

Vitamin D Levels and Risk of Dyslipidemia among US Children with Diabetes and
Obesity

A Thesis Presented

by

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Submitted to the Graduate School of the University of Massachusetts Amherst
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2011

School of Public Health Sciences

Epidemiology

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DEDICATION

This work is dedicated to The Almighty God, my dad and mom; Prof J.E. Hagan and Ms Angela S. Ahlijah and to all my siblings; Fransiwaa and Nana Leo (Paa Takyi) Vande-Pallen and Kojo Hagan

ACKNOWLEDGMENTS

To God be the all the glory, “through it all I came to depend on you Lord”. Thank you Father!, for taking me on this journey and seeing me through it to a successful end, I love you Lord.

I am immensely indebted to Dr. Lisa Chasan-Taber, who has been a great advisor and a source of help and encouragement to me. Thank you very much Lisa. To both my thesis committee members; Dr Reeves and Dr Pekow, you each enriched this journey for me in a special way, thank you for such a great learning opportunity, I learnt a lot.

I would also like to express my gratitude to all the UMass Amherst lecturers who taught me throughout this journey, each and every one of you contributed to the realization of this vision. Thank you especially to Dr Edward Stanek, Dr Elizabeth Bertone-Johnson, Dr Brian Whitcomb, Dr Shirley Mietlicki and Dr Lorraine Cordeiro. Thank you all! To my classmates and colleagues, I really appreciate your contribution to my academic, personal and social growth over these past two years.

To the physicians and staff at Baystate Pediatric Endocrinology and Pediatric Weight Management Clinics, especially my practicum supervisor Dr Chrystal Wittcopp (M.D), Chelsea Gordner, D.O., M.P.H and Paul Visintainer (PhD), thank you all for your contribution to my academic success, kindness, help and a fantastic internship opportunity. I really appreciate it!!.

To Dr Pamela Marsh-Williams (Asst Provost/Dean) and Kathy A. Weilerstein of the UMass Undergraduate Advising & Learning Communities, thank you both for all your encouragement, care and assistance in helping me successfully undertake this

journey. To someone very special to me, Nana Y. Asante (Jnr), I am grateful for all your contribution to this journey, thank you Nana!.

Thank you mum and dad and my dear siblings for your faith and trust in me and all the diverse ways you supported me. Dr Angela Owusu-Ansah, Mrs. Afia Bulley, Uncle Isaac Bempong, Aunty Andrea and family, Uncle Ababio and family, Uncle Lewis, thank you all so much for everything, each word of encouragement and all the care and financial support. Thank you Aunty Ama, Fiamor, Keziah, Raymond and all my cousins, aunties and uncles. Thank you grandma Tanty for all your love and prayers.

To Rev Samuel and Joyce Quaye and all my Liberty House International Church family especially Ricky and Lucy Frimpong and family, thank you! for the support, prayers and encouragement. To my friends Lawrence Borketey, Stephanie, Benedicta, Michael, Adolphina, Stanley, Robert, Serwaa, Adobea and Michael, Essie, Michiko, Kwame, Duke, just to mention a few I say thank you all!!!, especially to Peter Vanderpuije; thanks buddy for sticking with me. To all the friends I made here at UMass and anyone not mentioned here, who contributed one way or the other to this journey, I am grateful to you all and God bless!

ABSTRACT

VITAMIN D LEVELS AND RISK OF DYSLIPIDEMIA AMONG US CHILDREN WITH DIABETES AND OBESITY.

MAY 2011

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Dyslipidemia is increasing among U.S. children, and the prevalence is highest among children with diabetes and obesity. Recently, vitamin D deficiency has been suggested as a possible dietary risk factor for dyslipidemia. Despite the high prevalence of vitamin D deficiency amongst children, virtually no studies have evaluated the association between vitamin D and dyslipidemia among children. We evaluated the vitamin D and dyslipidemia relationship among 240 children and adolescents aged 2 through 21 years who were outpatients of a pediatric endocrinology unit at a large tertiary care facility in Western Massachusetts from April 2008 to April 2010. Eligible children were those with either obesity and/or type 1 or 2 diabetes mellitus. A total of 17.4% of children had severe (<15.0 ng/ml) vitamin D deficiency, 19.2% had moderate (15.0-19.9 ng/ml) deficiency, 36.3% were insufficient (20.0-29.9 ng/ml), and 27.1% had normal (≥ 30.0 ng/ml) levels. A total of 28.8% of children had high total cholesterol (TC ≥ 180 mg/dL), 19.6% had high triglycerides (TG; <10 years: ≥ 110 mg/dL, ≥ 10 years: ≥ 130 mg/dL), 21.3% had low high density lipoprotein (HDL <40 mg/dL), and 6.7% had high low density lipoprotein (LDL ≥ 130 mg/dL). Moderate vitamin D deficiency was associated with increased risk of high TC (adjusted odds ratio [OR_{adj}] = 2.9, 95%

confidence interval (CI): 1.0, 8.8) compared to children with normal vitamin D levels. Severe vitamin D deficiency was associated with an increased risk of low HDL ($OR_{adj} = 3.5$, 95% CI: 1.0-12.3) and high TG ($OR_{adj} = 11.7$, 95% CI: 1.9, 70.3) compared to children with normal vitamin D levels. Children with moderate vitamin D deficiency had approximately 3-fold increased risk of high TC compared to children with normal vitamin D levels. In comparison to children with normal vitamin D levels, severe vitamin D deficiency was associated with a strong and significant increased risk of low HDL and high TG; with a significant dose-response relationship. Additionally, in linear regression analyses, we found that an increase in vitamin D deficiency was associated with a significant mean increase in all four measures of dyslipidemia. Vitamin D adequacy may reduce the risk of dyslipidemia in children.

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CHAPTER I

INTRODUCTION

Dyslipidemia is a metabolic disorder of lipoprotein metabolism which results in abnormal excesses of: total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), or triglycerides, or a deficiency in high-density lipoprotein cholesterol (HDL-C) (1). Approximately 10% of US children are affected by hyperlipidemia; a type of dyslipidemia characterized by elevated levels of blood lipids (2, 3). Dyslipidemia is one of the key factors considered by the National Cholesterol Education Program Adult Treatment Panel (NCEP - ATP III) in defining metabolic syndrome. The US prevalence of metabolic syndrome in adults ≥ 20 years is 20% (3). In US adolescents ≤ 20 years, 6.4% can be classified as having metabolic syndrome based on age modified NCEP - ATP III criteria, out of which 50% are severely obese children and adolescents (3).

Childhood dyslipidemia is associated with the risk of developing CVD in adulthood (4). Familial hypercholesterolemia (FH), familial combined hyperlipidemia (FCHL), familial hyperglyceridemia and hyper-apoprotein – B, are some commonly inherited lipoprotein disorders observed in children of parents with premature cardiovascular disease (CVD) which makes these youth at high risk of developing CVD in the future (1, 2, 5).

The primary known risk factors for dyslipidemia include genetic disorders of lipid metabolism such as familial hypercholesterolemia. However secondary causes of dyslipidemia in adolescents are diabetes, cigarette smoking and anorexia nervosa. In view of the rising trends in dyslipidemia in children and adolescents, there is the need to

continually identify new risk factors for dyslipidemia in this population. Vitamin D deficiency is thought to be one of the newer risk factors for dyslipidemia in children and adolescents, especially for those with other underlying metabolic syndromes.

Vitamin D deficiency, mainly in the form of rickets, became epidemic in the US at the end of the 19th century. In children and adolescents, extreme cases of vitamin D deficiency result in rickets and, in adults it leads to osteomalacia. However, in recent years, there has been increasing interest in the association between vitamin D deficiency and diseases, such as cardiovascular disease (CVD) (6). There is substantial evidence suggesting that hypovitaminosis D (low levels of vitamin D) influences the development of CVD after age 50 (6). In vitamin D deficient youths, successful repletion of vitamin D has been shown to reduce the possibility of developing CVD in adulthood (6). Because pathological evidence suggests that precursors of CVD start from childhood (6), these findings support evaluating the association of vitamin D deficiency with CVD risk factors (6).

However, to date no study has evaluated the relationship of vitamin D deficiency with dyslipidemia, especially in children with obesity and / or diabetes. Therefore, we evaluated the association between vitamin D deficiency and blood lipids among children and adolescents, 2 through 21 years old, diagnosed with either diabetes and/or obesity. Specifically our aims were to evaluate the association between vitamin D deficiency and high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC) and triglycerides (TG), among these high risk children. We used a cross-sectional study design; data on serum 25(OH)Vitamin D levels as well as fasting (≥ 12 hours) blood lipid levels were obtained via laboratory analysis and chart review of medical record from the

Pediatric Endocrinology and Pediatric Weight Management Clinics, of the Baystate Medical Center's Children's Hospital in Springfield, Massachusetts. The American Academy of Pediatrics (AAP) and National Cholesterol Education Program (NCEP) guidelines, were used in classifying blood lipid levels. This study base represented an ethnically and socioeconomically diverse population of which majority were predominantly non-Hispanic white and Asian.

CHAPTER II

LITERATURE REVIEW

A. Biological Evidence (Physiology) of the Association between Vitamin D Levels and Dyslipidemia.

Dietary sources of vitamin D include oily fish, irradiated mushrooms, dairy foods such as milk and eggs, whole grains and cereals as well as other fortified foods such as juices (7). However, the bulk (90%) of vitamin D utilized by humans is obtained through the synthesis of vitamin D in the skin through a sturdy photolytic procedure. This process involves the action of sunlight (exposure to ultraviolet (UVB) radiation 290-315nm from the sun) on a cholesterol derivative i.e. 7-dehydrocholesterol, present in the plasma membrane of epidermal keratinocytes and dermal fibroblasts. Once the energy from the sun has been absorbed by the double bonds in the “B ring” of the 7-dehydrocholesterol, previtamin D is produced, which next gradually converted from one isomer to the other (rearrangement of the double bonds and opening of the B rings) into vitamin D₃ (7, 8). The natural form of vitamin D produced from this photosynthesis in the skin is vitamin D₃, (this compound, 25-hydroxyvitamin D₃ (25(OH)D₃)), which in its present form is metabolically inert or inactive, needs to be modified into its functional form in the body. The form in which vitamin D is commonly monitored to assess vitamin D status of patients is 25-hydroxyvitamin D₃ (25 (OH) D₃)) (8).

The exact mechanisms by which vitamin D deficiency influences dyslipidemia have not been fully elucidated (9). However, three indirect mechanisms have being suggested as possible ways in which vitamin D deficiency may influence blood lipid

metabolism or profiles. In terms of the first mechanism, Pittas *et al.* (2010), suggests a possible direct mechanism in which vitamin D deficiency may influence the risk of cardio-metabolic outcomes involving vitamin D receptor activation. This mechanism though, poorly understood in relation to the influence of vitamin D on dyslipidemia, is thought to be linked to how the vitamin D receptor influences other cardiometabolic outcomes such as hypertension (10). In terms of this mechanism, cardiac myocytes, endothelial, and smooth vascular muscle cells express both the vitamin D receptor and the 1- α -hydroxylase enzyme which then activates the inactive circulating vitamin D precursor (25(OH)D) into the active vitamin D metabolite and possibly increasing lipoprotein lipase enzyme levels and subsequently decreasing TG levels (10).

A second possible mechanism suggests that vitamin D may decrease blood lipid levels, specifically TG plasma levels, via regulation of vitamin D adipocytes (10). This mechanism is as a result of the disruption of the mevalonate pathway (HMG-CoA reductase pathway), by the effects of statin drug therapy, which function as inhibitors of the 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase enzyme and subsequently causes an increase in 7-dehydrocholesterol (10, 11). The excess 7-dehydrocholesterol in the human body is then converted to 25-hydroxycholecalciferol by the action of sunlight or the CYP11A1 enzyme (11). This action leads to an increase in vitamin D levels in the body and a corresponding decrease in TG levels possibly due to elevated levels of the lipoprotein lipase enzyme resulting from the disruption of the mevalonate pathway (10, 11).

The third proposed mechanism linking vitamin D deficiency with dyslipidemia, is via the elevation of peripheral insulin resistance in the body (11). This mechanism is also poorly understood.

In summary three possible indirect mechanisms have been proposed linking vitamin D to dyslipidemia: the first involves the activation of the vitamin D receptor, the second is as a result of the disruption of the mevalonate pathway (HMG-CoA reductase pathway) as a result of statin drug therapy, and the third mechanism is as a result of the elevation of peripheral insulin resistance in the body.

B. Epidemiology of Vitamin D Levels and Dyslipidemia (Abnormal Lipid Levels)

To the best of our knowledge, over the past five years eleven epidemiological studies have evaluated the association between vitamin D and cardiovascular risk factors including dyslipidemia (6, 12-21), eight of which were US based (6, 12-15, 18, 19, 21) and 3 international ones (16, 17, 20).

Out of the eight epidemiological studies conducted amongst older populations mainly ≥ 20 years, six were conducted cross-sectionally (12, 13, 16, 17, 19, 21), while only two (18, 20) prospectively evaluated the association between vitamin D deficiency and dyslipidemia. Two out of these eight studies in adults, found a negative association, (12, 21), another two found a positive association; (17, 18), while the remaining four; (13, 16, 19, 20) found null associations between dyslipidemia and vitamin D deficiency.

