The table identifies 100 g of dry milk as containing 0.1 mcg of vitamin D; this is not distinguished as being fortified. Our market analysis gave evidence to a wide range of vitamin D concentrations, many of which exceeded this level due to fortification. Because the value indicated in the Food Composition Table was used in our estimated contribution to vitamin D intake, we likely underestimate its importance. Similarly, the range that we found in vitamin D concentration makes it difficult to assert an accurate contribution; it would largely depend on the specific powdered milk product being consumed.

Conclusions

Vitamin D is important for fetal and maternal health outcomes and deficiency is prevalent in populations around the world, including those in South Asia. In contrast to the United States, where fortified dietary sources provide the predominant contribution to dietary vitamin D intake, naturally occurring sources are important foods for vitamin D in the Bangladeshi diet. Fish, especially large and medium sized, and eggs were frequently consumed and are rich in vitamin D; consumption frequency and vitamin D content were both factors in their importance. Still, improvements in vitamin D intake should be considered a public health concern, given the low status previously observed and extremely low dietary levels we observed in this study.

Continuing consumption of rich food sources should be continued. Added measures could be taken through public health efforts to promote the use of powdered milk as a simple, accessible way to increase vitamin D intake, given its existence in markets. Using already accepted foods in the Bangladeshi diet, industry and policy makers should explore the fortification of fresh milk.

The next step in this area of research will be to examine the relationships between diet, and sun exposure habits, and vitamin D status. Their relative contributions could further direct social marketing messages and other programs or policies to improve vitamin D status of pregnant women in low-resource settings.

Appendix A

Supplement to Food Frequency Questionnaire

Form 3C: Maternal Dietary Recall Supplement

Participant Identification

Participant ID	__ __ __ __
Research Personnel Name & ID	_____; ID __ __
Date and time of Interview	dd/mm/yy: __ __ / __ __ / __ __ Hour : minutes __ : ____
Which visit is this:	Baseline Visit ……..…… 1 6-Month Clinical Visit…..……2
6 Month Visit, Form 7 week # & attempt # completed today:	Week: _____ Visit attempt: _____ Baseline Visit….99.....99

Dietary Recall Instructions

I am going to ask you a few more questions, similar to the ones I just asked, about what you have eaten in the past 1 month. This still refers to any food or drinks that you consumed in the past four (4) weeks and includes items you ate on their own or as part of a dish or meal, foods that you ate raw or prepared, and those items you ate at home or outside the home. Please use the given pictures as examples of what you may have consumed.

Please ask me any questions at any time to ensure you understand the items.

1. Milk (powdered)	
Never	1 → Go to Q10
1 times per month	2
2-3 times per month	3
1 time per week	4
2-4 times per week	5
5-6 times per week	6
1 time per day	7
More than 1 time per day: ____time/day	8
Don't know	99

	When you consumed powdered milk in what way(s) did you use it? Please respond to each of the following:	**No**	**Yes**	**Unknown**
2.	Mixed with water to drink	1	2	9
3.	Mixed with tea or coffee	1	2	9
4.	Cooked with rice, porridge, or grains	1	2	9
5.	Cooked with vegetables	1	2	9
6.	Other _____			

	When you consumed powdered milk, were any products specifically for: *Show page of pictures for each of the following as examples.*	No	Yes	Unknown
7.	Pregnant or lactating women?	1	2	9
8.	Infants or children?	1	2	9

9. Each time you consumed powdered milk, what brand(s) did you consume? *Show page of pictures as examples to prompt response.*
1) _____ 2) _____ 3) _____ 4) _____ 5) _____ _____

10. Cereals (Cold, packaged, for breakfast)	
Never	1 → Go to Q12
1 time per month	2
2-3 times per month	3
1 time per week	4
2-4 times per week	5
5-6 times per week	6
1 time per day	7

More than 1 time per day: ____ times/day	8
Don't know	99

11. Each time you consumed cold breakfast cereals, what brand(s) did you consume? *Show page of pictures as examples to prompt response.*
1) _____ 2) _____ 3) _____ 4) _____ 5) _____

12. Cereals (Hot, packaged)	
Never	1 → **Go to Q16**
1 time per month	2
2-3 times per month	3
1time per week	4
2-4 times per week	5
5-6 times per week	6
1 time per week	7
More than 1 time per day: _____ times/day	8
Don't know	99

	When you consumed hot cereal, were any products specifically for: *Show page of pictures for each of the following as examples.*	**No**	**Yes**	**Unknown**

13.	Pregnant or lactating women?	1	2	9
14.	Infants or children?	1	2	9

15. Each time you consumed hot cereals, what brand(s) did you consume?

Show page of pictures for each of the following as examples.

