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Serum Vitamin Concentrations are Associated with Metabolic Syndrome and Insulin Resistance in US Children

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The Master's Thesis Advisory Committee, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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ABSTRACT

Background: Vitamin D deficiency is a concern in the US. Association between vitamin D status and metabolic syndrome (MetS), insulin resistance (IR), and inflammation is unclear in children.

Objective: The relationship between serum vitamin D and MetS, C-reactive protein (CRP), and Homeostatic Model Assessment-IR (HOMA-IR) was investigated.

Design: Data from 3 cycles of National Health and Nutrition Examination Survey, 2001-2006 for 3700 (1820, boys; 1880, girls) children and adolescents, aged 12-17 y were used to assess prevalence of vitamin D deficiency (<20 ng/mL) and association between serum vitamin D and prevalence of MetS, various components of MetS, CRP, and HOMA-IR using multivariate regression models.

Results: Overall, prevalences of MetS and vitamin D deficiency were 6.1% and 30.5%, respectively. Prevalence of vitamin D deficiency was higher in girls (52%), blacks (74%), non-supplement users (50%), persons who were examined in winter (56%), and persons in the low poverty income ratio group (57%) compared to their counterparts. Serum vitamin D was inversely associated with waist circumference ($P<0.001$), systolic blood pressure ($P=0.009$), and HOMA-IR ($P=0.003$) and positively associated with HDL-cholesterol ($P<0.001$). Children with lowest serum vitamin D are at increased risk for MetS ($P=0.04$; OR 2.26; 95% CI: 1.11, 4.61). Serum vitamin D was not related to CRP ($P<0.10$).

Conclusions: Children with poor vitamin D status are at increased risk for MetS and IR. Because of negative health outcomes associated with MetS and poor vitamin D status when existed

individually or in combination, early detection and intervention of these conditions are paramount, especially in children.

SERUM VITAMIN CONCENTRATIONS ARE ASSOICATED WITH METABOLIC SYNDROME AND INSULIN RESISTANCE IN US CHILDREN

By

Nida I Shaikh

A Thesis Presented in Partial Fulfillment of Requirements for the

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ABBREVIATIONS

NHANES	National Health and Nutrition Examination Survey
NCHS	National Center for Health Statistics
MEC	Mobile Examination Center
MetS	Metabolic Syndrome
T2DM	Type 2 Diabetes Mellitus
IR	Insulin Resistance
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
CRP	C-reactive protein
HDL-c	High-density lipoprotein cholesterol
CVD	Cardiovascular disease
BP	Blood pressure
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
IL	Interleukin
TNF	Tumor necrosis factor
NF-$_k$B	Nuclear factor kappa-light-enhancer of activated B cell
VDRs	Vitamin D receptor

CHAPTER I

INTRODUCTION

Health concerns associated with low serum vitamin D are on the rise in children and adolescents. Vitamin D is required not only for bone health [1] but also plays a role in a range of ailments such as autoimmune disease,[2, 3] cardiovascular disease (CVD), [4-6] type 2 diabetes mellitus (T2DM),[6] hypertension,[7] depression,[8, 9] and certain types of cancer [10]. Vitamin D deficiency is widespread in the US. There is inconsistency is defining vitamin D status using serum vitamin D concentration with vitamin D deficiency being defined at <11ng/mL,[11, 12] <15ng/mL[13, 14] or 20 ng/mL in different studies.[15-17] Recently, the prevalence of vitamin D deficiency (<15 ng/mL) and vitamin D insufficiency (15-29 ng/mL) was found to be 9% and 61% respectively in children and adolescents.[14]

Suboptimal circulating concentrations of serum vitamin D have been linked to metabolic syndrome (MetS),[18, 19] cardiometabolic risk factors [14] and insulin resistance (IR).[20] MetS is an established risk factor for CVD and T2DM.[6] While there is a lack of consistent criteria in identifying MetS given that various organizations have provided different working definitions, the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) definition is most widely used in the US. The prevalence of MetS increased among adolescents from 6.4% in the National Health and Nutrition Examination Survey (NHANES) 1999-2000 to 8.6 % in NHANES 2001-2006

. [21-23] Obesity and IR are the underlying cause of MetS. [24-26] IR is known to contribute to the pathogenesis of T2DM.[20, 27] Vitamin D reduces the risk for T2DM by preserving insulin secretion and increasing insulin sensitivity. [20, 27, 28] However, epidemiological evidence relating vitamin D status with IR and glucose homeostasis is not consistent. [27-32]

Recently, vitamin D has been linked to inflammation through the activation of vitamin D receptors (VDRs) on inflammatory cells.[16] C-reactive protein (CRP) is a predictor of elevated inflammation, [33, 34] which has been linked with increased risk for CVD, [35, 36] obesity, [32, 37] and MetS.[33, 38, 39] However, no epidemiological studies have investigated the association between vitamin D and inflammation using CRP as a marker.

Furthermore, few nationally representative studies investigated the relationship between vitamin D, MetS and its individual components, but did not include the association between vitamin D with inflammation and IR.[14, 18] In addition, the inverse association between serum vitamin D and IR has been confirmed in adults, but similar studies on children and adolescents are lacking. Due to clinical evidence confirming the presence of VDRs on pancreatic β cells [1] and inflammatory cells,[40] we suggest the plausible role of vitamin D in CVD, inflammation, and IR and hypothesize that an inverse association exists between serum vitamin D concentration and MetS, CRP, and IR. Therefore, we investigated the association between serum vitamin D concentrations and prevalence of MetS, individual components of MetS, CRP, and IR in children and adolescents from nationally representative sample surveys of the US population.

CHAPTER II

REVIEW OF LITERATURE

VITAMIN D

SYNTHESIS, METABOLISM & SOURCES

Vitamin D, a lipophilic vitamin, is obtained primarily upon exposure to ultra violet radiation of sunlight.[1, 41] Vitamin D precursors, 7-dehydrocholesterol in the skin is converted to vitamin D_3. The hydroxylation of vitamin D_3 results in the formation of vitamin D (25-hydroxyvitamin D) in the liver. Further vitamin D is converted to its active metabolite 1,25-dihydroxyvitamin D (1,25 $(OH)_2D$) or calcitriol by 1α-hydroxylase in the kidney.[1] Calcitriol is involved in regulating calcium homeostasis.[1]

There are limited natural dietary sources of vitamin D. A few foods such as fatty fish (mackerel, salmon, and sardines), cod liver oil, liver, and egg yolk contain vitamin D.[1, 6] In addition, vitamin D is found in fortified foods such as milk, yogurt, margarine, infant formulas, breakfast cereals, orange juice,[42] enriched rice, corn meal products, noodle products and macaroni products.[1, 43] Fortified milk (62%) and breakfast cereals (17%) are the main food sources of vitamin D in 1-5y old children.[44] Less than 10% of vitamin D is obtained from other foods and supplement sources.[45]

FUNCTIONS OF VITAMIN D

Vitamin D performs multitude of biological functions in the body. It regulates calcium and phosphorus homeostasis by enhancing calcium and phosphorus absorption in the gut and promoting bone mobilization.[1, 16, 41] The functions of vitamin D are mediate

through VDRs. Once the vitamin D enters the nucleus of the cell, it binds with the VDRs, and together they modulate gene expression at the cellular level.[1] VDRs are transcription factors expressed in most tissues including the skeletal muscle, vascular smooth muscle, osteoblasts, endothelium, cardiomyocytes, activated macrophages, pancreatic β-cells. VDRs are also mapped in various organs systems including the small intestine, skin, heart, brain, breast, gonads, and prostate gland. [1, 41] In addition to the role of vitamin D in calcium homeostasis, VDRs are also known to stimulate insulin production [1] and modulate immune function. [16, 46, 47]

VITAMIN D STATUS & RECOMMENDATIONS

Circulating serum vitamin D is used as a marker of vitamin D status because it reflects the total vitamin D production from endogenous and exogenous sources and has a longer half life than calcitriol.[1, 16, 48] Vitamin D deficiency was defined at serum vitamin D concentration <11 ng/mL (27.5 nmol/L) by the Institute of Medicine.[12] Recently, the same agency suggested serum vitamin D concentrations between 20-30 ng/mL as adequate to maintain bone health. Previous studies have demonstrated effects of vitamin D deficiency at serum vitamin D concentrations higher than 11 mg/mL.[11] The most consistent criteria used to categorize vitamin D in children are <20 ng/mL, 20-30 ng/mL, and ≥30 ng/mL for deficiency, insufficiency, and sufficiently, respectively.[15, 16, 44] However, Forouhi et al [30] classified vitamin D status in 4 categories: sufficiency (≥30 ng/mL or ≥75 nmol/L), hypovitaminosis D (<30 ng/mL or <75 nmol/L), insufficiency (<20 ng/mL or <50 nmol/L), and classical deficiency (<10 ng/mL or <25 nmol/L). Some investigators have also considered serum vitamin D deficiency concentrations ≤15ng/mL

(≤37.5 nmol/L) and >20 ng/ml (>50 nmol/L) to define deficiency and sufficiency, respectively.[13, 14] There is ambiguity in defining vitamin D status.