Despite immense recent interest in vitamin D deficiency and dyslipidemia in children and adolescents, only three out of the eleven epidemiologic studies investigated

this association in children and adolescents ≤ 20 years old; Kumar *et al* (2009), Reis *et al* (2009) and Ashraf *et al* (2009).

Due to the conflicting or inconsistent epidemiological evidence and the limited number of epidemiological studies relevant to our population, we included findings from studies in these eight older populations (12, 13, 16-21) that evaluated vitamin D deficiency and dyslipidemia in our literature review. To the best of our knowledge, there are no previous studies evaluating the relationship between vitamin D deficiency and dyslipidemia, specifically looking at abnormal blood lipid (high density lipoproteins (HDL), low density lipoproteins (LDL), total cholesterol (TC) and triglycerides (TG)) levels, in children with diabetes and obesity who are at high risk of developing cardiovascular disease in the US. However a number of studies have focused on the association between vitamin D deficiency and dyslipidemia in older populations (e.g. people 20 years and older) (12, 13, 16-21).

The first notable epidemiologic study of this association in children was a cross-sectional study designed and conducted by Kumar *et al* (2009), in a nationally representative sample of 6275 US children aged 1 to 21 years, in the National Health and Nutrition Examination Survey in 2001 to 2004. The Diasorin assay was used in assessing the serum vitamin D levels. Study subjects were categorized into three groups based on these vitamin D status: vitamin D deficient (< 15 ng/mL), and vitamin D insufficiency (15-29 ng/mL), vitamin D sufficiency (>30 ng/mL) (15, 22). Vitamin D supplement use was also accessed via self reported questionnaire and pill bottle review. Serum HDL and TC were assessed using the Beckman Synchron LX20 assay. Children with diabetes were eligible for the study if they met all other requirements.

In a multivariable-adjusted analysis children and adolescents, with vitamin D insufficiency or deficiency were more likely to have lower levels of high-density lipoprotein cholesterol (HDL-C) lipid levels in comparison to children with sufficient or normal vitamin D levels (difference: -2.29 mg/dL (95% CI -3.57 to -1.01) , $p = 0.001$) and (difference: -3.03 mg/dL (95% CI -5.02 to -1.04) , $p = 0.004$) respectively (15). Similarly total cholesterol was higher among vitamin D sufficient children compared to vitamin D insufficient; (difference: -3.66 mg/dL (95% CI -7.09 to -0.23) , $p = 0.04$) in a multivariable adjusted analysis (15). Hence this implied that vitamin D insufficiency and deficiency, was statistically significantly associated with lower levels of high density lipoprotein, additionally vitamin D insufficiency was also significantly associated with lower levels of total cholesterol in children and adolescents. After excluding all obese children from their analysis, as a means of controlling for residual confounding, the observed relationships between vitamin D insufficiency and deficiency with both TC and HDL levels were similar to that described previously for the entire study sample. This implied that irrespective of a child or adolescent's adiposity (being obese or non obese), vitamin D insufficiency and deficiency were significantly associated with low levels of HDL, while vitamin D insufficiency was also significantly associated with low levels of TC.

This study was limited by the lack of information on the seasons in which the vitamin D measures were taken. This is relevant because vitamin D levels vary greatly with sunlight exposure. In addition the measures obtained for 25(OH)D levels, may have been higher than the average 25(OH)D levels in the population, because screening and enrollment of participants from the northern states in the NHANES study occurred during

the summer. Finally, this study did not assess TG and LDL but only focused on HDL and TC which may limit its generalizability to adolescents whose dyslipidemia is as a result of an abnormality in the levels of LDL and TG.

In the second of the three studies conducted among children and adolescent populations to evaluate the association between vitamin D deficiency and blood lipid levels, Reis *et al.* (2009), conducted a cross-sectional study amongst 3,528 adolescents without a diagnosis of diabetes aged 12 through 19 years, who were participants in NHANES (6). This study differed from the prior study in that it focused primarily on adolescents but it was also based on the NHANES data and was conducted from 2001 to 2004. Serum vitamin D levels were assessed using radioimmunoassay techniques. Abnormal blood lipid levels for HDL-C and TG were ascertained using the National Cholesterol Education Program Adult Treatment Panel III definition modified for age: HDL-C < 40 mg/dL and TG > 110 mg/dL (6). For analytical purposes the vitamin D levels measured were classified into quartiles, with quartile I, II, III, and IV representing a vitamin D level of < 15.00 ng/mL, 15 to 21 ng/mL, 21 to 26 ng/mL and >26.00 ng/mL respectively. In comparison to adolescents in the highest quartile, those in the lowest quartile of vitamin D had a 50% non statistically significant increased risk for low high-density lipoprotein cholesterol levels (adjusted OR; 1.54, 95% CI: 0.99 to 2.39). Those in the highest quartile of vitamin D level classification did not have a statistically significant decreased risk of hypertriglyceridemia, when compared with those in the lowest quartile (adjusted OR: 1.00 (CI: 0.49 to 2.04)) (6), adjusting for age, gender, race or ethnicity, poverty-to-income ratio, and physical activity.

Overall this study failed to find any statistically significant association between low vitamin D levels or vitamin D deficiency and dyslipidemia as measured by variations in TG levels, but did observe the suggestion of an association with low HDL-C levels. This study also lacked information on season of vitamin D draw, therefore the observed vitamin D levels may not be representative of the average values for the study population. Finally, the study did not assess TC and LDL but only focused on HDL and TG which may limit its generalizability to adolescents whose dyslipidemia is as a result of an abnormality in the levels of LDL and TC.

The third study by Ashraf et al. (2009), conducted amongst 51 African American obese female adolescents, was the only study that specifically evaluated the association between vitamin D deficiency and cardiovascular risk factors, including the full blood lipid profile; (TC, TG, HDL and LDL) in adolescent obese females (14). Participants had a mean age of 14 ± 2 years and mean body mass index (BMI) of 43.3 ± 9.9 kg/m². This study utilized a cross-sectional study design, and collected two baseline serum samples of both the blood lipid levels and vitamin D levels. Total serum 25-(OH)D was measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology (14). A binary classification, with (25(OH)D < 15ng/mL) and (25(OH)D > 15ng/mL) as cutoffs, was used in grouping study participants into deficient or sufficient vitamin D levels respectively (14). This study failed to show an association between vitamin D deficiency and blood lipid levels. The vitamin D deficient groups (n= 31) had a mean value of 158.57 ± 26.00 and 83.63 ± 41.49 mg/dl for TC and TG respectively, 159.55 ± 18.85 and 88.80 ± 50.10 mg/dl for TC and TG for those in the vitamin D sufficient group (n= 20) (p=0.885 and p=0.693 respectively) (14). The vitamin D deficient groups (n= 31)

had a mean HDL and LDL of; 44.34 ± 8.56 and 95.69 ± 26.17 mg/dl, respectively, as compared to 43.35 ± 9.31 and 98.50 ± 21.02 mg/dl respectively for the subjects with sufficient vitamin D (n=20), ($p=0.701$ and $p=0.692$ respectively) (14). After adjusting for BMI, a non significant inverse correlation was noted for TC and vitamin D deficiency (n= 50, $r = -0.011$ $p=0.94$), whereas a positive but non significant correlation was noted for HDL (n = 49, $r = 0.012$ and $p=0.94$) and LDL (n= 49, $r = 0.037$ and $p=0.80$) as well as TG (n = 50, $r= 0.067$ and $p=0.64$) respectively (14). These findings imply that Ashraf *et al* (2009), found no significant association between serum 25 (OH) vitamin D and either; TC, HDL, LDL or TG.

The restriction of the study population to African Americans, limited its generalizability, to a racially diverse population of children with similar characteristics.

In summary, Kumar *et al* (2009), observed a significant difference in the decline in high density lipoprotein (HDL) and total cholesterol levels amongst vitamin D insufficient participants in comparison to vitamin D sufficient participants, additionally vitamin D deficiency was observed to be associated with low levels of HDL cholesterol irrespective of adiposity or obesity. Reis *et al* (2009), however, found no significant associations between levels (quartiles) of vitamin D with either low high density lipoprotein or high triglycerides levels. Similarly Ashraf *et al* (2009), found no significant association between serum 25 (OH) vitamin D and either; total cholesterol, HDL, low density lipoprotein or triglycerides. No previous studies have evaluated the association between vitamin D deficiency and abnormal blood lipids levels among multiracial children and adolescents with diabetes and or obesity, who are at high risk for cardiovascular disease (CVD). The studies which have been conducted primarily

assessed the association between vitamin D deficiency and blood lipid levels in mainly adult populations. To the best of our knowledge two out of the three studies conducted in children have failed to show any association between vitamin D and abnormal blood lipid levels. These studies are however limited by inadequate assessment of vitamin D deficiency because seasonal variations were not accounted for, and in some cases, had a small sample size (6, 14, 15).

C. Summary

The prevalence of dyslipidemia amongst children and adolescents, particularly those with prevailing metabolic syndrome such as obesity and diabetes, is steadily increasing in the US population. It is thought that there may be an association between childhood dyslipidemia and the risk of developing CVD in adulthood. For this reason studies have evaluated the possible role of vitamin D deficiency in the development of CVD in children. Mechanisms include the role of cardiac myocytes, endothelial, and smooth vascular muscle cells in the activation of both the vitamin D receptor and the 1- α -hydroxylase enzyme which then activates inactive circulating vitamin D precursor (25(OH)D) into active vitamin D metabolite and possibly increasing lipoprotein lipase enzyme levels and subsequently decreasing TG levels (10). Another possible mechanism is as a result of the disruption of the mevalonate pathway (HMG-CoA reductase pathway) by the effects of statin drug therapy, which function as inhibitors of the 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase enzyme and subsequently causes an increase in 7-dehydrocholesterol (10, 11). The excess 7-dehydrocholesterol in the human body is then converted to 25-hydroxycholecalciferol by the action of sunlight or the

CYP11A1 enzyme (11). This action leads to an increase in vitamin D levels in the body and a corresponding decrease in TG levels possibly due to elevated levels of the lipoprotein lipase enzyme resulting from the disruption of the mevalonate pathway (10, 11).

There is sparse epidemiologic data on the relationship between vitamin D deficiency and dyslipidemia, and majority of prior studies have examined this association among adults. Therefore we evaluated the association between vitamin D deficiency and blood lipids among children and adolescents, aged between 2 through 21 years, diagnosed with either diabetes and/or obesity, and at high risk for cardiovascular disease (CVD).

CHAPTER III

SPECIFIC AIM AND HYPOTHESES

The specific aim of our study was to evaluate the relationship between vitamin D deficiency and dyslipidemia among US children and adolescents at high risk for cardiovascular disease (CVD).

The specific hypotheses we addressed in the study were:

- 1) We hypothesized that vitamin D deficiency will be inversely related to high serum high density lipoprotein (HDL) levels among children and adolescents at high risk for CVD.
- 2) We also hypothesized that vitamin D deficiency will be positively related to high serum low density lipoprotein (LDL), total cholesterol (TC) and triglycerides (TG) levels among children and adolescents at high risk for CVD.

CHAPTER IV

METHODS

A. Cross-sectional Study Design, Setting and Population

We evaluated the relationship between vitamin D levels and blood lipid levels in children and adolescents with diabetes and/or obesity, in a cross-sectional study design. Two years (April 2008 through April 2010) of patient data were obtained from the Pediatric Endocrinology and Pediatric Weight Management Clinics, of the Baystate Medical Center. The data for this cross-sectional analysis were abstracted using a retrospective chart review of medical records. The data used for this study consisted of all data abstracted through January 24th, 2011.

B. Patient Ascertainment

Patients eligible for the study were identified from the medical charts and records using the International Classification of Diseases, 9th Revision (ICD-9) codes: ICD-9: 278.0 (obesity), 783.1 (abnormal weight gain), 250.02 (type 2 diabetes), 250.03 (IDDM Type 1 without complications uncontrolled), 250.00 (type 2 diabetes, controlled), 251.1(hyperinsulinism), 250.01 (juvenile diabetes uncomplicated), 253.5 (diabetes insipidus). This study was part of a larger study to evaluate the cardiovascular disease risk factors in children and adolescents with diabetes and/or obesity. The parent study also sought to investigate the effect of low vitamin D levels on levels of the parathyroid hormone. Children were referred to this clinic if they had a diagnosis of either obesity or diabetes that required medical intervention. Once referred to this specialized clinic,

children began a weight and/or diabetes management regime as part of their treatment. Data on exposure, outcome and covariates were collected from a routine screening procedure for each child during the initial physician visits. Data on both the exposure (vitamin D levels) and outcome (blood lipid profiles) as well as covariates such as parathyroid levels and serum calcium for each patient was obtained via laboratory testing, while data on some covariates such as age (verified from date of birth) gender, ethnicity and vitamin D supplementation were self reported by patients. Other covariates such as anthropometric measures (e.g. weight and height) were assessed by a trained nurse at the time of physician visit and documented in the medical records of each patient. Additionally, other covariates such as; body mass index (BMI), BMI- Z-score, glycated or glycosylated hemoglobin (HbA1C), and season were calculated from the relevant documented medical record data on patients. Physicians generally ordered a laboratory profile test for each patient at the initial visit and, when necessary, these tests may be repeated again during follow up visits at the physician's discretion for therapy and monitoring purposes. For each patient data for the first 25 (OH) D laboratory draw with accompanying lipid blood profile levels were utilized in this study.