1) _____
2) _____
3) _____
4) _____
5) _____

16. If you consumed calcium-fortified biscuits, what brand(s) did you consume?

Show page of pictures for each of the following as examples.

1) _____
2) _____
3) _____
4) _____
5) _____

Comments:

_____

_____

_____

_____

_____

_____

_____

End of interview.

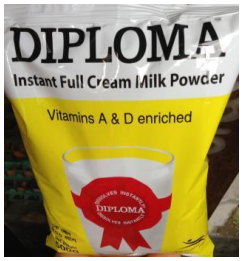
Form Verification

Supervisor name / ID	**_____ ID ___ ___**
Signature	**_____**
Date of Verification	**dd/mm/yy __ __ / __ __ / __ __**
Comments	

Appendix B

Food Frequency Questionnaire Supplement Picture Reference Page

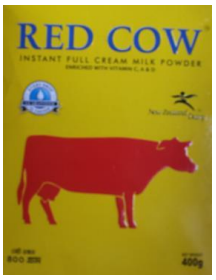
Powdered milk examples (Questions 7-9)



DIPLOMA

FRESH INSTA

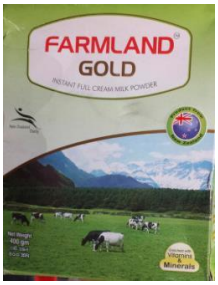
FRESH



RED COW

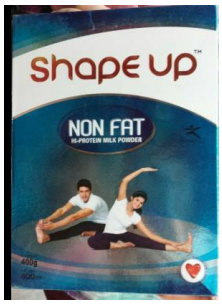
DANO

MARKS



FARMLAND GOLD

ANCHOR



SHAPE-UP

WOMEN'S HORLICKS

MOTHER'S HORLICKS

ORLICKS

JUNIOR'S HORLICKS

COMPLAN (any)

NIDO (Any)

INFANT FORMULA (any)

Cold cereal (Question 11)

CORN FLAKES

KOKO KRUNCH

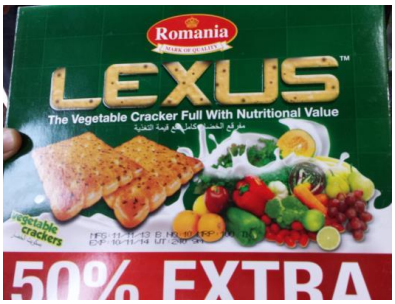
Hot cereal (Question 13-15)

CERELAC FRUIT

CERELAC KHICHURI

Biscuits (Question 16)

ENERGY PLUS



LEXUS

TIGER

COCOLA

Appendix C

Insights on Perceptions and Purchases of Vitamin D Foods from an Informal Focus Group

At the point of our departure, we were interested in learning about the contribution of food sources of vitamin D to vitamin D status [25(OH)D]. We suspected that sources of fortified vitamin D may play a role and could be important for public health nutrition. We therefore created a series of questions, similar to a focus group, aimed to understand the study population's intake of these vitamin D fortified foods. We had two discussions with CHWs at the field office after their days of conducting home visits; one of the research doctors translated for us. During this discussion, we asked the CHWs about their knowledge, use, and perceptions of powdered milks. We asked similar questions regarding yogurt, and hot and cold breakfast cereals, and also inquired about place of purchase. For all products, we were interested in knowing if pictures or written text on the food packages influenced purchase.