Excess serum vitamin D concentration is also undesirable. Serum vitamin D concentrations ≥80 ng/mL (200 nmol/L) are regarded as the upper limit of serum vitamin D.[41] Costello [16] reported vitamin D concentrations >200 ng/mL (>500 nmol/L) is considered potentially toxic.

Numerous factors such as skin pigmentation (melanin), excessive use of sunscreen, nature and amount of clothing, geographical location or latitude, season, and age affect vitamin *de novo* vitamin D synthesis in dermis. [31, 44, 49, 50] Although some investigators found that the prevalence of vitamin D deficiency was higher in children with darker skin pigmentation than those with lighter skin pigmentation,[44, 51] Cole et al [44] found that skin pigmentation did not significantly predict vitamin D status. Clemens et al [52] found that non-Hispanic white need 5 to 10 times lesser sun exposure to produce serum vitamin D as compared to non-Hispanic black. Season and geographical location affects synthesis of vitamin D in the skin. Vitamin D deficiency has been found more prevalent in winter than in summer and at higher latitudes than at lower latitudes.[51 43] Webb et al [53] reported endogenous vitamin D synthesis was reduced in persons living above 35° latitude in winter.

The Food and Nutrition Board of the National Academy of Sciences recommended 200 IU/d as the adequate intake of vitamin D for children to maintain serum vitamin D concentrations >11 ng/mL (27.5 nmol/L).[12] The American Academy of Pediatrics, a non-profit professional organization recommended 400 IU/d of vitamin D supplementation for children and adolescents that are unable to get adequate exposure to

sunlight or are unable to consume at least 500 mL of fortified milk daily.[54] However, with the failure to reach minimum serum vitamin D concentration of 20 ng/mL, the Institute of Medicine recently revised the recommended dietary allowance (RDA) and upper intake level of vitamin D in children and adolescents to 600 IU/d and 4000 IU/d, respectively. [55]

VITAMIN D INSUFFICIENCY AND DEFICIENCY

Vitamin D insufficiency and deficiency in children is an emerging public health concern in the US. Suboptimal vitamin D status hampers bone mineralization. Vitamin D deficiency prevents children from acquiring appropriate peak bone mass and attaining adequate height.[1] Vitamin D deficiency weakens the collagen matrix of bones leading to increased risk of bone fractures. Low serum vitamin D causes secondary hyperparathyroidism, which in turn decreases intestinal phosphorous absorption, resulting in impaired bone mineralization. Children develop rickets, adults develop osteomalacia from deranged mineralization of bones. [1, 41, 56]

It is estimated that 61% of children and adolescents are at risk for vitamin D insufficiency (15-29 ng/mL) in the US.[14] Using a definition of vitamin D deficiency <15 ng/mL, Kumar et al [14] found 9% of children 1-21 y old were vitamin D deficient in NHANES 2001-2004. A review of 11 studies on healthy US children revealed that prevalence of hypovitaminosis ranged from 15 to 78%. Low serum vitamin D concentrations were found in older children, in those with higher body mass index (BMI), in blacks, or in those measured in winter season.[43] In this review, vitamin D deficiency and insufficiency were defined as having serum vitamin D concentrations between 5 and 12 ng/mL and 10 and 32 ng/mL, respectively. Looker et al [51] assessed serum vitamin D

concentrations in adolescents and adults (aged ≥12 y) from NHANES III (1988-1994). They categorized the study sample into 2 groups based on the season of blood sample collection for measurement of serum vitamin D concentrations. The need for categorization into 2 such groups was due to NHANES survey design that involved collection of blood sample from a mobile examination center (MEC) during summer from northern latitudes (range 25-47°N) and during the winter from southern latitudes. In the group examined during winter (lower latitude), vitamin D deficiency (<17.5 nmol/L) and vitamin D insufficiency (<37.5-62.5 nmol/L) was <1% and 25-57%, respectively. In another study on NHANES III, Saintonge et al [11] investigated serum vitamin D in 12-19 y adolescents using vitamin D deficiency first as <11 ng/mL and then as <20 ng/mL. The investigators found vitamin D deficiency changed from 2% to 14% upon changing the cutoff. The risk of vitamin D deficiency was significantly higher in women, non-Hispanic black, and overweight adolescents as compared to their counterparts.

Other studies investigated the prevalence of vitamin D deficiency in both northern and southern parts of the US. In the south eastern US, Cole et al [44]found 23% of children aged 1-5 y old of low-income families were deficient in vitamin D (≤ 20 ng/mL). The researchers found 73.6% of children had serum vitamin D concentrations <30 ng/mL. In the northern US (latitude 42° N), Gordon et al [13] found 24% (n = 74) of 11-18 y old children were deficient (≤15 ng/mL) and 42% (n = 129) were insufficient (< 20 ng/mL) in serum vitamin D.

METABOLIC SYNDROME

A growing body of evidence suggests that suboptimal vitamin D concentrations are linked with an increasing number of health conditions such as MetS. Previously

known as Syndrome X [57] or Insulin Resistance Syndrome.[58] MetS is a constellation of factors that link obesity, hypertension, IR, T2DM, and CVD.[59] The World Health Organization (1998),[60] the European Group for the study of IR (EGIR) (1999),[61] National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) (2001),[62] and the International Diabetes Federation (IDF) (2005) [63] have established definitions for the diagnosis of MetS.

Of the numerous MetS definitions, the NCEP-ATP III is commonly used in research. According to this definition, the presence of at least 3 of the following 5 confirms having MetS for adults: WC ≥40 inches in men or ≥35 inches in women, serum triglycerides ≥150 mg/dL, serum high-density lipoprotein cholesterol (HDL-c) <40 mg/dL for men or <50 mg/dL for women, high BP ≥130/85 mm Hg, and fasting plasma glucose ≥100 mg/dL.[62]

There is no universally accepted definition of MetS in children and adolescents. Investigators have adapted the adult MetS definition for pediatric population.[22, 64] The modified MetS definition proposed by Cook et al [22] includes an individual meeting 3 of the 5 following criteria: (1) WC ≥90th percentile for age and sex, (2) triglycerides ≥110mg/dL (1.24 mmol/L), (3) HDL-c ≤40 mg/dL (1.03 mmol/L), (4) either systolic blood pressure (SBP) or diastolic blood pressure (DBP) ≥90th percentile for age, sex, and height, and (5) fasting glucose concentration ≥110 mg/dL (5.55 mmol/L). Ferranti et al [64] proposed a modified criteria for MetS in children. Children and adolescents were classified with having MetS if they met at least 3 or more of the following: (1) fasting triglycerides ≥100 mg/dL (1.1 mmol/L), (2) HDL-c <50 mg/dL (1.3 mmol/L), except in boys aged 15-19 y, in whom the cut off was <45 mg/dL (1.2 mmol/L), (3) fasting glucose

≥110 mg/dL (6.1 mmol/L), (4) waist circumference > 75th percentile for age and gender, and (5) SBP > 90th percentile for gender, age, and height or use of antihypertensive medications. A more recent modified NCEP-ATP III pediatric MetS criteria proposed by Ford et al [65] required that children and adolescents met 3 of the following: (1) triglyceride concentration ≥110 mg/dL, (2) HDL-c ≤40 mg/dL, (3) WC ≥ 90th percentile (sex-specific), (4) glucose concentration ≥100 mg/dL, and (5) SBP or DBP ≥90th percentile (age, height, and sex-specific).