The study is restricted to out-patients of the clinic, aged 2 through 21 years, diagnosed of obesity as defined by a BMI > 95th percentile, and/or type 1 diabetes mellitus, or type 2 diabetes mellitus (based on these ICD-9 codes:278.0 (obesity), 783.1 (abnormal weight gain), 250.02 (type 2 diabetes), 250.03 (IDDM Type 1 without complications uncontrolled), 250.00 (type 2 diabetes, controlled), 251.1(hyperinsulinism), 250.01 (juvenile diabetes uncomplicated), 253.5 (diabetes insipidus), who in addition also had a documented laboratory measurement of 25-

hydroxy vitamin D level (25(OH)D) during the study period. Exclusion criteria were known gastrointestinal malabsorptive disorder (i.e. celiac disease, cystic fibrosis), known parathyroid disease and pregnant adolescents. Also excluded were children and adolescents, with a diagnosis of non-essential hypertension and those without a lipid assessment within 1 month of the vitamin D assessment.

Data on exposure, outcome and covariates were obtained from three sources of the Pediatric Endocrinology and Pediatric Weight Management Clinic's patients computer database; laboratory data was abstracted from the 1) Clinical Information System (CIS) database and 2) Pediatric Endocrinology Dynamic Record Organizer (PEDRO) database. Demographic information on patients, was drawn from both the Baystate administrative computer database used by Baystate Medical Physician groups; 3) Centricity Business, and from CIS. The data obtained from these three sources; 1) CIS database, 2) Pediatric Endocrinology Dynamic Record Organizer (PEDRO) database and 3) Centricity Business were merged to create one database for our analysis. These data were entered into the database by trained nurses.

C. Vitamin D Levels (Exposure) Assessment

Vitamin D levels were assessed as serum 25 hydroxyvitamin D (25(OH) D) levels, collected from clinical data of laboratory results in CIS and PEDRO databases. All the study exposure data consisted of only vitamin D 25(OH) D assays conducted at the Baystate Hospital laboratory using the direct, competitive chemiluminescence immunoassay (CLIA) technique with a reference range of 32 - 100 ng/mL. Laboratory data was then entered into PEDRO, by a trained nurse and later abstracted for our data

analysis by a trained investigator and verified by a trained physician. Serum vitamin D levels was categorized into four groups, according to commonly utilized standard limits as: 1) severe vitamin D deficiency; less than 25.00 nmol/liter (< 15.00 ng/ml), 2) moderate vitamin D deficiency; 25.00–49.99 nmol/liter (15.00 –19.99 ng/ml), 3) vitamin D insufficiency, 50.00 –74.99 nmol/liter (20.00 –29.99 ng/ml) and 4) vitamin D optimal or normal range, at least 75.00 nmol/liter (\geq 30.00 ng/ml) similar to what has been done by other authors (15). In a separate analysis from the above, vitamin D levels were dichotomized as: (\geq 30.00ng/ml) representing normal vitamin D levels and <30.00ng/ml representing vitamin D deficiency. Additionally further analyses were conducted evaluating vitamin D levels as a continuous measure.

D. Validity of Exposure Assessment

The Baystate reference laboratory utilizes an externally and internally validated method; direct, competitive chemiluminescence assay (CLIA), for the 25(OH) vitamin D (also known as calcidiol, or cholecalciferol) metabolites analysis. The reference range for the CLIA analysis is 32-100 ng/mL.

E. Dyslipidemia (Outcome) Assessment

Based on general recommendations for blood lipid analysis, all outcomes were assessed using 12 hour fasting blood samples. All the lipid draws included in this study were analyzed at the Baystate reference laboratory; triglyceride (TG) was assessed by enzymatic colorimetric GPO/PAP technique. High and low density lipoprotein (HDL and

LDL) was by spectrophotometric; diet homogenous; enzymatic colorimetric technique. Total cholesterol (TC) was by enzymatic colorimetric techniques.

A binary classification of low (hypo) or high (hyper) was used in categorizing all the four individual blood lipid levels of interest; low density lipoproteins (LDL), high density lipoprotein - cholesterol (HDL-C), total cholesterol (TC) and triglycerides (TG). High density lipoprotein - cholesterol (HDL-C) and triglycerides (TG) were dichotomized using the National Cholesterol Education Program (NCEP), expert panel on cholesterol levels in children and adolescents cutoff as; low HDL-C; < 40 mg/dL and ≥ 40 mg/dL as sufficient (4, 5). Two reference levels were used in determining hypertriglyceridemia (high TG levels), based on age; the cutoff point in children aged 2 to < 10 years was ≥ 110 mg/dL and for children and adolescents aged 10 to 21 years the cut point was defined as ≥ 130 mg/dL, which was equivalent to the NCEP definition for high LDL (4, 5). Also based on the NCEP guidelines, high TC was defined as ≥ 180 mg/dL (4, 5). The NCEP cutoffs for high and low levels of the blood lipids represent approximately the 95th and 5th percentiles, respectively of the general population.

F. Validity of Outcome Assessment

The Baystate reference laboratory internally validates the manufacturer's (Roche) repeatability (within run precision, $n=21$) and intermediate precision (total precision/between run precision/between day precision) coefficient of variation (CV) periodically. The repeatability CV% for LDL, TC, TG and low HDL are approximately; 0.81, 0.8, 1.5 and 0.95, respectively whiles the intermediate precision CV% for LDL, TC, TG and low HDL are approximately; 1.18, 1.7, 1.8 and 1.3 respectively. For the

repeatability analysis, 21 human samples were run, while for each intermediate precision run, triplicate aliquots were analyzed for 21 days at one run per day.

G. Covariate Assessment

At the same time as the exposure and outcome assessments, data for covariates were also abstracted from the Centricity Business, CIS and PEDRO medical records database, for each eligible study participant (Table 1). The covariates of interests were age, sex (gender), serum calcium levels, weight in kilograms, and height in centimeters, body mass index (BMI), BMI Z-score, glycated or glycosylated hemoglobin (HbA1C), parathyroid hormone (PTH) levels, vitamin D supplementation, seasons (the four Northern American seasons; Winter, Spring, Summer and Fall) and ethnicity. Prior studies have indicated that age, gender and ethnicity are strong predictors of dyslipidemia in children and adolescents especially those with underlying chronic disease conditions such as diabetes and obesity (14).

H. Data Analysis Plan

Specific Aim: we evaluated the relationship between vitamin D levels and the risk dyslipidemia (abnormal blood lipid levels) among US children and adolescents with diabetes and obesity.

1. Univariate Analysis

The number and percent of study participants according to inclusion and exclusion criteria are presented in Table 2. The distribution (number and percent) of 25-OH vitamin

D levels (Table 3), and the distribution of blood lipid levels (Table 4), in our study population, are also presented. Both a Pearson's and Spearman's correlation analysis was conducted to evaluate the correlation between continuous exposure and continuous covariates (Table 8).

2. Bivariate Analysis

We used the two sample *t* test for continuous covariates and the chi-square test to assess categorical covariates as potential confounders by cross-tabulating them with binary coded exposure variables (Table 5) and exposure variables categorized into four sub-groups on the basis of the previously described standard cutoff values (Table 6), and a two sample *t* test and Analysis of Variance (ANOVA), for continuous exposure and categorical covariates (Table 7) to determine if the observed distribution within our 2X2 tables fits the expected distributions for large cell frequencies. A similar assessment was also done for the outcome variables coded as binary outcome variables (Table 9). For 2X2 tables with small cell frequencies an appropriate test such as the Fisher's exact test was used for this analysis. The statistical significance of the differences in the distributions for all categorical covariates are indicated by *P*-values. For continuous covariates, we calculated two-group *t* test, to determine the statistical differences in continuous outcome variables between levels of the covariates (Table 9). Pearson correlation coefficients were used to assess the correlation between the continuous exposure or covariate variables and blood lipid levels (Table 10). We also described the distribution; number and percentage of the categorical and continuous exposure variable within the outcome categories (Table 11).

3. Multivariable Analysis

We modeled the relationship between levels of vitamin D and all the four types of blood lipids, independently by levels within the individual types. This analysis was done separately for both categorical and continuous variables using unadjusted and adjusted multiple logistic regression and only adjusted multiple linear regression analysis respectively (Table 12 and 13). For the 25-OH vitamin D levels dichotomized based on widely used cutoffs, the normal level served as the referent group which we compared to the deficient group. Similarly the normal group within the second grouping of vitamin D levels into four widely accepted categories also served as the referent group for comparisons with the other three groups.

To evaluate confounders we used the backward elimination procedure; for both the logistic and linear regression model building respectively, to evaluate the association between vitamin D levels and the four blood lipid levels (TC, TG, HDL and LDL) adjusting for relevant covariates in separate models. Vitamin D levels were modeled as a dichotomous, a categorical and continuous variable respectively in separate models. Appropriate dummy variables were created for categorical covariates. All the previously mentioned variables (covariates) were evaluated for inclusion in the final multivariable logistic regression model as potential confounders, using the backward elimination selection method based on Likelihood ratio tests for logistic regression models and on Partial Fisher's (F) tests for the linear regression models. The assessment to determine if each covariate should be included in the model was done by initially building univariate logistic regression models for each covariate based on the assumption that all continuous variables were linear in the logit. Covariates with p -value < 0.25 from the univariable

logistic or linear regression model's likelihood ratio tests or Partial Fisher's (F) tests, as well as other variables of known clinical significance or of relevance to the association were included in the initial logistic and linear multivariable model respectively.

Next based on the Wald's test p -values from the initial multivariable model, the significance or relevance of each covariate in the model was tested, starting with the covariate or set of dummy variables with the highest p -value. A Likelihood ratio test with a p -value < 0.05 as the level of statistical significance was conducted to determine if the covariate being tested should be included in the logistic regression model, changes in the coefficients of the other covariates in the model was also considered in decision to retain a covariate in the model. A covariate that caused $>10\%$ change in the coefficients of the other covariates when exclude from the model was retained in the model to be evaluated as a confounder. In a similar way as described above for the logistic regression models a Partial Fisher's (F) tests with a p -value < 0.05 was used as the level of statistical significance in the linear regression model building procedure

This process was repeated until all the remaining covariates in the model were all of statistical significance or of known clinical significance to the association. Other covariates that were not included in the initial multivariable model due to lack of statistical significance were then also tested using the backward elimination selection process described above, any of these covariates that became statistically significant based on the likelihood ratio test or the Partial Fisher's (F) tests during these steps were included in the model, despite their initial exclusion to obtain the preliminary main effects model.

To assess the assumption that continuous variables in the preliminary main effects model, were linear in the logit scale, the design variable method was used, when this yielded non linear graphs, next fractional polynomials were utilized to confirm the linearity. In some instances in this analysis the assumption of continuous variables being linear in the logit was met, but in instances where it was not met for some continuous variables, these covariates were recoded using either quartiles or categorizations based on cutoff points of inflections on the non linear graph. This recoding process yielded multiple linear variables for the specific covariate that had been recorded which was now included into the model as quartiles or categorical variables in the final main effects multivariable model.

Possible effect modification by the interactions of pairs of covariates was also tested. To evaluate the significance of hypothesized interactions in the logistic regression models the Likelihood ratio tests was used and for the linear regression models the Partial Fisher's (F) tests was used, for comparison of the model including the interaction term to the main effects model. An analysis that indicated that there were no plausible significant effect modifiers of the association, hence no interaction terms were included in any of our final multivariable models for the final analysis. Goodness of fit of the models were assessed using the Hosmer-Lemeshow test. To assess model discrimination an Area under ROC curve was generated. In addition the Hosmer-Lemeshow's delta deviance test was used to assess poorly fit points which were identified as those having significant influence on the model deviance, in a similar way for the parameter estimates the Pregibon's delta beta test was used to detect influential points.

To assess the effect of the previously identified influential observations on our observed results a Sensitivity analysis was conducted to exclude these influential observations from the model prior to refitting the model. However the identified influential observations represented missing data hence the original final model was maintained. Further sensitivity analysis was done to determine if the exclusion of younger children (<10) or obese children and adolescents, from the final model changed the estimated effects. This analysis also did not yield any different results, hence the original final model was maintained.

Odds ratios and 95% confidence intervals were calculated for the logistic regression analysis, while regression coefficients and 95% confidence intervals were estimated for the linear regression analysis.

Stata version 11.0 was used for all analyses (Stata Corporation College Station, TX). Two-sided *p-values* ≤ 0.05 were considered statistically significant, with no adjustment for multiple comparisons.

I. Significance

To the best of our knowledge, to date no study has evaluated the relationship between vitamin D levels or deficiency, and abnormal blood lipid levels (dyslipidemia), in children with metabolic syndromes such as obesity and diabetes. Given the growing incidence of cardiovascular disease risk factors including dyslipidemia amongst children, findings could inform more aggressive lifestyle and dietary interventions with vitamin D supplementation to reduce the risk of dyslipidemia in high risk children. Other potential benefits include the possibility to estimate the prevalence of vitamin D deficiency in this

unique population of children in relation to their risk of dyslipidemia. An added benefit includes determination of whether the PEDRO database can be supported for future clinical research purposes.