The CHWs reported perception and practices regarding liquid and dry milk. Liquid or fresh milk is referred to as a "health drink," recommended by doctors for children and pregnant women. Pregnant women are encouraged to drink one glass per day, and kids may have one glass of a mixed malted milk product, like Complan or Jr. Horlicks. One participant in the group said that she sometimes drinks these products herself, rather than adult equivalents, because they taste better. Outside of the Old Dhaka area, liquid milk is not commonly consumed. Still, CHWs reported usually keeping dry milk in their homes, in 500 g or 1 kg packages, purchased on a monthly basis, to be used for tea and making sweets. It is rarely used for drinking purposes

because of barriers related to taste. When dry or malted milk products are used, they are rarely measured out according to a serving size, but are often diluted more than suggested on the package directions. Additionally, the CHWs perceived dry milk as more expensive than fresh or liquid milk. This perception was one impetus for the cost analysis.

Yogurt is generally purchased on a bi-monthly basis, as opposed to being made in the home. In contrast with milk, the entire family will consume yogurt. Similar to milk, yogurt is not usually eaten on its own, but is used to prepare meat dishes and sweets for special occasions.

With regards to fortified breakfast cereals, CHWs reported that mothers may buy Cerelac, an infant cereal mix served warm, for infants of up to 9 months of age, but, because it is expensive and does not last for many meals, they will not eat it or buy it often.

Regardless of the form or product, CHWs do not look for "vitamin D" written as text on packages. Instead, they are more influenced by key messages, such as "complete nutrition," "vitamins and minerals," or "energy," or by pictures of children when seeking a product for their children.

Taken together, this information can give way to future research for public health nutrition intervention or policy. With the information collected, we have a better understanding of what influences the purchase of vitamin D fortified foods. If these products are, in fact, important contributors to vitamin D status, social marketing, public health initiatives, and education can be shaped around some of the barriers and motivators we identified. Most importantly for my thesis, these points of discussion helped to tailor our supplemental FFQ to be simplified and shortened, so to we could capture the most important questions.

Appendix D

Trial Observations

Trial observations during the fieldwork included data collection, study staff structure, and office processes. At MCHTI, observations included the administration of baseline forms, which required a blood draw, a food frequency questionnaire, a survey to gather information on demographics and clothing practices, and measurement of height and weight. CHWs and FRAs completed most of these tasks. CHWs also collected data throughout participant pregnancy by conducting weekly home visits to provide the supplement and to complete a survey. The thesis author and advisor observed two home visits. At the end of the day CHWs and FRAs gather at the field office to organize participant files. Documents are double checked for accuracy and then stored before being sent to data management.

Other observations included the sampling of placenta tissue, and the transport and storage of biospecimens and supplements.

Appendix E

Complete list of frequency of food intake in pregnant women, Dhaka Bangladesh, 2014 (n=561)

Food	Never	Less than once per week	Once per week	2-6 times per week	Once per day	More than once per day
	n (%)					
Milk (goat or cow, whole of skim)	127 (22.6)	156 (27.8)	26 (4.6)	145 (25.9)	105 (18.7)	2 (0.4)
Milk (condensed and powdered)	416 (74.2)	41 (7.3)	12 (2.1)	43 (7.7)	39 (7.0)	10 (1.8)
Yogurt and curd	314 (56.0)	210 (37.4)	21 (3.7)	14 (2.5)	2 (0.4)	0 (0)
Ice cream	212 (37.8)	233 (41.5)	46 (8.2)	63 (11.2)	6 (1.1)	1 (0.2)
Cheese (cottage)	522 (93.1)	35 (6.2)	2 (0.4)	2 (0.4)	0 (0)	0 (0)
Rice (white, boiled)	2 (0.4)	0 (0)	0 (0)	0 (0)	18 (3.2)	541 (96.4)
Rice (popped and puffed)	107 (19.1)	170 (30.3)	60 (10.7)	175 (31.2)	49 (8.7)	0 (0)
Rice (flaked)	534 (95.2)	0 (0)	6 (1.1)	18 (3.2)	3 (0.5)	0 (0)
Barley	542 (96.6)	15 (2.7)	1 (0.2)	3 (0.5)	0 (0)	0 (0)
Ruti	86 (15.3)	122 (21.8)	27 (4.8)	112 (20.0)	211 (37.6)	3 (0.5)
Bread (white)	230 (41.0)	181 (32.3)	38 (6.8)	90 (16.0)	21 (3.7)	1 (0.2)
Biscuits (not fortified)	242 (43.1)	120 (21.4)	30 (5.4)	102 (18.2)	66 (11.8)	1 (0.2)
Biscuits (calcium fortified)	402 (71.7)	94 (16.8)	0 (0)	52 (9.3)	12 (2.1)	1 (0.2)
Peanuts (groundnuts)	339 (60.4)	169 (30.1)	21 (3.7)	25 (4.5)	7 (1.3)	0 (0)
Other nut	510 (90.9)	43 (7.7)	3 (0.5)	4 (0.7)	1 (0.2)	0 (0)
Pumpkin and squash seeds	365 (65.1)	158 (28.2)	0 (0)	35 (6.2)	3 (0.5)	0 (0)
Sesame seeds	525 (93.6)	32 (5.7)	2 (0.4)	2 (0.4)	0 (0)	0 (0)
Chickpeas (Bengal gram)	280 (49.9)	147 (26.2)	26 (4.6)	87 (15.5)	21 (3.7)	0 (0)
Lentils (red and yellow)	28 (5.0)	41 (7.3)	24 (4.3)	180 (32.1)	132 (23.5)	156 (27.8)