PATHOPHYSIOLOGY OF METABOLIC SYNDROME

Evidence suggests obesity, particularly visceral obesity is an important characteristic of the MetS [23, 24, 59, 66]. Adipose tissue is not only an energy reservoir, but also an endocrine organ that secretes inflammatory adipocytokines and all components of the renin-angiotensin system (RAS).[59] The RAS regulates BP and blood volume homeostasis and excessive RAS stimulation can lead to hypertension.[67, 68] The RAS is upregulated in obesity.[66] Adipose tissue secretes adipocytokines such as leptin, resistin, interleukin (IL)-6, plasminogen activator inhibitor-1, and tumor necrosis factor (TNF)-α.[24, 66] Elevated concentrations of IL-6 and TNF-α stimulate the production of acute phase protein called CRP in the liver.[35, 59] Higher circulating CRP concentrations indicate low-grade systemic inflammation and along with elevated pro-inflammatory cytokines, CRP contribute to the development of atherosclerotic plaques.[69, 70] Thus, increase in adiposity is likely to trigger a cascade of events that includes activation of the RAS and increased secretion of pro-inflammatory adipocytokine leading to IR s causing atherogenic dylipidemia , systemic inflammation through increased secretion of inflammatory marker CRP, and decreased insulin sensitivity resulting in increased insulin

resistance [24, 71]. Other factors such as the oxidative stress, heredity, and lifestyle factors also contribute to the development of MetS.[59] Hence, there exists a complex relationship between obesity, IR and various components of MetS.

PREVALENCE OF METABOLIC SYNDROME

The increasing prevalence of MetS in children and adolescents has become a public health concern.[71] Epidemiological studies using the NCEP-ATP III definition have found the prevalence of MetS in NHANES vary from 4.2 to 12.7% [18, 21-23, 39]. Cook et al [22] reported a prevalence of MetS in 4% adolescents and a prevalence of 30% in overweight adolescents using the data from the NHANES III (1988-1994). Using the modified MetS definition proposed by Cook et al, [22] Duncan et al [21] found the prevalence of MetS further increased to 6.4% in adolescents (n = 991) in NHANES 1999-2000. In the same data, they found that the prevalence of MetS was 32.1% in overweight adolescents. DeFerranti et al [64] compared the prevalence of MetS in NHANES 1988-1994 with those in NHANES 1999-2000 in adolescents. They found that the prevalence of MetS increased from 9.2% in NHANES 1988-1994 to 12.7% in NHANES 1999-2000. An assessment of MetS in 2456 adolescents (12-19 y) in a more recent NHANES (2001-2006) using the modified MetS definition proposed by Ford et al,[65] Johnson et al [23] reported a prevalence of MetS was 8.6% overall and 28.7% in overweight adolescents. The prevalence of MetS differed by age and race. The prevalence of MetS was more common in men than in women [21-23, 72] and is more common in Mexican-Americans than in non-Hispanic whites and non-Hispanic blacks. [22, 64]

VITAMIN D, METABOLIC SYNDROME AND OBESITY

It has been reported that serum vitamin D concentrations decrease with increasing body weight or BMI.[11, 73, 74] Numerous mechanisms have been proposed for low serum vitamin D concentrations in obese individuals. One mechanism is the sequestering of vitamin D in adipose tissue leading to low circulating vitamin D concentrations decreases its bioavailability. [73, 74] Worstman et al [74] suggested increased vitamin D storage with increase in adipose tissue, although they found no difference in the endogenous synthesis of vitamin D and vitamin D precursor (7-dehydrocholesterol) between obese and non-obese people. This negates the theory that obese people could possibly have lesser vitamin D precursors in the skin, which could result in less vitamin D production than that in their non-obese counterparts. Another theory is whether there is a difference in the way obese people utilize vitamin D production either through from skin or oral route. Wortsman et al [74] investigated obese individuals' serum vitamin D concentrations from the skin as compared from an oral route and intestinal absorption in obese compared to non-obese subjects. The investigators reported that after a 24 hr sunlight exposure, there was a 57% lesser increase in serum vitamin D concentrations in obese compared to non-obese individuals. This suggests that the release of vitamin D may be affected in older persons.

In children, WC is associated with visceral adiposity, while BMI is more likely to predict subcutaneous body fat.[75] A BMI percentile between 85th- 95th percentiles is considered as overweight, while a BMI $\geq 95^{th}$ percentile is considered as being obese [21] (CDC chart) for children. Szmitko et al [36] suggested that WC and skinfold thickness are independent predictors of MetS in early adulthood. Increased risk of vitamin D

deficiency was found in overweight adolescents as compared with normal-weight adolescents. Tangorra et al [73] investigated the prevalence of vitamin D deficiency (<20 ng/mL) and components of MetS in 217 obese children, aged 7-18 y old. Insulin sensitivity was calculated by the quantitative insulin sensitivity check index (QUICKI). The prevalence of vitamin D deficiency was 55.2% in obese children. They found that serum vitamin D was inversely related with BMI and positively related with HDL-c. They did not investigate the association between serum vitamin D and season, exposure to sunlight, season, race-ethnicity, diet or exercise. Consistent with previous studies, the researchers reported that obesity is a risk factor for vitamin D deficiency and that vitamin D deficiency more prevalent in obese children than in non-obese children.[11, 74]

There is emerging evidence that low serum vitamin D concentration is associated with the development of CVD and mortality.[4, 5] An inverse association between the prevalence of MetS and vitamin D status has been established. [19, 76] Also, low serum vitamin D concentrations have been associated with various components of MetS. Maki et al [4] found low serum vitamin D independently associated with MetS and with HDL-c in 257 men and women.[4] Ford et al [19] reported an inverse association between vitamin D and MetS, abdominal obesity, hyperglycemia, and hypertriglyceridemia in NHANES III. Reis et al [76] investigated the association of serum vitamin D and PTH with MetS in adults (≥20 y) in NHANES 2003-2004. They found a significant inverse association between serum vitamin D and prevalences of MetS independent of BMI, parathyroid hormone, and total calcium intake. Reis et al [18] investigated the association between serum vitamin D and cardiometabolic risk factors in 3577 adolescents in NHANES 2001-2004. Using the NCEP-ATP III definition modified by Cook et al [22] found that low serum vitamin D

was significantly associated with MetS and components of the MetS such as elevated BP and elevated fasting glucose.

In another study using the NHANES 2001-2004 data, Kumar et al [14] found serum vitamin D deficiency (<15 ng/mL) was significantly associated with elevated BP and with depressed HDL-c in 1-21 y old children and adolescents. They found only 4% of the children had taken 400 IU/d of vitamin D in the month prior to the data collection. Alberti et al [71] reported that individuals with MetS are twice as likely to develop CVD over the next 5-10 y as compared with individuals without MetS. Thus, MetS can lead to an atherogenic profile and increase the risk for CVD.

VITAMIN D, METABOLIC SYNDROME, AND BLOOD PRESSURE

A newly recognized role of vitamin D is its ability to regulate BP by inhibiting renin gene expression.[77] Renin is an enzyme and a key component of the RAS. The RAS, known to regulate BP and blood volume [68], is upregulated in obesity.[66] This leads to sodium retention in the kidney.[66] All components of the RAS are produced in the adipose tissue.[78] For instance, angiotensin receptors are expressed in adipocytes.[79] Studies have suggested that hypertension is caused by many interrelated factors such as disrupted lipid metabolism, activation of the RAS, and hyperleptinemia. [66, 68] Morse et al [66] reviewed literature on different components related to hypertension and suggested that excess weight gain is the underlying cause of elevated BP. Increased adiposity triggers increased activity of the sympathetic nervous system (SNS), RAS, and increased adipocytokine production . It has also been reported that IR from hyperinsulinemia contributes to the development of hypertension.[58]