J. Human Subjects Protection

This vitamin D study was approved by the Institutional Review Board (IRB) of the Baystate Medical Center. A secondary analysis on the data collected was also approved by the Institutional Review Board (IRB) of the School of Public Health Sciences, University of Massachusetts Amherst.

Strict measures were put in place to ensure confidentiality of patient data and adherence to the IRB protocol. Patient data were coded and de-identified after abstracting from the medical records into our database. The study database was securely stored on a password protected Baystate Health Center computer, to which only approved study staff had access to. Paper charts were immediately returned to the medical records office after patient data was obtained in a Baystate office. All study personnel were trained in privacy protocols.

With the exception of the unlikely occurrence of an accidental breach of confidentiality or data sharing, there were no known potential risks to study participants because there was no direct patient contact and no sensitive patient information was accessed. Also the training of study personnel ensured the adherence to privacy protocols. There also was minimal to no risks to the physical, psychological, legal, economic and social aspects of study subjects. Study participants had no additional benefits, however study findings had potential benefits to science and society including; the potential to

estimate the prevalence of vitamin D deficiency in children with diabetes or obesity and an attempt to relate the level of vitamin D to dyslipidemia in this population.

K. Permission to Access Data

Permission was sought from the Institutional Review Board (IRB) of the Baystate Medical Center, to share the de-identified data with study personnel at the University of Massachusetts, Amherst.

CHAPTER V

RESULTS

From the original study population of 743 participants, a total of 503 (67.7%) were excluded and 240 (32.3%) included in the final study sample (Table 2). The majority (n = 492, 66.2%) of the exclusions were due to a lack of vitamin D draw during the study time period (Table 2). The majority of the participants (n = 175, 72.9%) were vitamin D deficient with vitamin D levels ranging from 4.0 to 69.3 ng/ml, with a mean of 24.65 (standard deviation (SD) 10.33) (Table 3).

The majority of participants (60.8%) had normal total cholesterol (TC) measures (mean TC; 167 mg/dL (34.68 SD)) (Table 4). Overall, across all age groups (2 to 21 years), half of the study sample (50%), had normal TG levels and 47 (19.6%) had high TG levels. Half of the participants had sufficient HDL levels (49.6%) and (21.3%) had low levels. Only 16 (6.7%) of had high LDL with the majority (62.1%) having normal levels (Table 4).

The vitamin D deficient participants were more likely to be obese (42.2% body mass index (BMI) \geq 29.0) and (BMIZ score > 1.5) as compared to the patients with normal vitamin D levels (26.2% obese) although this was not statistically significant (p=0.121). Compared to patients with normal vitamin D levels mean weight, categorical BMIZ, mean serum calcium (Ca), mean parathyroid hormone (PTH) levels and ethnicity were significantly higher among vitamin D deficient patients (Table 5). On the contrary age, sex (gender), height, body mass index (BMI), mean BMI Z-score (BMIZ), vitamin D supplementation, glycated hemoglobin (HbA1C) and season did not differ significantly

between patients with normal vitamin D levels and those with vitamin D deficiency (Table 5).

We then evaluated whether covariates differed according to category of vitamin D deficiency (Table 6). Our observations were similar to those previously described with dichotomized vitamin D levels (Table 5), however, contrary to our prior observation age, BMI, and height, differed significantly among patients according to categories of vitamin D (Table 6). Specifically, severely vitamin D deficient participants were the oldest, tallest and had the highest PTH levels (Table 6).

We then evaluated the distribution of covariates according to continuous vitamin D levels; mean vitamin D levels differed significantly across categories of BMI, BMIZ, season and ethnicity (Table 7). The lowest mean vitamin D level occurred in winter, while the highest occurred in the summer season. Mean vitamin D levels were highest and almost equivalent amongst Caucasian and Asian patients, as compared to African Americans / Blacks (Table 7).

We then evaluated Pearson's and Spearman's correlation between continuous covariates and vitamin D levels. Weight, body mass index Z-score (BMIZ) and parathyroid hormone (PTH) were negatively and significantly correlated with vitamin D, while age, height, serum calcium (Ca) and glycated hemoglobin (HbA1C), were not significantly correlated (Table 8).

We then evaluated the distribution of covariates, according to normal vs. abnormal blood lipid levels (Table 9). Patients with high mean glycated hemoglobin (HbA1C) (poor control) were significantly more likely to have high TC as compared to those with low HbA1C ($P = 0.004$) (Table 9). Patients who were younger and heavier

were more likely to have high TG. Children who were Caucasian or Hispanic were more likely to have high TG as compared to children who were African American/Black. Patients who were heavier and those with acceptable glycated hemoglobin (HbA1C) levels were more likely to have low HDL.

We then evaluated the correlation between vitamin D and covariates with blood lipid levels (Table 10). Both vitamin D level and HbA1C were positively and significantly correlated with total cholesterol (TC); ($r = 0.18, P = 0.0084$) and ($r = 0.21, P = 0.0049$) respectively. Each unit increase in vitamin D level (vdl), resulted in a 21% decrease in TG; ($r = -0.21, P = 0.006$), on the contrary a one kilogram increase in weight and a unit increase in BMIZ lead to a corresponding increase in TG; ($r = 0.22, P = 0.0038$) and ($r = 0.32, P < 0.0001$) respectively (Table 10). Each unit increase in vitamin D level (vdl), age or HbA1C, corresponded to an increase in HDL (Table 10). The relationship between both weight and BMIZ with HDL were reversed and in both cases the observed inverse correlation was statistically significant; ($r = -0.35, P < 0.0001$) and; ($r = -0.21, P = 0.0074$) respectively (Table 10). Additionally each unit increase in vitamin D level (vdl), or height corresponded to a weak reverse effect on LDL levels; ($r = -0.17, P = 0.0265$) and ($r = -0.16, P = 0.0424$) respectively (Table 10).

In a bivariate analyses we assessed the crude relationship between our exposure variable (vitamin D dichotomized, categorized and continuous) and dichotomized levels of each of the four outcome variables (blood lipids) (Table 11). Participants with deficient levels of vitamin D were significantly more likely to have high TG, and low HDL but less likely to have high TC and high LDL compared to those with normal vitamin D levels (Table 11). Compared to patients with normal vitamin D levels, patients

with either vitamin D insufficiency, moderate or severe vitamin D deficiency were more likely to have low HDL, but less likely to have high TC, high TG and high LDL (Table 11). Mean vitamin D levels were more likely to lower among patients with high TG and low HDL but similar among patients with high TC and high LDL in comparison to vitamin D levels among patients with normal blood lipids (Table 11).

We then evaluated the association between vitamin D levels and the risk of dyslipidemia (Table 12). In an unadjusted logistic regression model, moderately deficient vitamin D patients had 1.68 times the odds of high total cholesterol compared to patients with normal vitamin D levels (95% Confidence Interval (95% CI; 0.72 to 3.94). After adjusting for age, BMIZ and HbA1C, findings were strengthened (OR = 2.9, 95% CI; 0.95 to 8.82) (Table 12). In an unadjusted logistic regression model, severely vitamin D deficient patients had 3.5 times the odds of high triglycerides, compared to patients with normal vitamin D levels (OR 3.50, 95CI% 1.25 to 9.79) (Table 12). A *P*-value for trend; 0.012 indicated a significant association between decreasing levels of vitamin D and the odds of high triglycerides. Adjustment for PTH, BMIZ and ethnicity strengthened the association, but confidence intervals were wide (OR 11.65, 95CI% 1.93 to 70.27) (Table 12). An overall trend of a positive association between decreased levels of vitamin D in relation to the risk of high triglyceride was observed (*P*-value for trend = 0.006) (Table 12).

In an unadjusted logistic regression model, a protective effect of high triglyceride was observed amongst patients with vitamin D levels above the mean value; irrespective of adjustments for potential confounding; unadjusted OR; 0.95 (95% CI: 0.91 to 0.98) and multivariable OR (0.91) adjusting for PTH, BMIZ and ethnicity 95% CI: 0.86 to 0.97

(Table 12). In an unadjusted logistic analysis vitamin D deficient patients had 3.06 times the odds of low high density lipoprotein as compared to patients with normal vitamin D levels. This observed risk was attenuated after adjusting for BMIZ and ethnicity (OR = 2.51, 95% CI: 0.94 to 6.69) (Table 12). In an adjusted logistic regression analysis moderately and severely vitamin D deficient patients had 4.02 and 5.24 times the odds of low high density lipoprotein respectively, as compared to patients with normal vitamin D levels (Table 12). After adjusting for confounding by BMIZ and ethnicity the risk amongst moderately vitamin D deficient patients was elevated while an attenuated risk was observed amongst severely vitamin D deficient patients (Table 12).

Finally, we evaluated the association between vitamin D levels and risk of dyslipidemia in a linear regression analysis (Table 13). After adjusting for potential confounding by gender, HbA1C, ethnicity and season, a one ng/mL change in vitamin D deficiency, vitamin D insufficiency, moderate or severe vitamin deficiency was associated with a mean increase of 14.82 mg/dL, 11.8 mg/dL, 21.75 mg/dL and 17.68 mg/dL in total cholesterol levels respectively (Table 13). On the contrary a one unit (ng/mL) change in continuous vitamin D levels was associated with a mean decrease of 0.58 mg/dL, in total cholesterol levels (Table 13). After adjusting for BMIZ, ethnicity and parathyroid hormone (PTH), in comparison to patients with normal vitamin D, deficiency in patients was positively associated with the risk of high triglycerides (TG) (Table 13). A positive and significant association was observed between categorical vitamin D levels and the risk of high TG, although the trend was not uniform across strata; *P* value for trend = 0.002 (Table 13). Each one unit (ng/mL) reduction in vitamin D levels was associated with a 2.14 mg/dL increase in high TG levels, *P* - value for trend

= <0.001) (Table 13). Across strata of categorical vitamin D levels the magnitude of association between vitamin D levels and the risk of low HDL, increased with decreasing levels of vitamin D deficiency (Table 13). Similarly each one ng/mL increase in vitamin D levels was associated with a 0.37 increase in the risk of low HDL, *P* - value for trend (0.004) (Table 13). Lastly, each one ng/mL decrease in vitamin D levels was associated with a mean increase of 0.51 mg/dL in the risk of high LDL, *P* - value for trend (0.019), although there was no consistent association across increasing category of vitamin D deficiency.

CHAPTER VI

DISCUSSION

In this cross-sectional analysis of the relationship between vitamin D levels and dyslipidemia, we observed that children and adolescents with moderate vitamin D deficiency had an almost 3-fold increased risk of high TC compared to children with normal vitamin D levels. Severe vitamin D deficiency was associated with a strong and significant increased risk of low HDL as well as high TG as compared to children with normal vitamin D levels; with a significant dose-response relationship. We did not observe a statistically significant relationship between vitamin D deficiency and neither high TC nor high LDL, however there was the suggestion of an increased risk. The lack of association with high LDL could possibly be due to the small number of cases of high LDL and therefore limited power. In addition, in linear regression analyses, we did find that an increase in vitamin D deficiency was associated with a significant mean increase in all four measures of dyslipidemia.

A. Study Limitations

1. Nondifferential Misclassification of Exposure

Although our measures of 25-hydroxy vitamin D levels (exposure) came from validated standardized laboratory tests done at the Baystate Health Center's laboratory, the exposure assessment was via laboratory assays, for which there could have been a slight chance of random measurement inaccuracies occurring as a result of human factors such as analysis setup and interpretation and recording of results, which could have led to

slight variations or inaccuracies in our 25-hydroxy vitamin D measures. These random errors however should not have differed amongst our blood lipid levels in a systematic way, hence it is unlikely that some non-differential misclassification of exposure could have occurred via this avenue, which would have potentially led to an underestimation of our effects estimates and biased our results towards the null value, by equally distorting the true vitamin D levels amongst dyslipidemia cases and non-cases. We categorized our exposure (vitamin D levels), using widely used cutoff points to minimize the impact of non-differential misclassification of exposure on our effect estimates. Hence within these categories each participant's, exposure level was accurately represented and a comparison between these categories could then help minimize possible misclassification between vitamin D categories in relation to dyslipidemia status. Additionally this error was minimized by using trained laboratory personnel in obtaining our laboratory measures on exposure.

Secondly, due to the observational measure of our study we used single measurements of serum 25-hydroxy vitamin D as a proxy for the vitamin D status of our study participants, although this is thought to be the best measure of vitamin D status and hence is widely used. This measure however may not reflect long term vitamin D status, because serum 25(OH) D, which indicates vitamin D made cutaneously as well as that derived from diet and supplements has a half – life of 15 days (23). Hence, although we collected data on vitamin D supplementation, our reliance on serum 25-hydroxy vitamin D may have introduced some nondifferential misclassification in our evaluation of vitamin D status. The occurrence of this nondifferential misclassification could have potentially biased our results towards the null and thereby resulted in a reduction of the

effect estimates for our relationship between 25-hydroxy vitamin D levels (25(OH) D) and dyslipidemia in these children. The effect of this misclassification would have been minimal, because proxy measures such as those we used are considered the best measure of vitamin D status and is widely used and accepted in analysis.