Beans (mung, adzuki, jack, lima, navy, velvet, etc.) and cowpeas (seeds and pods)	437 (77.9)	91 (16.2)	9 (1.6)	24 (4.3)	0 (0)	0 (0)
Gram (red, green, black) and other peas	458 (81.6)	82 (14.6)	9 (1.6)	11 (2.0)	1 (0.2)	0 (0)
Coconut meat	401 (71.5)	129 (23.0)	12 (2.1)	16 (2.9)	2 (0.4)	1 (0.2)
Potato	8 (1.4)	23 (4.1)	8 (1.4)	225 (40.1)	116 (20.7)	181 (32.3)
Sweet potato (orange, yellow, and white flesh)	424 (75.6)	117 (20.9)	12 (2.1)	6 (1.1)	2 (0.4)	0 (0)
Amaranth (spiny) and Agathi Leaves	556 (99.1)	2 (0.4)	1 (0.2)	1 (0.2)	1 (0.2)	0 (0)
Amaranth (red and green), cowpea, drumstick, colocasia, and fenugreek leaves	236 (42.1)	160 (28.5)	38 (6.8)	132 (21.9)	2 (0.4)	2 (0.4)
Other leafy greens (spinach, Indian spinach, jute, turnip, and radish leaves, etc.)	172 (30.7)	208 (37.1)	85 (15.2)	91 (16.2)	5 (0.9)	0 (0)
Okra (lady finger)	139 (24.8)	207 (36.9))	74 (13.2)	141 (25.1)	0 (0)	0 (0)
Other vegetables (bitter, bottle, teasle, and pointed gourds, carrots, brinjal, cabbage, etc.)	26 (4.6)	109 (19.4)	36 (6.4)	364 (64.9)	23 (4.1)	3 (0.5)
Wood apple	481 (85.7)	62 (11.1)	7 (1.3)	11 (2.0)	0 (0)	0 (0)
Tamarind	333 (59.4)	146 (26.0)	27 (4.8)	41 (7.3)	14 (2.5)	0 (0)

Other fresh fruits (mango, pineapple, etc.)	72 (12.8)	105 (18.7)	41 (7.3)	230 (41.0)	106 (18.9)	7 (1.3)
Egg	51 (9.1)	95 (19.9)	52 (9.3)	229 (40.8)	131 (23.4)	3 (0.5)
Poultry	76 (13.6)	176 (31.4)	129 (23.0)	176 (31.4)	3 (0.5)	1 (0.2)
Beef, mutton, and pork	92 (16.4)	202 (36.0)	108 (19.3)	150 (26.7)	9 (1.6)	0 (0)
Organ meats (all animals: liver, brain, etc.)	309 (55.1)	198 (35.3)	27 (4.8)	21 (3.7)	6 (1.1)	0 (0)
Shrimp	174 (31.0)	191 (34.1)	67 (11.9)	124 (22.1)	5 (0.9)	0 (0)
Small fish (bata, bacha, gura, mola, pamfret, poa/bele, and fry fish)	188 (33.5)	187 (33.3)	61 (10.9)	116 (20.7)	8 (1.4)	1 (0.2)
Medium Fish (scorpion, aire, folui,chapila, khalshe, butter,climbibg, tengra, and catfish)	252 (44.9)	160 (28.5)	50 (8.9)	94 (16.8)	4 (0.7)	1 (0.2)
Large Fish (Carp, ruhit, mrigal, sar, magur, dragon, bocha, hilsha and flat fish)	119 (21.1)	213 (38.0)	58 (10.3)	163 (29.1)	6 (1.1)	2 (0.4)
Dried Fish (magur, pata, tapse, tengra, lota and chapila dried fish)	0 (0)	463 (82.5)	44 (7.8)	48 (8.6)	5 (0.9)	1 (0.2)