Elevated BP is one of deranged abnormalities of the MetS.[66, 68] Recent studies have implicated low serum vitamin D concentrations as a risk factor for elevated BP.Vitamin D affects BP through regulation of the RAS, as well as its calcium mobilizing properties.[80-82] Zittermann et al [68] postulated that vitamin D may affect vascular smooth muscle, cell proliferation, inflammation, vascular calcification, and BP through the RAS [68]. Animal studies show that vitamin D is an important regulator of the RAS system and that 1,25-dihydroxyvitamin D suppresses renin gene expression.[67] Disruption of the VDR gene leads to elevated renin production, cardiac hypertrophy, and elevated BP in mice.[83]

Epidemiological studies have investigated the relationship between serum vitamin D concentrations and BP. In the Framingham Offspring longitudinal study, Wang et al [48] assessed CVD risk and found twice the risk for CVD events in vitamin D deficient individuals that had hypertension than those that did not have hypertension. The authors also reported 28% of the participants had vitamin D deficiency (<15 ng/mL) and confirmed evidence that vitamin D deficiency is a risk factor for CVD. Judd et al [7] found obese children with serum vitamin D <20 ng/ml were found to have significantly higher SBP than obese children with serum vitamin D ≥20 ng/mL, in NHANES III (1988-1992). The investigators classified BP into 5 categories with a sixth category added to distinguish participants with normal SBP (<110 mm Hg) from those with high-normal SBP (110-119 mm Hg). In contrast, Maki et al [4] did not find an association between serum vitamin D and BP. They suggested that such an association was not found because a majority of the subjects in the study were vitamin D sufficient (>30 ng/mL) and were

non-Hispanic white. In order to reduce elevated BP, studies suggest vitamin D supplementation [7, 48] and weight loss, especially in obese individuals.[84]

INSULIN RESISTANCE

Pancreatic β-cells promote the uptake, utilization, and storage of glucose by liver and peripheral tissues by producing insulin. The cells also express VDRs and1α-hydroxylase which is required for the conversion of vitamin D to 1,25(OH)2D. [85] Decreased insulin sensitivity or increased IR, a physiological state that results from impaired ability of plasma insulin to control altered glucose metabolism. It has been found that IR can also be present in individuals independent of excessive adiposity.[72] However, in the obese state, higher secretion of adipocytokines can lead to hypertension, endothelial dysfunction, and deranged lipid metabolism.[58] Thus, IR precedes the development of MetS [24, 66] and is associated with increased risk for T2DM and CVD [15, 24].

IR and pancreatic β-cell function are measured both by direct and indirect methods. The euglycaemic insulin clamp is considered the gold standard because this method is used to measure the IR directly and is regarded as highly sensitive.[86, 87] Other indirect indices such as the Homeostatic Model of IR (HOMA-IR) and the QUICKI are also used to measure IR and insulin sensitivity. The HOMA-IR method, based on fasting glucose and insulin concentrations, is determined using a mathematical model.[88] The value of IR is calculated as the product of insulin concentration (μU/mL) and fasting glucose concentration (mg/dL) divided by 405.[88] Unlike the euglycaemic insulin clamp method, the HOMA-IR is a less invasive and labor-intensive method. This method is widely used for the measurement of IR in large epidemiological studies.[87, 89] On

evaluating 55 hospitalized Japanese patients with T2DM before and after a diet and exercise therapy treatment for 6 weeks, Katzuki et al [87] found HOMA-IR to be useful in identifying IR. The investigators came to this conclusion after having found significant correlation between HOMA-IR and the euglycemic clamp both before and after treatment in T2DM patients. Several other epidemiological studies have shown HOMA-IR to be a reliable marker of IR. [32, 88]

VITAMIN D, METABOLIC SYNDROME, AND INSULIN RESISTANCE

Vitamin D is proposed to alter insulin action on the adipocyte, affect the release of insulin from the pancreatic β-cell, or regulate the RAS. [27, 90] The mapping of VDRs on pancreatic β-cells and binding of 1,25 $(OH)_2D$ to it in the cell nucleus suggests a physiological role of vitamin D in regulating pancreatic β-cells functioning.[27] Because vitamin D deficiency causes increased renin-angiotensin II expression,[90] adequate serum vitamin D concentrations may help improve insulin sensitivity by preventing increase in renin-angiotensin II expression and its resultant stimulation of nuclear factor kappa-light-enhancer of the activated B cells ($NF\text{-}_kB$). [40, 91, 92]

Alvarez et al [20] summarized the literature related to potential vitamin D influences on glucose homeostasis and insulin sensitivity. There is evidence that circulating serum vitamin D is inversely associated with IR, and a complex vitamin D action with these VDRs in the adipocyte and pancreatic β cell mediates insulin sensitivity and insulin secretion. In addition, glucose homeostasis and insulin metabolism are influenced by polymorphisms in VDR gene.[20, 89] Stunff et al [89] studied the association of a common *p110 β* single nucleotide polymorphism (SNP) with IR using HOMA-IR in 580 severely obese (BMI > 99.6th percentile) and 606 non-obese European children. The investigators

identified that the SNP was linked to elevated fasting glucose, insulin, and HOMA-IR index. Although the precise mechanism by which genetic factors affect insulin action on liver and muscle has not been elucidated, the possibility of targeting genetic VDR markers may help drive interventions to prevent development of IR and its progression to T2DM in children and adolescents.

In epidemiological studies, Forouhi et al [30] found baseline serum vitamin D inversely associated with fasting glucose, fasting insulin, and HOMA-IR in a10-year follow up study. Caceres et al [72] studied the association between IR and MetS components in 61 Bolivian obese children and adolescents (age 5-18y) using the NCEP-ATP III modified definition. In this sample, the prevalence of MetS and IR were 36% and 39.4%, respectively. IR was significantly associated with components of MetS such as hypertension and high triglyceride concentrations. A study by Alemzadeh et al [31] found low vitamin D status associated with increased risk of impaired glucose metabolism, independent of body adiposity, in 127 obese children and adolescents (mean age ± SD: 13.0 ± 3.0 y) living in northern climate (43° N). The investigators found 32.3% of vitamin D deficiency overall, with suboptimal vitamin D concentrations (<30 ng/mL) in 74% of obese subjects. Using the QUICKI, serum vitamin D was directly associated with insulin sensitivity. There was overrepresentation of women in the study (62.2%).

Chiu et al [28] investigated the relation of serum vitamin D concentrations to insulin sensitivity and β-cell function in 126 healthy adults using both hyperglycemic clamp and oral glucose tolerance test and found that hypovitaminosis D was a risk factor for MetS, and was directly associated with insulin sensitivity and inversely associated with β-cell function. Evliyaoğlu et al [93] evaluated IR using oral glucose tolerance test (OGTT),

HOMA-IR, glucose/insulin ratio, and serum lipid profiles in 19 pre-pubertal girls with premature pubarche (mean age=6.9 ± 1.7) as compared to 10 age- and Tanner stage-matched controls (mean age=7.5±1.3) using indirect IR parameters. Using HOMA-IR and glucose/insulin ratio methods with 95% CI of 2.96 as the cut off for HOMA-IR, IR was found as 42.1% and 31.6%, respectively. Mean baseline insulin and HOMA-IR were significantly higher and glucose/insulin ratio was lower in the girls with premature pubarche than in the control group. In contrast, Reinehr et al [32] did not find a relationship between vitamin D, and insulin sensitivity based on the HOMA index in obese children. The study was based on a small sample size. The majority of subjects had normal 1,25 $(OH)_2D$ concentrations. These may explain the lack of relation between vitamin D and insulin sensitivity.

Thus, evidence linking vitamin D and IR is equivocal. Adequate serum vitamin D concentrations may improve insulin sensitivity and reduce IR.[20, 27] However, the exact mechanism through which vitamin D influences the pathophysiology associated with T2DM is not clear.

VITAMIN D, METABOLIC SYNDROME, & CRP

The role of CRP, an inflammatory biomarker, in hypertension and CVD has been explored recently [36, 94]. Several epidemiological studies suggest high CRP concentrations are associated with increased risk for obesity, hypertension, endothelial dysfunction, MetS [38, 39], and CVD [33]. In children, there is evidence that CRP is significantly associated with obesity [33, 34, 37, 39], and MetS, and components of MetS [33, 38, 39]. Oliviera et al [33] investigated the association between CRP and MetS and components of MetS in 407 overweight and obese Brazilian children. They reported that children with MetS had the

most elevated CRP concentrations and that elevated CRP was significantly associated with IR and MetS.[33] Ford et al [38] studied low-grade inflammation in 1366 children aged 12-17 y in NHANES 1999-2000. Significantly higher CRP concentrations (> 3.0 mg/L) were found in 38.4% of children with MetS as compared to 10.3% of children without MetS. Abdominal obesity was independently associated with CRP.