2. Nondifferential Misclassification of Outcome

Blood lipid levels were abstracted from laboratory results documented in medical records for patients with a diagnosis of obesity and or type 1 and 2 diabetes mellitus using specific International Classification of Disease version 9 (ICD 9) codes for these two disease conditions. Also all cases of blood lipid levels were confirmed by a trained investigator through chart review. Two trained physicians checked and verified the outcome measures in the database. If during the data abstraction process however, cases of dyslipidemia were incorrectly entered into the database, this could have attenuated our effect estimates and biased our results towards the null, but the occurrence of this is highly unlikely, because of the data verification steps employed to ensure that the data was accurately abstracted from the medical charts and entered correctly into our database.

If some study participants did not fast 12 hours prior to their laboratory testing as was required, their measurements of the abnormal blood lipid may have been artificially elevated and not reflective of their true values and as such could have led to an underestimation of the effect estimates and biased our results towards the null value. The occurrence of this situation was highly unlikely because participants were advised to fast before the laboratory testing and their fasting status was verbally ascertained prior to testing via self report. Studies show that TG in particular is elevated postprandial

although the physiological mechanism is poorly understood (24, 25). In contrast, in comparison to fasting lipid profiles of the general population, Langsted *et al.* (2008) observed negligible decreases in levels of TC, HDL and LDL measured postprandial (25).

We relied on a single measurement of blood lipids, but both analytical inaccuracies and imprecision resulting from laboratory methods of blood lipid assessment as well as biologic or physiologic variation can influence blood lipid levels (26-28). In terms of inaccuracy resulting from laboratory methods, previous repeatability (within-run precision) and intermediate precision (total precision / between run precision / between day precision) validation analyses done at the Baystate laboratory have indicated relatively small coefficients of variation.

In terms of inaccuracy resulting from use of a single blood lipid measure to accurately represent an individual's long term blood lipid profile, Kafonek *et al.* (1992), evaluated the biological variability in TC, HDL, LDL and TG. The authors calculated physiological coefficients of variations (CVp) to take into account the total physiological and analytical variations per patient from triplicate samples, taken at baseline from each patient prior to lipid control treatment. They observed the following median physiological coefficients of variation: 5.0% for TC, 17.8% for TG, 7.1% for HDL and 7.8% for LDL and concluded that when triplicate blood samples are obtained in sequence, a single measure of blood lipids obtained from these analysis will be representative of an individual's blood lipid levels within these ranges of precision (± 1 , ± 4 , ± 11 and ± 8 CV% for TC, TG, HDL and LDL respectively) (27). It has been reported that physiologic variation could result in a 3%, or 5% or 10% change in serum TC levels (26). Shumak *et*

al (1993) suggests that a non linear decline in the CVs is noted with multiple lipid sampling per person; the authors observed CVs ranging between approximately 4% to 10% for TC, 7% to 14% for LDL, 6% to 13% for HDL and 19% to 51% for TG (26). Additionally, for example, the CV in females change from 8.6% to 6.5% after duplicate cholesterol sampling done individually, and a 50% reduction in the CV can be obtained from calculations from five cholesterol measures. Based on these observations Shumak *et al* (1993), concluded that although repeated lipid measures prior to lipid control therapy decisions have been suggested, very little precision is achieved via repeated measurements.

In an evaluation of the impact of physiologic and analytical variations on the use of a single lipid measure, to accurately assign risk categories in 51 individuals from triplicate lipid samples, Bookstein *et al.* (1990) observed 5% day-to-day variability in TC, 20% in TG, 10% in HDL and 8% in calculated LDL respectively (28). However, Bookstein *et al.* (1990) also observed that, reliable TC and LDL measures, based on the NCEP guidelines may be obtained from a single lipid measurement, if measured TC levels were < 185 mg/dL or ranged between 215 to 225 mg/dL or was >225 mg/dL, likewise for LDL, reliable measures could be obtained for LDL levels < 116 mg/dL or > 174 mg/dL from single lipid measures (28). Thus misclassification due to variations in lipid levels may be unavoidable in the context of single or duplicate lipid level sampling, and particularly when lipid levels fall within 10% of the documented NCEP cutoff points (28).

In summary, our use of single measurements of lipid values, which are susceptible to physiologic (natural) variations, may have led to misclassification of patients risk categories (26).

3. Selection Bias

Due to the uniform admission of participants into our study, using a uniformly applied selection criteria as defined by the availability of data on the first measured value of 25 (OH) D levels within the study period, selection bias was highly unlikely in our study. Also knowledge of the dyslipidemia of study participants was masked or unknown to the physicians and investigator in the selection of study participants into the study. For example, selection bias could only have occurred, if study participants were selected into the study based on their diagnosis of dyslipidemia, which in this case was not the situation, or if patients with vitamin D deficiency and dyslipidemia were sicker than other patients and as such were more motivated to follow through with the physician referral and hence had a higher chance of being selected into our study compared to other participants. Although this was not the case, if this had happened it would have led us, to miss some participants who had normal vitamin D levels or normal blood lipid levels. This then could have led to an overestimation of our effects estimates and our results would have been biased away from the null value, this we believe was an unlikely occurrence in our study.

Another avenue for selection bias to have occurred in our study was due to the fact that 492 patients (66.2%), out of the total original study sample of 743 (100%) patients, were excluded from our final study sample due to lack of vitamin D draw in the

study period. Ideally we would have liked to conduct a sensitivity analysis comparing the characteristics of the excluded patients to those included in the final study sample, to assess the potential of selection bias in our study via patient inclusion or exclusion criteria. However, we were unable to do this because our study database lacked data on the excluded patients.

4. Information Bias

The physicians were blinded to the vitamin D and dyslipidemia disease status of our study participants thus reducing the possible occurrence of information bias, through the data validation process. Surveillance bias was also very unlikely in our study design since the data collected was from laboratory records. No information was gathered from the participants hence there was no possibility of recall bias in the exposure data collection from our study participants. Another possibility of information bias would have been due to variations in the assessments of individual measures of 25(OH) D levels and blood lipid levels due to variations in the time at which each participant had these measure taken. The time varied within our study period, hence some patients may have been visiting the diabetes management clinic and receiving some counseling and if they were compliant to the recommendations for a healthy lifestyle and as such had changed their diet and lifestyle prior to the assessment of 25(OH) D) and blood lipid levels, the additional counseling could have led these patients to modify lifestyle and diet which would have impacted the blood test results. If this had happened then these patients could for example have taken unprescribed vitamin D supplements or multivitamins or intentionally eaten foods known to be vitamin D rich as well as low fat foods as a way of

managing their vitamin D deficiency and dyslipidemia status which they had become aware of after consulting with a physician. This could then have led to an underestimation of their true effects estimates within our study period and biased our results towards the null value, because they would have already started managing their vitamin D levels and dyslipidemia status before data assessment was done for our study.

5. Temporal Bias

One major limitation of our cross-sectional study design, due to its observational nature was our lack of temporality between vitamin D levels and the risk of dyslipidemia (temporal relationship between exposure and the occurrence of the disease). Hence causality could not be established due to the difficulty in demonstrating if the vitamin D deficiency preceded the dyslipidemia. Despite this inability to establish a cause-and-effect relationship the study granted us a hypothesis generating ability. In addition the extreme obesity of the study participants may have masked the relevant associations between vitamin D deficiency and dyslipidemia. This could have happened because vitamin D is a fat soluble vitamin, hence when in excess it is stored in body fat, therefore for individuals who were obese and had excess fat stores, the bulk of their vitamin D may have been trapped and stored in the excess fat hence, increasing their risk of vitamin D deficiency (7). As such, the observed significant increase in odds of high TC, low HDL as well as high TG amongst children and adolescents with below optimal vitamin D levels compared to children with normal vitamin D levels, could possibly be due to the fact that some of their serum vitamin D was being trapped within the elevated serum blood lipid levels, implying a possible reverse causality. A randomized clinical trial

would be needed to assess this possible reverse causality and thus ascertain the relationship between vitamin D and dyslipidemia in these children and adolescents.

6. Confounding

Within our multivariable linear and logistic regression model, we controlled for and evaluated many known risk factors for dyslipidemia and other cardiovascular disease risk factors recognized in literature as potential confounders of our vitamin D and lipid levels association, using a Directed Acyclic Graph (DAG).

Adequate sunlight exposure and normal vitamin D levels are highly positively correlated. Both vitamin D deficiency (exposure) and dyslipidemia (high TC, high TG, high LDL and low HDL - disease) are independently and negatively associated with adequate sunlight exposure. Yamazaki *et al.* (1998), observed significant increases (10.0 ± 6.7 to 38.9 ± 38.0 pmol/cm²) in the levels of cholesterol hydro peroxides (Ch 7-OOHs) in human skins after three hours of 10 – 40mJ/cm²/min of sunlight exposure (29). Hence if adequate sunlight exposure was not accurately controlled for, it could have acted as a confounder and would have led to an overestimation of our crude estimates (results) in relation to the null value. We minimized this concern by evaluating data on seasons of vitamin D level measurements within our models, this we did by collecting data on exposure and outcome for participants within a two year period that encompassed duplicates of the four seasons of the Northeastern US. Thereby reducing concerns regarding for variations in vitamin D draws due to change in sunlight exposure as a result of seasonal variations. Hence we believe that the occurrence of confounding due to inadequate sunlight exposure evaluation in our study was highly unlikely.

Our dataset did not include information on the physical activity of participants, which is negatively and independently associated with both vitamin D deficiency and dyslipidemia (high TC, high TG, high LDL and low HDL disease). Therefore not controlling for it in our multivariable analysis could have led to an overestimation of the association between vitamin D deficiency and dyslipidemia. We believe the effect of not controlling for physical activity as a variable was minimized because we included BMI and BMI Z-scores in our analysis and BMI is highly correlated with physical activity. However, there is still likely residual confounding due to physical activity which we were unable to adjust for because our dataset had no information on directly measured physical activity of our patients.

7. Generalizability

Our results may not be generalizable to the general population of children and adolescents, but may only be generalized to children and adolescents aged 2 to 21 years, with a diagnosis of type 1 and type 2 diabetes mellitus and / or obesity (BMI >95th percentile), living in geographical regions or temperate countries with seasonal variations similar to the Northeast US, with no gastrointestinal malabsorptive disorder (i.e. celiac disease, cystic fibrosis) and no known parathyroid disease.

It has been established that vitamin D levels are lower in darker pigmented people than light skinned people (7, 30). Hence, differences in skin pigmentation may cause biological differences to exist in how vitamin D deficiency influences dyslipidemia and thus possibly limit the generalizability of our findings to children and adolescents with

skin pigmentations similar to that of our study population (mainly light skinned participants).

B. Consistency with Prior Literature

Despite immense recent interest in vitamin D deficiency and dyslipidemia in children and adolescents, only three out of the eleven epidemiologic studies investigated this association in children and adolescents ≤ 20 years old; Kumar *et al* (2009), Reis *et al* (2009) and Ashraf *et al* (2009). Our findings were inconsistent with that of Kumar *et al* (2009), who in their cross-sectional study design, observed amongst vitamin D insufficient participants in comparison to vitamin D sufficient participants, a significant difference in the decline in high density lipoprotein (HDL) (difference: -2.29 mg/dL; 95% CI -3.57 to -1.01, $p = 0.001$) and total cholesterol levels, (difference: -3.66 mg/dL; 95% CI -7.09 to -0.23, $p = 0.04$) respectively, additionally vitamin D deficiency was observed to be associated with low levels of HDL cholesterol (difference: -3.03 mg/dL, 95% CI -5.02 to -1.04, $p = 0.004$) irrespective of adiposity or obesity. In contrast, we found statistically nonsignificant odds ratios (OR) of 1.52 (95% CI 0.50 to 4.56) and OR of 2.51 (95% CI 0.94 to 6.69) amongst vitamin D insufficient and deficient participants respectively, in comparison to vitamin D sufficient participants in relation to the risk of low HDL. We also observed no significant associations between vitamin D deficiency amongst patients in comparison to normal vitamin D levels and the risk of high TC (OR = 1.95; 95% CI: 0.85 to 4.46) .

Both Reis *et al.* and Ashraf *et al.* observed null findings in their cross-sectional design studies. Despite their relatively large study sample, Reis *et al.* did not observe

significant associations between levels (lowest quartiles compared to highest) of vitamin D with either low high density lipoprotein (adjusted OR: 1.54, 95% CI: 0.99 to 2.39) or high triglycerides levels (adjusted OR: 1.00, 95% CI: 0.49 to 2.04). Similarly, Ashraf *et al* (2009), found no significant association between serum 25 (OH) vitamin D and either; total cholesterol ($r = -0.011$, $p = 0.94$), triglycerides ($r = 0.067$, $p = 0.64$), HDL ($r = 0.012$, $p = 0.94$), or low density lipoprotein ($r = 0.037$, $p = 0.80$). In contrast we observed: ($r = 0.18$, $p = 0.0084$), ($r = -0.21$, $p = 0.006$), ($r = 0.22$, $p = 0.0039$) and ($r = 0.17$, $p = 0.0265$) for the same comparisons. Differences in findings between our study and that of these 3 prior studies among youth may be due to lack of consistency in the categorization of both exposure and outcome, variations in covariate assessments such as inadequate adjustment of confounding leading to the possibility of residual confounding, or unevaluated effect modification and differences in study population characteristics and distribution.