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ACADEMIC **VITA**

Morgan Cooper
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EDUCATION Pennsylvania State University, University Park, Pennsylvania
Schreyer Honors College, Class of 2015
B.S. Nutritional Science, Applied Option
Minor: Global Health

ACADEMIC HONORS THESIS *Availability and intake of foods with naturally occurring or added vitamin D in a setting of high vitamin D deficiency*
Fall 2013-Present

- Piloted Food Frequency Questionnaire related to Vitamin-D consumption in Dhaka, Bangladesh
- Responsibilities included: development of standard operating procedures (SOPs); coordination with collaborators from diverse fields; cross-cultural communication

TEACHING ASSISTANT *Nutrition 251: Introductory Principles of Nutrition*
Spring 2014

- Accomplishments: Create usable course material (quiz) on fat and water soluble vitamins
- Skills developed: Communicate with students in didactic manner verbally (review session) and written (grading/evaluating assignments)

AWARDS John E. Smith Outstanding Senior in Nutrition Award by Nutrition and Dietetics Alumni Society of Penn State
Finalist in ASN's Emerging Leaders in Nutrition Science Poster Competition at Experimental Biology Conference, 2015

INVOLVEMENT Student Nutrition Association *President*
Fall 2011-Present

- Lead general membership and board meetings and facilitate chair roles
- New initiatives included Career Night in which local nutrition professionals present job responsibilities and give insights on field, implementation of *Seniors Eating Well* curriculum at local senior center, exhibition at Centre County Super Fair on proper serving sizes and portion distortion, distribution of weekly club newsletter, &"SNA Study Hours" for students to convene for studying and SNA projects
- Recruited new members through involvement fairs and blog posts
- Communicate with nutrition students about outside learning and professional opportunities
- Correspond with Nutrition and Dietetics Alumni Association

EMPLOYMENT NUTRITION TRACKS *Evaluation Research Assistant* (State College, Pennsylvania)
September 2014-Present

- Create interactive map using ArcGIS to display state SNAP-Ed partners and service sites
- Receive and track surveys from partners; enter and recheck data collected
- Complete other assorted tasks as needed

BETUMI, Regional Ghanaian Cookbook Project *Recipe Tester Team Manager* (State College, Pennsylvania)
March-May, 2014

- Correspond with recipe testers: distribute recipes to be tested, receive feedback, maintained social media presence, organize participant files
- Skills developed: Ability to systematically organize and file recipe tester correspondence and feedback, project schedules, recipes, etc.; understand nutrition and its relationship to cuisine in a global context

Camp Nejeda *Senior Counselor, Assistant to Dietician, Kitchen Staff* (Stillwater, New Jersey)
Summer 2012, 2013

- Learned from campers/counselors about diabetes management, pumps/pens, insulin treatment, daily life with type I diabetes
- Responsibilities included: Monitor and treat blood glucose; implementation of food safety standards, especially related to special diets; lead nutrition education activities

VOLUNTEER Greenmoore Gardens *Student Community Supported Agriculture (CSA) Promoter & Farm Hand* (Port Matilda, Pennsylvania)
October 2012 to May 2014

- Serve as student ambassador; formally present to Penn State students the advantages and purposes of the student CSA option and other involvement/internship opportunities
- Engage with farmers and CSA members on important food and environment related issues

MEMBERSHIPS Member of Academy of Nutrition and Dietetics (Spring 2012-Present) and American Society for Nutrition (Fall 2014-present)

ADDITIONAL CREDENTIALS National Restaurant Association ServSafe Certified, CITI IRB Trained
Proficient in Microsoft Word, PowerPoint, and Excel; Experience in Stata, CulinarE-Companion™ Recipe Management, and ArcGIS