To test a hypothesis that an increase in the prevalence of multiple inflammatory markers is associated with increase in body weight, Skinner et al [37] investigated CRP, absolute neutrophil count, and ferritin level in children aged 1-17 y using the data from NHANES 1999-2006. They found that obese children, aged 3-5y old, had elevated CRP concentrations (>1.0 mg/L). Gillum [34] also found that BMI was strongly associated with CRP concentrations in 6-11 y old Mexican-American children in the NHANES III. Sesso et al [94] investigated IL-6 and CRP concentrations in 400 women that developed hypertension during 10 y period of follow-up in the Women's Health Study. The investigators found significantly higher risk of developing hypertension with higher CRP concentrations. They concluded that inflammation had a definite role to play in identifying individuals at risk for hypertension. DeFerranti et al [39] reported that 12-19 y old adolescents with MetS had significantly higher CRP concentrations in NHANES 1999-2000.

Recent evidence links VDRs with inflammation suggesting a role for vitamin D in inflammation. The location of VDRs on inflammatory cells such as the peripheral blood mononuclear cells, T-lymphocyte, and macrophages suggests the role of vitamin D in mediating immune system response and inflammatory process. [40 68, 95] But, the exact mechanism through which vitamin D deficiency affects CRP is not clear. Sun et al [40]

confirmed that the expression of various cytokines such as as interleukin (IL)-1, IL-6, IL-8, and TNF are down-regulated by 1,25$(OH)_2$D and that VDR is involved in the inflammatory response pathway through its regulation of the NF-$_k$B. It is plausible that central obesity plays an integral role in inflammatory process by triggering the inflammatory cascade.

There are limited data on the relationship between CRP and vitamin D status. In a study by Michos et al,[15] a weak inverse relation between CRP and serum vitamin D was found (*n* = 650). However, this association was not significant after adjusting the analysis for BMI. The mechanism through which inflammatory markers such as CRP affects vitamin D concentration or vice versa is unclear. Thus, CRP is not only an independent predictor of CVD risk [36] and marker of low-grade inflammatory state in obesity, [96] but high CRP concentration indicates inflammation that is likely to contribute to components of MetS. Clinical findings make it reasonable to support an anti-inflammatory role of vitamin D in immune system, but it is supported by limited epidemiological evidence.

CONCLUSION

Newer roles of vitamin D in addition to its function in bone health have implications in the development of many chronic diseases. It appears that there is a role for vitamin D in adipocytes. Children and adolescents with low vitamin D concentrations are at risk for rickets, obesity, MetS, and its related health conditions. Evidence stresses the need for standardization of an universally accepted definition for both MetS and vitamin D status. Much remains to be learned about the complex role of vitamin D in MetS, IR, and inflammation, and the consequences of vitamin D deficiency from childhood to adulthood

CHAPTER III

SUBJECTS AND METHODS

STUDY DESIGN AND SUBJECTS

The National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) conducts large nationally representative sample surveys known as NHANES on civilian, non-institutionalized US population, using a stratified, multistage, probability sample survey design. Data on demographic characteristics, diet, and health are collected from personal interviews. Physical examinations and collection of blood and urine samples are done in the MEC. Low-income persons, adolescents (12-19 y), individuals ≥60 y, African Americans, and Mexican Americans were over sampled to yield reliable estimates for these groups. The detailed description of the survey methods and analytic guidelines are reported elsewhere. [97] All NHANES protocols were approved by NCHS Ethics Review Board.

For this study, data on children and adolescents aged 12-17 y from NHANES 2001-2002 (n = 1905), 2003-2004 (n = 1724), and 2005-2006 (n = 1704) were combined into one master database, NHANES 2001-2006. Of 5333 eligible participants, 5186 completed the interview and the examination components of the survey. Subjects with missing values for serum vitamin D (25-hydroxy cholecalciferol or calcidiol) concentration and potential confounding variables of MetS were excluded from the data

analysis. The study was limited to children over the age of 17 because fasting plasma glucose and serum insulin was measured only in NHANES participants over the age of

DESCRIPTION OF STUDY VARIABLES

Potential confounding variables included in the data analysis were age, sex, race-ethnicity, BMI, poverty income ratio (PIR), time of examination, and use of supplements. The age of the subjects was categorized into12-14 y and 15-17y age groups. Race-ethnicity was reported as non-Hispanic white, non-Hispanic black, Mexican-American, and others (included multiracial). PIR is the ratio of income to the family's appropriate poverty threshold, given by the US Census Bureau to define poverty status in accordance with Office of Management and Budget (OMB) Statistical Policy Directive14.[100] PIR values were categorized as below poverty (<1.0), middle income (1.0-2.5), and higher income (≥2.5).[100] In NHANES, data were collected in 2 time periods. In the North, data were collected during summer (May 1 - October 31), whilst in the South, data were collected during winter (November 1 - April 30).

Measurements for waist circumference (WC), BMI, systolic blood pressure (SBP), and diastolic blood pressure (DBP) measurements were obtained from the examination component of NHANES. For children and adolescents aged 12-17 y, WC was measured at the high point of the iliac crest at the end of a normal expiration to the nearest 0.1 cm by trained personnel. Age- and sex-specific 90th percentiles of WC were derived for the study population. BMI was categorized as normal weight (<85th percentile) and overweight and obese (≥85th percentile) for age and sex. For each subject, up to 4 SBP and DBP measurements were obtained after resting quietly in a sitting

position for 5 min and determining the maximum inflation level using a mercury sphygmomanometer. These values were used to calculate mean BP.[101] Age- and sex-adjusted 90th percentile of SBP and DBP measurements were derived. The participants who answered 'yes' to the question "did you use supplements in the past 30 d" were regarded as supplement users.

BIOCHEMICAL MEASUREMENTS

Blood was collected by venipuncture at MEC according to standard protocol. [98, 99, 102] Serum vitamin D concentrations were determined using the Diasorin RIA kit assay at the NCHS, CDC. Serum HDL-C was analyzed using the heparin manganese precipitation method in NHANES 2001-2002 and a direct HDL-C immunoassay method in NHANES 2005-06. Triglyceride was measured enzymatically at Johns Hopkins Hospital, Baltimore, MD. Glucose concentration was determined enzymatically in NHANES 2001-2002 and with the Hexokinase method in NHANES 2003-2006. To trend the fasting glucose data and to be able to compare NHANES 2005-2006 to the NHANES 2003-2004 glucose data, we used the following glucose regression equation as per NCHS guidelines. [98, 99]

Y (2003-2004) = 0.9835*X (2005-2006), n=92, r=0.9993, intercept not significant.

Y (2005-2006) = 0.9815*X (2003-2004) + 3.5707, n=92, r=0.9919.

Serum insulin was measured with a Pharmacia method in NHANES 2001-2002, with a Tosoh immunoenzymometric method in NHANES 2003-04, and with a Mercodia ELISA immunoassay method in 2005-2006. The mean for the Tosoh method was ≈ 11% lower than the mean for Pharmacia method . To account for the change in laboratory measurement procedure, CDC recommended the following linear regression to adjust the

NHANES 1999-2002 Pharmacia values when comparing them to NHANES 2003-2004 values:

Y (Tosoh) = (1.0027 * Pharmacia) - 2.2934

Upon adjustment, one observation with negative insulin values was excluded from the analysis. To trend the serum insulin data and to be able to compare the NHANES 2005-2006 (Mercodia) method to the NHANES 2003-2004 (Tosoh) method, we used the following insulin regression equation to compare NHANES 2005-2006 data to NHANES 2003-2004 data as per NCHS guidelines:

Y (Tosoh) = 1.0526 *X (Mercodia) – 1.5674, n=189, r=0.9870 [98, 99]

Both fasting glucose and serum insulin were measured at the Diabetes Diagnostic Laboratory, University of Missouri, (Columbia, MO) for NHANES 2001-2004 and at the Fairview Medical Center Laboratory, University of Minnesota (Minneapolis, MN) for NHANES 2005-2006. IR was assessed using Homeostatic Model Assessment-Insulin Resistance index (HOMA-IR) calculated using fasting insulin (μU/mL) × fasting glucose (mg/dL)/405 [88]. Serum CRP was measured with latex-enhanced nephelometry method at University of Washington, Seattle, WA.