Although the biological mechanism linking vitamin deficiency to the risk of dyslipidemia is still poorly understood, there are three proposed possible indirect mechanisms. The first mechanism involves the activation of the vitamin D receptor; the second mechanism is a possible consequence of the effect of statin drug therapy on the disruption of the mevalonate pathway (HMG-CoA reductase pathway); and the third mechanism is as a result of the elevation of peripheral insulin resistance in the body.

C. Significance

Our findings relate vitamin D deficiency to dyslipidemia in children with diabetes and obesity and add to the sparse body of literature in this area. We found that children

and adolescents with varying levels of vitamin D deficiency had significantly increased risk of high TC, low HDL, and high TG. Findings can inform more aggressive lifestyle and dietary interventions with vitamin D supplementation to reduce the risk of dyslipidemia in high risk children. Findings also shed light on the prevalence of vitamin D deficiency in this unique population of children and adolescents.

Table 1. Classification of study variables: Baystate Medical Children’s Hospital vitamin D deficiency and dyslipidemia study, 2008 to 2010, (n=240).

Name	Description	Type
Outcome variables		
TCcat	Total Cholesterol ≥ 180 mg/dL (high) < 180 mg/dL (normal)	Dichotomous
TC	continuous TC	continuous
TGcatlo	Triglycerides (cut points for 2 to <10 years) ≥ 110mg/dL (high) < 110mg/dL (normal)	Dichotomous
TGcathi	Triglycerides (cut points for 10 to 21 years) ≥ 130mg/dL (high) < 130mg/dL (normal)	Dichotomous
Tgcatagett	Triglycerides: binary classification for all ages (2 to 21 years) High Normal	Dichotomous
TG	continuous TG	continuous
HDLcat	High density lipoprotein cholesterol < 40mg/dL (low) ≥ 40mg/dL (sufficient)	Dichotomous
HDL	continuous HDL	continuous
LDLcat	Low density lipoprotein – cholesterol ≥ 130mg/dL (high) < 130mg/dL (normal)	Dichotomous
LDL	continuous LDL	continuous
Exposure variable		
VDLcat4	25-OH vitamin D levels < 15.00 ng/ml (severe deficiency) 15.00 – 19.99 ng/ml (moderate deficiency) 20.00 – 29.99 ng/ml (25(OH)D insufficiency) ≥30.00 ng/ml (normal or optimal)	Categorical
VDLcat2	25-OH vitamin D deficiency <30.00 ng/ml (Deficient) ≥30.00 ng/ml (Normal)	Dichotomous
VDL	Continuous vitamin D levels	Continuous

Table 1. contd.

Covariates

Agecat2	Age at study enrollment and assessment 1= 2 to <10 years 2 = 10 to 21 years	Categorical
Age continuous	Individual ages at study enrollment and assessment	continuous
Sex	Gender 1 = Male 2 = Female	Dichotomous
Ca	Serum Calcium	Continuous
Wgt	Weight in kg.	Continuous
Hgt	Height in cm	Continuous
BMIcat4	Body Mass Index 1 = BMI < 19.8 kg/cm ² (Underweight) 2 = BMI 19.8 - 26.0 kg/cm ² (Normal) 3 = BMI > 26.0 - 29.0 kg/cm ² (Overweight) 4 = BMI ≥ 29.0 kg/cm ² (Obese)	Categorical
BMIZ	BMI Z score	Continuous
BMIZcat	BMI Z score categories 1 = BMIZ >1.5 (Overweight / Obese) 0 = BMIZ ≤1.5 (Normal)	Categorical
A1C	Glycated or glycosylated hemoglobin (HbA1C)	Continuous
A1Ccat	Glycated or glycosylated hemoglobin 1 = HbA1C >8.0 (Bad HbA1C control) 0 = BMIZ ≤8.0 (Good HbA1C control)	Categorical
PTH	Parathyroid Hormone	Continuous
VDS	Vitamin D supplementation 1= Yes 2= No	Categorical
SEASON	Seasons of the year in US 1= Winter 2= Spring 3= Summer 4= Fall	Categorical (Nominal)
ETH	Ethnicity 1 = Caucasian 2 = Asian 3 = African American / Black 4 = Hispanic / Latino 5 = Other	Categorical (Nominal)

Table 2. Characteristics of Baystate vitamin D deficiency and dyslipidemia
2008- 2010 study population: inclusion and exclusion criteria (n=240)

	N	%
Original study sample	743	100
Exclusion criteria		
No vitamin D draw in study date range	492	66.2
Medical visits outside study dates	1	0.1
Medical based exclusion	7	0.9
Age exclusion (<2 age >21 years)	3	0.4
Multiple exclusion criteria	5	0.7
Total Excluded	503	67.7
Final study sample	240	32.3
By diagnostic group		
Type I Diabetes	128	53.3
Type II Diabetes	13	5.4
Obesity	99	41.3

Table 3. Distribution of 25-OH vitamin D measures and levels among Baystate vitamin D, 2008- 2010 study population (n=240).

	N	%
25-OH Vitamin D levels		
<30.00 ng/ml (Deficient)	175	72.9
≥30.00 ng/ml (Normal)	65	27.1
	N	%
25-OH Vitamin D levels		
< 15.00 ng/ml (Severe deficiency)	42	17.5
15.00 – 19.99 ng/ml (Moderate deficiency)	46	19.2
20.00 – 29.99 ng/ml (Insufficiency)	87	36.3
≥30.00 ng/ml (Normal or Optimal)	65	27.1
	Mean (SD)	Median (Range)
	24.65	
25-OH Vitamin D measures (ng/ml)	(10.33)	23.2 (4 - 69.3)

Table 4. Distribution of blood lipid levels among Baystate vitamin D, 2008- 2010 study population.

	N	%
Total Cholesterol (TC)		
≥ 180 mg/dL (high)	69	28.8
< 180 mg/dL (normal)	146	60.8
Total	215	89.6
	Mean (SD)	Median (Range)
TC - continuous	167.0 (34.68)	166 (77 -336)
Triglyceride (TG 2 to <10 years)		
≥ 110mg/dL (high)	10	29.4
< 110mg/dL (normal)	10	29.4
Total	20	58.8
Triglyceride (TG 10 to 21 years)		
≥ 130mg/dL (high)	37	18
< 130mg/dL (normal)	110	53.4
Total	147	71.4
Triglyceride (TG 2 to 21 years)		
High	47	19.6
Normal	120	50
Total	167	69.6
	Mean (SD)	Median (Range)
TG - continuous	109.0 (60.85)	91 (20 - 518)
High density Lipoprotein cholesterol (HDL-C)		
< 40mg/dL (low)	51	21.3
≥ 40mg/dL (sufficient)	119	49.6
Total	170	70.8
	Mean (SD)	Median (Range)
HDL - continuous	48.5 (13.46)	46 (20 - 88)
Low density Lipoprotein cholesterol (LDL-C)		
≥ 130mg/dL (high)	16	6.7
< 130mg/dL (normal)	149	62.1
Total	165	68.8
	Mean (SD)	Median (Range)
LDL - continuous	95.9 (29.93)	94 (30 - 251)

Table 5. Distribution of covariates according to dichotomous 25-OH vitamin D levels: Baystate vitamin D 2008- 2010 study population (n = 240).

	25-OH Vitamin D levels		p value*
	Deficient	Normal	
n	175 (72.9%)	65(27.1%)	
Age, categorized (Agecat2)			
2 to <10 years	23(13.1%)	11(16.9%)	0.455
10 to 21 years	152 (86.9%)	54 (83.1%)	
Age	13.92 (3.55 SD)	14.76 (3.66 SD)	0.1055
Sex			0.985
Male	81 (46.3%)	30 (46.2%)	
Female	94 (53.7%)	35(53.9%)	
Weight	76.35 (32.71SD)	68.01 (22.29SD)	0.0589
Height	158.10 (16.14SD)	159.94 (13.07SD)	0.4121
BMI			0.121
BMI < 19.8 (Underweight)	28 (16.2%)	11 (16.9%)	
BMI 19.8 - 26.0 (Normal)	52 (30.1%)	28 (43.1%)	
BMI > 26.0 - 29.0 (Overweight)	20 (11.6%)	9 (13.9%)	
BMI ≥ 29.0 (Obese)	73(42.2%)	17(26.2%)	
BMI Z-score (BMIZ)	2.25(7.54SD)	1.31 (0.90SD)	0.3195
BMIZ categorical			0.016
BMIZ > 1.5 (Obese)	104(60.5%)	28(43.1%)	
BMIZ ≤ 1.5 (Normal)	68(39.5%)	37(56.9%)	
Vitamin D supplementation			0.341
Yes	4 (2.3%)	3 (4.6%)	
No	171 (97.7%)	62 (95.4%)	
Serum Calcium (Ca)	9.83 (0.37SD)	9.70 (0.48 SD)	0.0479
Glycated hemoglobin (HbA1C)			0.704
HbA1C ≤ 8 (Acceptable)	85 (60.7%)	30 (57.7%)	
HbA1C > 8 (Bad control)	55 (39.3%)	22 (42.3%)	
Glycated hemoglobin (HbA1C)	7.80 (2.11 SD)	7.84 (1.47 SD)	0.9023
Parathyroid Hormone (PTH)	36.81 (14.99SD)	31.95 (8.70SD)	0.022
Seasons of the year in US			0.103
Winter	34 (19.4%)	5 (7.7%)	
Spring	33 (18.9%)	10 (15.4%)	
Summer	59 (33.7%)	29 (44.6%)	
Fall	49 (28.0%)	21 (32.3%)	
Ethnicity			0.029
Caucasian	117 (77.0%)	56 (93.3%)	
Asian	6 (4.0%)	3 (5.0%)	
African American / Black	23 (15.0%)	1 (1.7%)	
Hispanic / Latino	3 (2.0%)	0 (0.0%)	
Other	3 (2.0%)	0 (0.0%)	

* P value derived from two sample *t* test for continuous variables and from chi - square test for dichotomous or categorical variables.

Table 6. Distribution of covariates according to categorical 25-OH Vitamin D levels: Baystate vitamin D, 2008-2010 study (n = 240).

	25-OH Vitamin D levels				p value*
	Severe deficiency	Moderate deficiency	Insufficiency	Normal	
	N(%)	N(%)	N(%)	N(%)	
n	42 (17.5%)	46 (19.2%)	87 (36.3%)	65 (27.1%)	
Age					0.025
2 to <10 years	1 (2.4%)	4 (8.7%)	18 (20.7%)	11 (16.9%)	
10 to 21 years	41 (97.6%)	42 (91.3%)	69 (79.3%)	54 (83.1%)	
Sex					0.985
Male	20 (47.6%)	22 (47.8%)	39 (44.8%)	30 (46.2%)	
Female	22 (52.4%)	24 (52.2%)	48 (55.2%)	35 (53.9%)	
BMI					<0.001
BMI < 19.8 (Underweight)	4 (9.5%)	5 (11.1%)	19 (22.1%)	11 (16.9%)	
BMI 19.8 - 26.0 (Normal)	6 (14.3%)	11 (24.4%)	35 (40.7%)	28 (43.1%)	
BMI > 26.0 - 29.0 (Overweight)	4 (9.5%)	6 (13.3%)	10 (11.6%)	9 (13.9%)	
BMI ≥ 29.0 (Obese)	28 (66.7%)	23 (51.1%)	22 (25.6%)	17 (26.2%)	
BMIZ categorical					<0.001
BMIZ > 1.5 (Obese)	34 (81.0%)	30 (68.2%)	40 (46.5%)	28 (43.1%)	
BMIZ ≤ 1.5 (Normal)	8 (19.1%)	14 (31.8%)	46 (53.5%)	37 (56.9%)	
Vitamin D supplementation					0.446
Yes	2 (4.7%)	0 (0.0%)	2 (2.3%)	3 (4.6%)	
No	40 (95.2%)	46 (100.0%)	85 (97.7%)	62 (95.4%)	

Table 6 continued on next page.

Table 6 continued from previous page. Distribution of covariates according to categorical 25-OH Vitamin D levels: Baystate vitamin D, 2008-2010 study (n = 240).

	25-OH Vitamin D levels				p value*
	Severe deficiency	Moderate deficiency	Insufficiency	Normal	
	N(%)	N(%)	N(%)	N(%)	
Glycated hemoglobin (HbA1C)					0.209
HbA1C ≤ 8 (Acceptable)	22 (68.8%)	15 (45.5%)	48 (64.0%)	30 (57.7%)	
HbA1C > 8 (Bad control)	10 (31.3%)	18 (54.6%)	27 (36.0%)	22 (42.3%)	
Seasons of the year in US					0.343
Winter	8 (19.1%)	12 (26.1%)	14 (16.1%)	5 (7.7%)	
Spring	10 (23.8%)	8 (17.4%)	15 (17.2%)	10 (15.4%)	
Summer	12 (28.6%)	16 (34.8%)	31 (35.6%)	29 (44.6%)	
Fall	12 (28.6%)	10 (21.7%)	27 (31.0%)	21 (32.3%)	
Ethnicity					0.013
Caucasian	23 (62.2%)	25 (71.3%)	69 (86.3 %)	56 (93.3%)	
Asian	2 (5.4 %)	1 (2.9%)	3 (3.8%)	3 (5.0%)	
African American / Black	9 (24.3%)	7 (20.0%)	7 (8.8%)	1 (1.7%)	
Hispanic / Latino	2 (5.4%)	1 (2.9%)	0 (0.0%)	0 (0.0%)	
Other	1 (2.7%)	1 (2.9%)	1 (1.3%)	0 (0.0%)	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Age	15.26 (±2.87)	13.89 (±2.87)	13.29 (±4.00)	14.76 (±3.66)	0.01
Weight	95.32 (±36.07)	80.51 (±30.78)	65.01 (±27.10)	68.01 (±22.29)	<0.0001
Height	163.20 (±12.46)	159.32 (±14.01)	154.99 (±18.09)	159.94 (±13.07)	0.0264
BMIZ	4.42 (±15.06)	1.74 (±1.02)	1.45 (±1.09)	1.31 (±0.90)	0.0608
Serum Calcium (Ca)	9.85 (±0.32)	9.90 (±0.39)	9.79 (±0.38)	9.70 (±0.48)	0.1268
Glycated hemoglobin (HbA1C)	7.43 (±2.03)	8.51 (±2.69)	7.64 (±1.80)	7.84 (±1.47)	0.1137
Parathyroid Hormone (PTH)	46.63 (±18.89)	37.91 (±12.60)	31.78 (±11.59)	31.95 (±8.70)	<0.0001

* P- value derived from ANOVA for continuous variables and from chi square test for dichotomous or categorical variables

Table 7. Distribution of covariates according to continuous 25-OH Vitamin D measures: Baystate vitamin D, 2008- 2010 study population (n=240).

	25-OH Vitamin D continuous		
	Mean	SE	p value*
Age			0.2131
2 to <10 years	26.70	1.18	
10 to 21 years	24.32	0.75	
Sex			0.766
Male	24.44	0.96	
Female	24.84	0.92	
BMI			0.0001
BMI < 19.8 (Underweight)	27.23	1.81	
BMI 19.8 - 26.0 (Normal)	27.53	1.05	
BMI > 26.0 - 29.0 (Overweight)	25.72	2.02	
BMI \geq 29.0 (Obese)	20.77	0.99	
BMIZ categorical			<0.0001
BMIZ > 1.5 (Obese)	22.22	0.83	
BMIZ \leq 1.5 (Normal)	27.90	1.03	
Vitamin D supplementation			0.6113
Yes	26.61	4.29	
No	24.60	0.68	
Glycated hemoglobin (HbA1C)			0.8744
HbA1C \leq 8 (Acceptable)	25.20	1.05	
HbA1C > 8 (Bad control)	24.95	1.07	
Seasons of the year in US			0.0066
Winter	20.58	1.33	
Spring	22.88	1.30	
Summer	27.02	1.28	
Fall	25.03	1.11	
Ethnicity			0.0001
Caucasian	26.12	0.75	
Asian	26.23	3.76	
African American / Black	17.14	1.49	
Hispanic / Latino	13.13	3.23	
Other	17.93	3.38	

* P value derived from ANOVA for continuous variables and from chi square test for dichotomous or categorical variables. * ^a P value testing for statistical significance in trends.

Table 8. Distribution of continuous covariates according to continuous 25-OH Vitamin D measures: Baystate vitamin D 2008-2010 study (n = 240).

	25-OH Vitamin D continuous			
	Pearson correlation (r)	p value*	Spearman correlation (r)	p value*
Age	0.03	0.6025 ^a	-0.01	0.9154
Weight	-0.27	<0.0001 ^a	-0.28	<0.0001
Height	-0.03	0.6542 ^a	-0.05	0.4734
BMIZ	-0.11	0.0917 ^a	-0.32	<0.0001
Serum Calcium (Ca)	-0.1	0.152 ^a	-0.1	0.1493
Glycated hemoglobin (HbA1C)	-0.03	0.7305 ^a	0.04	0.5415
Parathyroid Hormone (PTH)	-0.31	<0.0001 ^a	-0.29	<0.0001

* ^a P value testing for statistical significance in trends (Pearson's)

* P value testing for statistical significance in trends (Spearman's)

Table 9 for TC and TG. Distribution of covariates according to dichotomous blood lipid levels: Baystate vitamin D 2008- 2010 study population (n = 240).

	Total Cholesterol (TC mg/dL)			Triglyceride (TG mg/dL)		
	High	Normal	p value*	High	Normal	p value*
N	69 (28.8%)	146 (60.8%)		47 (19.6%)	120 (50.0%)	
Age			0.896			0.021
2 to <10 years	9 (13.0%)	20 (13.7%)		10 (21.3%)	10 (8.3%)	
10 to 21 years	60 (87.0%)	126 (86.3%)		37 (78.7%)	110 (91.7%)	
Age	14.83 (3.60SD)	13.92 (3.51 SD)	0.0789	13.61 (3.84 SD)	14.52 (3.25 SD)	0.1218
Sex			0.187			0.482
Male	26 (37.7%)	69(47.3%)		22 (46.8%)	49 (40.8%)	
Female	43 (62.3%)	77 (52.7%)		25 (53.2%)	71 (59.2%)	
Weight	75.48 (32.57 SD)	72.70 (27.46 SD)	0.5149	84.99 (33.70 SD)	76.12 (27.82SD)	0.0835
Height	158.78 (13.98 SD)	158.76 (15.40 SD)	0.996	157.67 (14.68 SD)	160.24 (13.67 SD)	0.2865
BMI			0.847			0.024
BMI < 19.8 (Underweight)	10 (14.7%)	22 (15.1%)		2 (4.4%)	13 (10.8%)	
BMI 19.8 - 26.0 (Normal)	24 (35.3%)	51 (34.9%)		8 (17.4%)	43 (35.8%)	
BMI > 26.0 - 29.0 (Overweight)	7 (10.3%)	21 (14.4%)		7 (15.2%)	17 (14.2%)	
BMI ≥ 29.0 (Obese)	27 (39.7%)	52 (35.6%)		29 (63.0%)	47 (39.2%)	
BMIZ	2.99 (11.93 SD)	1.59 (1.01 SD)	0.1599	4.31 (14.38 SD)	1.60 (1.00 SD)	0.0418
BMIZ categorical			0.43			0.002
BMIZ > 1.5 (Obese)	35 (51.5%)	83 (57.2%)		38 (82.6%)	68 (57.1%)	
BMIZ ≤ 1.5 (Normal)	33 (48.5%)	62 (42.8%)		8 (17.4%)	51 (42.9%)	

Table 9. Continued on next page:

Table 9 for TC and TG. Continued from previous page: Distribution of covariates according to dichotomous blood lipid levels: Baystate vitamin D 2008- 2010 study population (n = 240).

	Total Cholesterol (TC mg/dL)			Triglyceride (TG mg/dL)		
	High	Normal	p value*	High	Normal	p value*
Vitamin D supplementation			0.412			0.773
Yes	1 (1.5%)	5 (3.4%)		2 (4.3%)	4 (3.3%)	
No	68 (98.6%)	141 (96.6%)		45 (95.7%)	116 (96.7%)	
Serum Calcium (Ca)	9.84 (0.39 SD)	9.77 (0.42 SD)	0.2644	9.84 (0.43 SD)	9.77 (0.44 SD)	0.393
Glycated hemoglobin (HbA1C)			0.175			0.371
HbA1C ≤ 8 (Acceptable)	32 (55.2%)	75 (65.8%)		23 (76.7%)	64 (68.1%)	
HbA1C > 8 (Bad control)	26 (44.8%)	39 (34.2%)		7 (23.3%)	30 (31.9%)	
Glycated hemoglobin (HbA1C)	8.35 (2.41 SD)	7.43 (1.68 SD)	0.004	6.81 (1.62 SD)	7.40 (1.82 SD)	0.1197
Parathyroid Hormone (PTH)	35.46 (12.51 SD)	35.71 (14.38 SD)	0.9065	35.12 (17.67 SD)	37.22 (12.79 SD)	0.4219
Seasons of the year in US			0.151			0.484
Winter	10 (14.5%)	27 (18.5%)		9 (19.2%)	18 (15.0%)	
Spring	9 (13.0%)	29 (19.9%)		6 (12.8%)	23 (19.2%)	
Summer	24 (34.8%)	56 (38.4%)		17 (36.2%)	51 (42.5%)	
Fall	26 (37.7%)	34 (23.3%)		15 (31.9%)	28 (23.3%)	
Ethnicity			0.418			0.044
Caucasian	50 (82.0%)	107 (83.0%)		38 (88.4%)	80 (76.9%)	
Asian	4 (6.6%)	3 (2.3%)		1 (2.3%)	5 (4.8%)	
African American / Black	7 (11.5%)	15 (11.6%)		2 (4.7%)	18 (17.3%)	
Hispanic / Latino	0 (0.0%)	2 (1.6%)		2 (4.7%)	0 (0.0%)	
Other	0(0.0%)	2 (1.6%)		0 (0.0%)	1 (1.0%)	

* P value derived from two sample *t* test for continuous variables and from chi square test for dichotomous or categorical variables

Table 9. Continued on next page:

Table 9 for HDL and LDL. Continued on next page: Distribution of covariates according to dichotomous blood lipid levels: Baystate vitamin D 2008- 2010 study population (n = 240).

	High Density Lipoprotein (HDL mg/dL)			Low Density Lipoprotein (LDL mg/dL)		
	Low	Sufficient	P value*	High	Normal	p value*
n	51 (21.3%)	119 (49.6%)		16 (6.7%)	149 (62.1%)	
Age			0.603			0.449
2 to <10 years	5 (9.8%)	15 (12.6%)		1 (6.3%)	19 (12.8%)	
10 to 21 years	46 (90.2%)	104 (87.4%)		15 (93.8%)	130 (87.3%)	
Age	13.88 (3.29 SD)	14.47 (3.49 SD)	0.3059	14.46 (3.60SD)	14.23 (3.45SD)	0.8026
Sex			0.478			0.553
Male	24 (47.1%)	49 (41.2%)		8 (50.0%)	63 (42.3%)	
Female	27 (52.9%)	70 (58.8%)		8 (50.0%)	86 (57.7%)	
Weight	92.75 (35.21SD)	71.89 (24.46SD)	<0.0001	87.31 (38.16SD)	77.62 (28.80SD)	0.2177
Height	159.54 (14.59 SD)	159.69 (13.58 SD)	0.9505	159.34 (13.14SD)	159.53 (14.18SD)	0.9576
BMI			<0.001			0.401
BMI < 19.8 (Underweight)	1 (2.0%)	16 (13.6%)		0 (0.0%)	15 (10.1%)	
BMI 19.8 - 26.0 (Normal)	8 (15.7%)	45 (38.1%)		4 (25.0%)	46 (31.1%)	
BMI > 26.0 - 29.0 (Overweight)	6 (11.8%)	17 (14.4%)		2 (12.5%)	22 (14.9%)	
BMI ≥ 29.0 (Obese)	36 (70.6%)	40 (33.9%)		10 (62.5%)	65 (43.9%)	
BMIZ	4.21 (13.79SD)	1.50 (1.01SD)	0.0346	2.05 (0.83SD)	2.40 (8.13SD)	0.8648
BMIZ categorical			<0.001			0.352
BMIZ > 1.5 (Obese)	44 (88.0%)	61 (51.7%)		12 (75.0%)	93 (63.3%)	
BMIZ ≤ 1.5 (Normal)	6 (12.0%)	57 (48.3%)		4 (25.0%)	54 (36.7%)	

Table 9 for HDL and LDL continued on next page:

Table 9. Continued from previous page for HDL and LDL : Distribution of covariates according to dichotomous blood lipid levels: Baystate vitamin D 2008- 2010 study population (n = 240).