DEFINITION OF METABOLIC SYNDROME VARIABLES

The prevalence of MetS in children and adolescents was defined according to the modified National Cholesterol Education Program –Adult Treatment Panel III age-adjusted criteria.[65] According to these criteria, the presence of at least 3 of the following will be regarded as having MetS: (1) WC ≥90th percentile for age and sex, (2) triglycerides ≥110 mg/dL (1.24 mmol/L), (3) HDL-C ≤40 mg/dL (1.03 mmol/L), (4) either SBP or DBP ≥90th percentile for age and sex /use of blood pressure

medication/previous diagnosis of BP, and (5) fasting glucose ≥100 mg/dL (5.55 mmol/L)/current diabetes status/ current insulin use or hypoglycemic medication use.

STATISTICAL ANALYSIS

SAS-callable SUDAAN (version 10.0.1) and SAS (version 9.2) statistical software packages were used in the data analysis. A 6-year sampling weight and secondary sampling units, i.e., Masked Variance Pseudo-PSU and Masked Variance Pseudo-Stratum were used to produce statistically reliable estimates and correct for unequal probability of selection due to oversampling of certain study subgroups. The Taylor Linearization method was used to compute variance estimates assuming a design with replacement. Detailed descriptions on sample weights and variance estimation methods are provided in the NHANES Analytic Guidelines.[97]

Combining the data from 3 NHANES cycles (2001-2002, 2003-2004, and 2005-2006) provided us more stable estimates of means, percentiles, and prevalence estimates for serum vitamin D. The status of vitamin D was classified as deficient, insufficient, and sufficient if subjects had serum vitamin D concentrations <20 ng/mL, 20-30 ng/mL, and ≥30 ng/dL, respectively. Serum vitamin D of subjects was stratified into tertiles. The tertile ranges were <19.5 ng/dL, 19.5-26.2 ng/dL, and ≥ 26.2 ng/dL. The difference in proportion of subjects with vitamin D deficiency, insufficiency, and sufficiency was determined using Rao-Scott chi-square test. The multivariate- adjusted means and 95% confidence interval (CI) for indicators of MetS , HOMA-IR, and CRP across tertiles of serum vitamin D concentrations were generated using regression analysis .

Association between prevalence of MetS and serum vitamin D concentration was analyzed with multivariate-adjusted logistic regression analysis. This analysis included sex, age, race-ethnicity, smoking, alcohol, supplement use, timing of examination, BMI, and PIR as confounding variables. Non-significant variables such as PIR, time of the examination, use of supplements, alcohol use, and smoking were dropped from the model. Multivariate-adjusted odds ratios (OR) and 95% CI were calculated for the presence of MetS for each serum vitamin D tertile category after adjusting the analysis for age, sex, race-ethnicity, and BMI. ORs for tertile 1 and 2 in relation to tertile 3 (referent category) were compared. Additionally, the association between prevalence of MetS in subjects with BMI $\geq 85^{th}$ percentile and serum vitamin D was analyzed.

Individual multivariate linear regression models were developed to ascertain the association between serum vitamin D and WC, fasting blood glucose, HDL-C, triglyceride, SBP, DBP, CRP, and HOMA-IR. Because the distribution of fasting glucose, triglycerides, CRP, and HOMA-IR were skewed, these variables were log-transformed in the analysis. Multiple comparisons ($P < 0.0167$) were performed to determine significant difference between the adjusted means for serum vitamin D tertiles for each indicator of MetS with Bonferroni adjustment after testing the hypothesis with unpaired 2-tailed t-test. We determined interactions between vitamin D and confounding variables. A P of ≤ 0.05 was considered statistically significant in all analyses.

CHAPTER IV

RESULTS

Sample sizes and health characteristics of the study population are given in **Table 1.** The study population consisted of 49.1% boys and 50.9% girls. Mean age of the sample was 14.5 y. Of the 3700 subjects, 61.8% (*n* = 985) were non-Hispanic white, 14.6% (*n* = 1226) were non-Hispanic black, and 17.5% (*n* = 1333) were Hispanic/Mexican American. The subjects were uniformly distributed between 12-14y (50.2%) and 15-17y (49.8%) age groups. Approximately 24% of the study population reported having taken a supplement in 1 month prior to the survey. The majority of the subjects were examined in summer (≈ 59%). Detailed sample sizes and health characteristics of study population are reported in Appendix A.

The prevalence of MetS in12-17 y old children and adolescents in NHANES 2001-2006 was 6.1% (**Table 2).** The prevalence of MetS was significantly higher in boys than in girls (7.9% vs. 4.3%; P <0.001) and in overweight and obese subjects (BMI ≥85th percentile) than in non-overweight subjects (BMI <85th percentile) (39.1% vs. 1.8%; P <0.001). Additionally, MetS was significantly different across vitamin D status categories (8.7% vs. 6.4% and 3.5%; P <0.001). Detailed descriptive statistics of the prevalence of MetS is reported in Appendix B.

The prevalence of vitamin D deficiency in 12-17 y old children and adolescents in NHANES 2001-2006 is presented in **Table 3**. Of the total study population, 30.5% had

vitamin D concentrations <20 ng/mL. Suboptimal serum vitamin D concentrations were higher in girls than in boys (51.9% vs. 42.5%), in non-Hispanic black than in multiracial ethnicity and Hispanic/Mexican American (73.6% vs. 51.9% and 46.8%), in subjects in the PIR <1.0 category than in those in the PIR 1.0-2.5 or ≥2.5 category (57.2% vs. 50.9% and 35.3%), in subjects

Table 1: Sample sizes and health characteristics of 12-17 y old children and adolescents in the National Health and Nutrition Examination Surveys (NHANESs) 2001-2006[1]

Characteristic	Values [2]
Sex	
Boys	1820 (49.1)
Girls	1880 (50.9)
Race-ethnicity	
Non-Hispanic white	985 (61.8)
Non-Hispanic black	1226 (14.6)
Hispanic/Mexican American	1333 (17.6)
Other	156 (6.0)
Age	
12-14 y	1859 (50.2)
15-17 y	1841 (49.8)

Poverty income ratio [3]	
<1.0	989 (17.4)
1.0-2.5	1259 (28.8)
≥2.5	1280 (50.0)
Time of examination [4]	
Summer	1749 (58.9)
Winter	1951 (41.1)
Use of supplements [3, 5]	
Yes	714 (24.1)
No	2984 (75.8)
Body mass index	22.3 ± 0.1
Vitamin D status	
Deficiency (<20 ng/mL)	1748 (30.5)
Insufficiency (20-30 ng/mL)	1541 (49.8)
Sufficiency (≥30 ng/mL)	411 (19.7)
Positive for metabolic syndrome	213 (6.1)
Waist circumference (cm) [3]	
Boys	78.6 ± 0.4
Girls	78.2 ± 0.5
Blood pressure (mm Hg)	
Systolic [6]	107.1 ± 0.3
Diastolic [6]	59. ± 0.4
HDL-cholesterol (mg/dL) [6]	51.4 ± 0.3

Fasting triglyceride (mg/dL) [7]	77.8 ± 1.4
Fasting glucose (mg/dL) [7]	93.0 ± 0.4
Serum insulin (µU/mL) [7]	10.5 ± 0.2
C-reactive protein (µg/dL) [7]	43 ± 0.001
HOMA-Insulin Resistance [7, 8]	2.4 ± 0.06

[1]*n* = 3700, weighted *n* = 17681659. NHANES 2001-2002, 2003-2004, and 2005-2006 were combined into one master database, NHANESs 2001-2006.

[2]Values are *n* and weighted percentages in parentheses or mean ± SE.

[3]Sample sizes are slightly lower due to missing responses.

[4]The examination was performed during summer (May 1 - October 31) and winter (November 1 - April 30).

[5]Participants who took supplements 1mo before the survey was conducted.

[6]Arithmetic means ± SE because data are normal.

[7]Geometric mean ± SE because data are skewed.

[8]Derived using the formula: fasting insulin (µU/mL) × fasting glucose (mg/dL)/405.

Table 2: Characteristics of 12-17 y old children and adolescents with metabolic syndrome in the National Health and Nutrition Examination Surveys (NHANESs) 2001-2006[1]

Characteristic	Prevalence of Metabolic syndrome, *n* (%)[2]	*P*-value[3]
All subjects	213 (6.1)	
Sex		<0.001
Boys	127 (7.9)	
Girls	86 (4.3)	
Race-ethnicity		0.11
Non-Hispanic white	65 (6.6)	
Non-Hispanic black	49 (4.2)	
Hispanic/Mexican American	92 (6.6)	
Other	7 (3.7)	
Age		0.74
12-14 y	106 (5.9)	
15-17 y	107 (6.3)	
Poverty income ratio[4]		0.67
<1.0	56 (6.5)	
1.0-2.5	74 (6.4)	
≥2.5	72 (5.6)	
Time of examination[5]		0.18
Summer	91 (6.7)	
Winter	122 (5.2)	

Use of supplements [4, 6]		0.01
Yes	29 (4.2)	
No	183 (6.6)	
Body mass index [4]		<0.001
<85th percentile	41 (1.8)	
≥85th percentile	172 (39.1)	
Vitamin D status		<0.001
Deficiency (<20 ng/mL)	131 (8.7)	
Insufficiency (20-30 ng/mL)	56 (6.4)	
Sufficiency (≥30 ng/mL)	26 (3.5)	

[1] *n* = 3700, weighted *n* = 17681659. NHANES 2001-2002, 2003-2004, and 2005-2006 were combined into one master database, NHANESs 2001-2006.

[2] Metabolic syndrome was defined according to the modified National Cholesterol Education Program Adult Treatment Panel III criteria, the presence of at least 3 of the following criteria (waist circumference > 90th percentile for age and sex, triglycerides > 110 mg/dL (1.24 mmol/l), HDL < 40 mg/dL (1.03 mmol/l) for gender, either systolic or diastolic blood pressure >90th percentile for age, sex and height, or use of blood pressure medication, and fasting glucose >100 mg/dL (5.55 mmol/l). Values are *n* and weighted percentages in parentheses.

[3] Significance in Rao-Scott χ^2 test.

[4] Sample sizes are slightly lower due to missing responses.

[5] The examination was performed during summer (May 1 - October 31) and winter (November 1 - April 30).

[6]Participants who took supplements 1mo before the survey was conducted.

Table 3: Prevalence of vitamin D deficiency in 12-17 y old children and adolescents in the National Health and Nutrition Examination Surveys (NHANESs) 2001-2006[1]

Characteristic	Serum vitamin D status			*P*-value[2]
	Deficiency (<20 ng/mL)	Insufficiency (20-30 ng/mL)	Sufficiency (≥30 ng/mL)	
All subjects (*n* =3700)	1748 (30.5)	1541 (49.8)	411 (19.6)	
Sex				<0.004
Boys (*n* =1820)	773 (42.5)	821 (45.1)	226 (12.4)	
Girls (*n* =1880)	975 (51.9)	720 (38.3)	185 (9.8)	
Race-ethnicity				<0.001
Non-Hispanic white (*n* =985)	141 (14.3)	550 (55.8)	294 (29.8)	
Non-Hispanic black (*n* =1226)	902 (73.6)	296 (24.1)	28 (2.3)	
Hispanic/Mexican American (*n* =1333)	624 (46.8)	628 (47.1)	81 (6.1)	
Other (*n* =156)	81 (51.9)	67 (43.0)	8 (5.1)	
Age				0.91
12-14 y (*n* =1859)	869 (46.7)	790 (42.5)	200 (10.8)	
15-17 y (*n* =1841)	879 (47.8)	751 (40.8)	211 (11.5)	
Poverty income ratio [3]				<0.001
<1.0 (*n* = 989)	566 (57.2)	367 (37.1)	56 (5.7)	
1.0-2.5 (*n* =1259)	641 (50.9)	498 (39.6)	120 (9.5)	
≥2.5 (*n* =1280)	452 (35.3)	620 (48.4)	27 (2.1)	
Time of examination [4]				<0.001
Summer (*n* =1749)	654 (37.4)	798 (45.6)	297 (17.0)	

Winter (*n* =1951)	1093 (56.0)	743 (38.1)	114 (5.8)	
Use of supplements [3, 5]				<0.001
Yes (*n* =714)	247 (34.6)	360 (50.4)	107 (15.0)	
No (*n* =2984)	1499 (50.2)	1181 (39.6)	304 (10.2)	
Body mass index [3]				<0.001
<85th percentile(*n* =3181)	1408 (44.3)	1391 (43.7)	382 (12.0)	
≥85th percentile (*n* =491)	319 (65.0)	143 (29.1)	29 (5.9)	

[1] *n* = 3700, weighted *n* = 17681659. NHANES 2001-2002, 2003-2004, and 2005-2006 were combined into one master database, NHANESs 2001-2006. Prevalence based on actual cases.

[2] Significance in Rao-Scott χ^2 test.

[3] Sample sizes are slightly lower due to missing responses.

[4] Examination was performed during summer (May 1 - October 31) and during winter (November 1- April 30).

[5] Participants who took supplements 1mo before the survey was conducted.

examined in the winter than those examined in the summer (56.0% vs. 37.4%), in non-supplement users than in supplement users (50.2 vs. 34.6%)and in children with BMI ≥85th percentile than in children <85th percentile (65.0% vs. 44.3%). Sex, race-ethnicity, PIR, time of examination, use of supplements, and BMI were significantly different for serum vitamin D concentrations across the 3 categories (*P* <0.004). Detailed descriptive statistics are reported in Appendix C.

Sample sizes and serum D concentrations for descriptive variables are given in **Table 4.** Sample sizes of girls (51.9%), non-Hispanic black (73.6%), subjects in the PIR<1.0 category (57.2%), subjects examined in the winter (56.0%), and in non-supplement users (50.2%) were higher in the lowest serum vitamin D tertile category (tertile 1 <19.5 ng/mL) as compared with the highest serum vitamin D tertile category (tertile 3 ≥ 26.2 ng/mL) and as compared to their counterparts. Sex, race-ethnicity, PIR, time of examination, use of supplements, and BMI were significantly different for serum vitamin D across the tertile categories (*P* <0.001). Detailed sample sizes of descriptive variables according to vitamin D concentration categories can be found in Appendix D.

The association between serum vitamin D concentrations and prevalence of MetS is presented in **Table 5**. In the multivariate adjusted analysis, serum vitamin D was significantly associated with prevalence of MetS (*P* for trend = 0.04). There was a significant trend in the likelihood of having MetS across the serum vitamin D tertile categories. The likelihood of children having MetS in the lowest serum vitamin D tertile category was 2.26 (95% CI: 1.11, 4.61) compared with subjects in the highest serum vitamin D tertile category. Although there was no association between serum vitamin D and MetS in children and adolescents with BMI ≥85th percentile (*P* for trend = 0.33), a

trend of increasing likelihood of MetS with decreasing vitamin D concentrations was found. Detailed results of the association between serum vitamin D concentrations and prevalence of MetS are reported in Appendix E.