	High Density Lipoprotein (HDL mg/dL)		P value*	Low Density Lipoprotein (LDL mg/dL)		p value*
	Low	Sufficient		High	Normal	
Vitamin D supplementation			0.856			0.557
Yes	2 (3.9%)	4 (3.4%)		1 (6.3%)	5 (3.4%)	
No	49 (96.1%)	115 (96.6%)		15 (93.8%)	144 (96.6%)	
Serum Calcium (Ca)	9.81 (0.41SD)	9.79 (0.45SD)	0.7235	9.94 (0.42SD)	9.79 (0.44SD)	0.2902
Glycated hemoglobin (HbA1C)			0.005			0.487
HbA1C ≤ 8 (Acceptable)	29 (87.9%)	58 (61.7%)		6 (60.0%)	79 (70.5%)	
HbA1C > 8 (Bad control)	4 (12.1%)	36 (38.3%)		4 (40.0%)	33 (29.5%)	
Glycated hemoglobin (HbA1C)	6.26 (1.17SD)	7.65 (1.82SD)	0.0001	6.71 (1.52SD)	7.30 (1.82SD)	0.3242
Parathyroid Hormone (PTH)	35.90 (14.25SD)	36.27 (13.60SD)	0.8833	38.27 (6.47SD)	36.70 (14.79SD)	0.7283
Seasons of the year in US			0.187			0.214
Winter	12 (23.5%)	16 (13.5%)		1 (6.3%)	26 (17.5%)	
Spring	8 (15.7%)	20 (16.8%)		1 (6.3%)	27 (18.1%)	
Summer	22 (43.1%)	46 (38.7%)		7 (43.8%)	60 (40.3%)	
Fall	9 (17.7%)	37 (31.1%)		7 (43.8%)	36 (24.5%)	
Ethnicity			0.441			0.805
Caucasian	38 (86.4%)	83 (78.3%)		11 (78.6%)	105 (80.2%)	
Asian	0 (0.0%)	6 (5.7%)		0 (0.0%)	6 (4.6%)	
African American / Black	5 (11.4%)	15 (14.2%)		3 (21.4%)	17 (13.0%)	
Hispanic / Latino	1 (2.3%)	1 (1.0%)		0 (0.0%)	2 (1.5%)	
Other	0 (0.0%)	1 (1.0%)		0 (0.0%)	1 (0.8%)	

* P value derived from two sample *t* test for continuous variables and from chi square test for dichotomous or categorical variables

Table 10. Pearson correlation of 25(OH)D and covariates with blood lipid levels: Baystate vitamin D, 2008- 2010 study population (n = 240).

	Total Cholesterol (TC mg/dL)		Triglyceride (TG mg/dL)		High Density Lipoprotein (HDL mg/dL)		Low Density Lipoprotein (LDL mg/dL)	
	r	p value*	r	p value*	r	p value*	r	p value*
Vitamin D levels (vdl)	0.18	0.0084	-0.21	0.006	0.22	0.0039	0.17	0.0265
Age	0.09	0.1978	-0.05	0.5221	0.28	0.0002	0.02	0.7605
Weight	0.01	0.8804	0.22	0.0038	-0.35	<0.0001	0.01	0.8846
Height	0.05	0.4875	-0.07	0.3595	0.13	0.0805	0.16	0.0424
BMIZ	0.04	0.5921	0.32	<0.0001	-0.21	0.0074	0.02	0.8453
Serum Calcium (Ca)	0.11	0.1315	0.05	0.5464	-0.12	0.1512	0.15	0.0852
Glycated hemoglobin (HbA1C)	0.21	0.0049	-0.13	0.1575	0.43	<0.0001	-0.1	0.2947
Parathyroid Hormone (PTH)	0.01	0.9128	-0.1	0.2355	-0.01	0.9391	0.13	0.1278

* P value testing for statistical significance in trends

Table 11. Distribution of 25-OH Vitamin D levels among blood lipid levels: Baystate vitamin D 2008- 2010 study (n = 240).

	TC				p-value	TG				p-value
	High		Normal			High		Normal		
	N	%	N	%		N	%	N	%	
25-OH vitamin D - dichotomized					0.39					0.057
<30.00 ng/ml (Deficient)	53	76.8	104	71.2		39	83.0	82	68.3	
≥30.00 ng/ml (Normal)	16	23.2	42	28.8		8	17.0	38	31.7	
Total	69	100.0	146	100.0		47	100.0	120	100.0	
25-OH vitamin D - categories					0.402					0.089
< 15.00 ng/ml (Severe deficiency)	15	21.7	23	15.8		14	30	19	15.8	
15.00 – 19.99 ng/ml (Moderate deficiency)	16	23.2	25	17.1		10	21.3	20	16.7	
20.00 – 29.99 ng/ml (Insufficiency)	22	31.9	56	38.4		15	31.9	43	35.8	
≥30.00 ng/ml (Normal or Optimal)	16	23.2	42	28.8		8	17	38	31.7	
Total	69	100.0	146	100.0		47	100.0	120	100.0	
	Mean	SE	Mean	SE		Mean	SE	Mean	SE	
25-OH Vitamin D - continuous (ng/ml)	22.97	1.30	25.39	0.85	0.1139	20.72	1.20	25.94	1.02	0.0041
	Median	Range	Median	Range		Median	Range	Median	Range	
25-OH Vitamin D - continuous (ng/ml)	21	4.0 - 57.6	25	5.7 - 69.3		19.5	8.1 - 40.9	24.45	4.6 - 69.3	

* P - value derived from chi square test for dichotomous or categorical variables and from t test for continuous variables

Table 11. Continued on next page for HDL and LDL

Table 11 continued from previous page for HDL and LDL. Distribution of 25-OH Vitamin D levels among blood lipid levels: Baystate vitamin D 2008- 2010 study (n = 240).

	HDL-C				p-value	LDL-C				p-value
	Low		Sufficient			High		Normal		
	N	%	N	%		N	%	N	%	
25-OH vitamin D - dichotomized					0.01					0.391
<30.00 ng/ml (Deficient)	44	86.3	80	67.2		13	81.3	106	71.1	
≥30.00 ng/ml (Normal)	7	13.7	39	32.8		3	18.8	43	28.9	
Total	51	100.0	119	100.0		16	100.0	149	100.0	
25-OH vitamin D - categories					0.005					0.418
< 15.00 ng/ml (Severe deficiency)	16	31.4	17	14.3		4	25.0	29	19.5	
15.00 – 19.99 ng/ml (Moderate deficiency)	13	25.5	18	15.1		5	31.3	25	16.8	
20.00 – 29.99 ng/ml (Insufficiency)	15	29.4	45	37.8		4	25.0	52	34.9	
≥30.00 ng/ml (Normal or Optimal)	7	13.7	39	32.8		3	18.8	43	28.9	
Total	51	100.0	119	100.0		16	100.0	149	100.0	
	Mean	SE	Mean	SE		Mean	SE	Mean	SE	
25-OH Vitamin D - continuous (ng/ml)	20.92	1.25	25.96	1.00	0.0042	21.46	2.67	24.8	0.88	0.237
25-OH Vitamin D - continuous (ng/ml)	Median	Range	Median	Range		Median	Range	Median	Range	
	18.7	11.0 - 46.4	25	4.6 - 69.3		18.5	6.7 - 45.9	23.3	4.6 - 69.3	

* P value derived from chi square test for dichotomous or categorical variables and from t test for continuous variables

Table 12. Odds Ratio of dyslipidemia by 25-OH Vitamin D levels: Bay State vitamin D study, 2008 -2010 (n = 240).

	Cases		Unadjusted		<i>P</i> <i>trend</i>	Multivariate		<i>P</i> <i>trend</i>
	No.	%	OR	95% CI		OR	95% CI	
High Total Cholesterol (TC)								
25-OH Vitamin D Normal	16	23.2	1	Referent	0.39	1	Referent	0.114
25-OH Vitamin D Deficient	53	76.8	1.34	0.69 - 2.60		1.95 ^a	0.85 - 4.46	
25-OH Vitamin D Normal or Optimal	16	23.2	1	Referent	0.12	1	Referent	0.062
25-OH Vitamin D Insufficiency	22	31.9	1.03	0.48 - 2.20		1.53 ^a	0.62 - 3.83	
25-OH Vitamin D Moderate deficiency	16	23.2	1.68	0.72 - 3.94		2.90 ^a	0.95 - 8.82	
25-OH Vitamin D Severe deficiency	15	21.7	1.71	0.72 - 4.08		2.41 ^a	0.78 - 7.44	
25-OH Vitamin D - continuous (Mean (SD))	22.97	10.84	0.98	0.95 - 1.01	0.115	0.97	0.93 - 1.01	0.094
High Triglyceride (TG)								
25-OH Vitamin D Normal	8	17.0	1	Referent	0.061	1	Referent	0.231
25-OH Vitamin D Deficient	39	83.0	2.26	0.96 - 5.30		1.91 ^b	0.66 - 5.47	
25-OH Vitamin D Normal or Optimal	8	17.0	1	Referent	0.012	1	Referent	0.006
25-OH Vitamin D Insufficiency	15	31.9	1.66	0.63 - 4.34		1.03 ^b	0.31 - 3.45	
25-OH Vitamin D Moderate deficiency	10	21.3	2.38	0.81 - 6.96		3.30 ^b	0.74 - 14.81	
25-OH Vitamin D Severe deficiency	14	30.0	3.50	1.25 - 9.79		11.65 ^b	1.93 - 70.27	
25-OH Vitamin D - continuous (Mean (SD))	20.72	8.20	0.95	0.91 - 0.98	0.005	0.91 ^b	0.86 - 0.97	0.004
Low High density Lipoprotein (HDL)								
25-OH Vitamin D Normal	7	13.7	1	Referent	0.013	1	Referent	0.066
25-OH Vitamin D Deficient	44	86.3	3.06	1.27 - 7.42		2.51 ^c	0.94 - 6.69	
25-OH Vitamin D Normal or Optimal	7	13.7	1	Referent	0.001	1	Referent	0.013
25-OH Vitamin D Insufficiency	15	29.4	1.86	0.69 - 5.02		1.52 ^c	0.50 - 4.56	
25-OH Vitamin D Moderate deficiency	13	25.5	4.02	1.37 - 11.79		5.06 ^c	1.43 - 17.88	
25-OH Vitamin D Severe deficiency	16	31.4	5.24	1.83 - 15.06		3.56 ^c	1.03 - 12.33	
25-OH Vitamin D - continuous (Mean (SD))	20.92	8.92	0.95	0.91 - 0.98	0.005	0.96 ^c	0.92 - 1.00	0.062
High Low density Lipoprotein (LDL)								
25-OH Vitamin D Normal	3	18.8	1	Referent	0.397	1	Referent	-
25-OH Vitamin D Deficient	13	81.3	1.76	0.48 - 6.48		-	-	
25-OH Vitamin D Normal or Optimal	3	18.8	1	Referent	0.215	1	Referent	-
25-OH Vitamin D Insufficiency	4	25.0	1.10	0.23 - 5.20		-	-	
25-OH Vitamin D Moderate deficiency	5	31.3	2.87	0.63 - 13.03		-	-	
25-OH Vitamin D Severe deficiency	4	25.0	1.98	0.41 - 9.50		-	-	
25-OH Vitamin D - continuous (Mean (SD))	21.46	10.67	0.97	0.92 - 1.02	0.236	-	-	-

^a adjusted for age, BMIZ and HbA1C

^b adjusted for PTH, BMIZ and ethnicity

^c adjusted for bmiz and ethnicity

P value testing for trends

* (-) due to lack of statistical power (small number of cases in each category), no covariates were found to be confounding the observed relationship

Table 13. Association between dyslipidemia by 25-OH Vitamin D levels: Baystate vitamin D study, 2008 -2010 (n=240).

	Regression coefficient	Standard error (SE)	<i>P trend</i>
High Total Cholesterol (TC)			
25-OH Vitamin D Normal	1	Referent	0.007
25-OH Vitamin D Deficient	14.82 ^a	5.37	
25-OH Vitamin D Normal or Optimal	1	Referent	0.007
25-OH Vitamin D Insufficiency	11.8 ^a	5.78	
25-OH Vitamin D Moderate deficiency	21.75 ^a	7.66	
25-OH Vitamin D Severe deficiency	17.68 ^a	7.76	
25-OH Vitamin D - continuous	-0.58 ^a	0.25	0.022
High Triglyceride (TG)			
25-OH Vitamin D Normal	1	Referent	0.013
25-OH Vitamin D Deficient	30.91 ^b	12.22	
25-OH Vitamin D Normal or Optimal	1	Referent	0.002
25-OH Vitamin D Insufficiency	22.44 ^b	13.06	
25-OH Vitamin D Moderate deficiency	41.07 ^b	17.63	
25-OH Vitamin D Severe deficiency	56.30 ^b	19.22	
25-OH Vitamin D - continuous	-2.14 ^b	0.59	<0.001
Low High density Lipoprotein (HDL)			
25-OH Vitamin D Normal	1	Referent	0.188
25-OH Vitamin D Deficient	-3.78 ^c	2.85	
25-OH Vitamin D Normal or Optimal	1	Referent	0.005
25-OH Vitamin D Insufficiency	-0.61 ^c	2.99	
25-OH Vitamin D Moderate deficiency	-7.99 ^c	4.00	
25-OH Vitamin D Severe deficiency	-9.67 ^c	3.91	
25-OH Vitamin D - continuous	0.37 ^c	0.13	0.004
High Low density Lipoprotein (LDL)			
25-OH Vitamin D Normal	1	Referent	0.197
25-OH Vitamin D Deficient	6.57 ^d	5.08	
25-OH Vitamin D Normal or Optimal	1	Referent	0.093
25-OH Vitamin D Insufficiency	2.18 ^d	5.83	
25-OH Vitamin D Moderate deficiency	13.35 ^d	6.88	
25-OH Vitamin D Severe deficiency	8.11 ^d	6.59	
25-OH Vitamin D - continuous	-0.50 ^d	0.21	0.019

^a Multivariate model includes: gender, glycated hemoglobin (HbA1C), ethnicity and season.

^b Multivariate model includes: bmiz, ethnicity and parathyroid hormone (PTH).

^c Multivariate model includes: age, gender, bmiz, glycated hemoglobin (HbA1C), ethnicity and season.

^d Multivariate model includes: age, height and season. * *P* - value testing for statistical significance in trend

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