The associations between serum vitamin D concentrations and individual components of MetS are presented in **Table 6.** An inverse association between serum vitamin D and WC (*P* for linear trend <0.001), SBP (*P* for linear trend = 0.009), and HOMA-IR score (*P* for linear trend = 0.003) and a positive association between serum vitamin D and HDL-c (*P* for linear trend <0.001) were found in the multivariate-adjusted regression analysis. WC, SBP, and HOMA-IR were significantly higher and HDL-c was significantly lower in the lowest serum vitamin D tertile category compared with the highest serum vitamin D tertile category (*P* < 0.0167). The adjusted mean HOMA-IR of subjects was significantly higher in the lowest serum vitamin D tertile category (OR 2.59; 95% CI: 2.44, 2.75) as compared with the highest serum vitamin D tertile category (OR 2.25; 95% CI: 2.12, 2.39). There was no association between serum vitamin D and fasting plasma glucose (*P* = 0.38), serum triglyceride (*P* = 0.55), DBP (*P* = 0.60), and CRP (*P* for linear trend = 0.18). Also, no association was found between serum vitamin D concentration and CRP in children with BMI $\geq 85^{th}$ percentile (*P* = 0.24) (data not shown). Detailed associations between serum vitamin D concentrations and individual components of MetS are reported in Appendix F**.**

Table 4: Sample sizes for serum vitamin D concentrations by age, sex, race-ethnicity, PIR, BMI, use of supplements, and time-period of examination in 12-17 y children and adolescents in the National Health and Nutrition Examination Surveys (NHANESs) 2001-2006[1]

Characteristic	Serum vitamin D status			*P*-value [2]
	Tertile 1 (<19.5 ng/mL) (*n* = 1748)	Tertile 2 (19.5-26.2 ng/mL) (*n* = 1133)	Tertile 3 (≥26.2 ng/mL) (*n* = 819)	
Sex				<0.001
Boys (*n* =1820)	773 (42.5)	588 (32.3)	459 (25.2)	
Girls (*n* =1880)	975 (51.9)	545 (29.0)	360 (19.1)	
Race-ethnicity				<0.001
Non-Hispanic white (*n* =985)	141 (14.3)	353 (38.5)	491(49.8)	
Non-Hispanic black (*n* =1226)	902 (73.6)	239 (19.5)	85 (6.9)	
Hispanic/Mexican (*n* =1333)	624 (46.8)	492 (36.9)	217 (16.3)	
Other (*n* =156)	81 (51.9)	49 (31.4)	26 (16.7)	
Age				0.99
12-14 y (*n* =1859)	869 (46.7)	589 (31.7)	401 (21.6)	
15-17 y (*n* =1841)	879 (47.7)	544 (29.5)	418 (22.7)	
Poverty income ratio [3]				<0.001
<1.0 (*n* =989)	566 (57.2)	291 (29.4)	132 (13.3)	
1.0-2.5 (*n* =1259)	641 (50.9)	366 (29.1)	252 (20.0)	

≥2.5 (*n* =1280)	452 (35.3)	433 (33.8)	395 (30.9)	
Time of examination [4]				<0.001
Summer (*n* =1749)	654 (37.4)	556 (31.8)	539 (30.8)	
Winter (*n* =1951)	1094 (56.1)	577 (29.6)	280 (14.4)	
Use of supplements [3, 5]				<0.001
Yes (*n* =714)	247 (34.6)	255 (35.7)	212 (29.7)	
No (*n* =2984)	1499 (50.2)	878 (29.4)	607 (20.3)	
Body mass index [3]				<0.001
<85th percentile (*n* =3181)	1408 (44.2)	1011 (31.8)	762 (24.0)	
≥85th percentile (*n* =491)	319 (65.0)	118 (24.0)	54 (11.0)	

[1]*n* = 3700, weighted *n* = 17681659. NHANES 2001-2002, 2003-2004, and 2005-2006 were combined into one master database, NHANESs 2001-2006. Sample sized based on actual cases. Percentages are given in parentheses.

[2]Significance for Rao-Scott χ^2 test.

[3]Sample sizes are slightly lower due to missing responses.

[4]Examination was performed during summer (May 1 - October 31) and during winter (November 1 - April 30).

[5]Participants who took supplements 1mo before the survey was conducted.

Table 5: Multivariate-adjusted odds ratio (OR) and 95 % confidence interval (CI) for metabolic syndrome according to tertiles of serum vitamin D concentrations in 12-17 y old children and adolescents in the National Health and Nutrition Examination Surveys (NHANESs) 2001-2006[1]

Variable	Serum vitamin D status			*P* for trend[2]
	Tertile 1 (<19.5 ng/mL)	Tertile 2 (19.5-26.2 ng/mL)	Tertile 3 (≥26.2 ng/mL)	
All subjects				
Positive for metabolic syndrome *n* (%)	131 (8.7)	56 (6.4)	26 (3.5)	0.04
Multivariate-adjusted OR (95% CI) [3]	2.26 (1.11, 4.61)	2.15 (1.14, 4.05)	1.0 [4]	
Subjects with body mass index ≥85th percentile	118 (42.0)	37(38.6)	17 (32.9)	0.33
Positive for metabolic syndrome *n* (%) [5]	2.05 (0.77, 5.49)	1.5 (0.57, 3.97)	1.0[4]	
Multivariate-adjusted OR (95% CI) [6]				

[1] *n* = 3444, weighted *n* = 16630443. NHANES 2001-2002, 2003-2004, and 2005-2006 were combined into one master database, NHANESs 2001-2006. Metabolic syndrome was defined according to the modified National Cholesterol Education

Program- Adult Treatment Panel III criteria, the presence of at least 3 of the criteria: waist circumference >90th percentile for age and sex, triglycerides >110 mg/dL (1.24 mmol/l), HDL < 40 mg/dL (1.03 mmol/l), either systolic or diastolic blood pressure >90th percentile for age, sex and height/use of blood pressure medication, and fasting glucose >100 mg/dL (5.55 mmol/l)/current diabetes diagnosis/ current use of insulin or oral hypoglycemic drugs.

[2]Significance for the effect of metabolic syndrome variable in the multivariate logistic regression analysis.

[3]Logistic regression was adjusted for age, sex, race-ethnicity, and BMI. There was interaction between race-ethnicity and BMI (P = 0.02). Poverty income ratio, time of examination, use of supplements, smoking status, and alcohol consumption were dropped because these variable were not significant in the model.

[4]Referent category.

[5]n = 491. Subjects with BMI ≥85th percentile.

[6]Logistic regression was adjusted for age, sex, and race-ethnicity. Poverty income ratio, time of examination, use of supplements, smoking status, and alcohol consumption were dropped because these variables were not significant in the model.

Table 6: Adjusted concentrations of indicators of metabolic syndrome according to tertiles of serum vitamin D in 12-17 y old children and adolescents in the National Health and Nutrition Examination Surveys (NHANESs) 2001-2006[1]

Variable	Serum vitamin D status[2]			P-value[3]
	Tertile 1 (<19.5 ng/mL)	Tertile 2 (19.5-26.2 ng/mL)	Tertile 3 (≥26.2 ng/mL)	
Waist circumference (cm)[4]	84.4 (83.1, 87,8) [a]	79.8 (79.0, 80,6) [b]	76.3 (75.4, 77.2) [c]	<0.001
Fasting glucose (mg/dL)[5]	93.7 (91.8, 95.6)	93.7 (91.8, 95.6)	91.8 (90.0, 93.7)	0.38
HDL-cholesterol (mg/dL)[6]	48.9 (48.4, 49.9) [a]	48.9 (48.4, 49.9) [a]	51.9 (50.9, 53.0) [b]	<0.001
Triglyceride (mg/dL)[7]	76.7 (72.2, 81.5)	79.8 (54.1, 83.9)	77.5 (73.0, 82.3)	0.55
Blood pressure				
Systolic (mm Hg)[8]	109.5 (108.5, 110.6) [a]	108.3 (107.5, 109.0) [a, b]	107.6 (106.8, 108.3) [b]	0.009
Diastolic (mm Hg)[9]	59.6 (58.7, 60.5)	59.9 (58.8, 61.0)	60.2 (59.1, 61.2)	0.60
C-reactive protein (µg/dL)[10]	43 (96, 48)	48 (44, 52)	49 (48, 50)	0.18
HOMA-Insulin Resistance [11]	2.59 (2.44, 2.75) [a]	2.39 (2.25, 2.53) [a, b]	2.25 (2.12, 2.39) [b]	0.003

[1] *n* = 3444, weighted *n* = 16630443. NHANES 2001-2002, 2003-2004, and 2005-2006 were combined into one master database, NHANESs 2001-2006.

[2]Means and 95% confidence intervals. Multiple comparisons were made with Bonferroni correction after testing the hypothesis with unpaired, 2-tailed t-test. Means with different superscript letters are significantly different from each other within the row, $P < 0.0167$.

[3]Significance of metabolic syndrome indicator variable in the multivariate regression analysis.

[4]Adjusted for age, sex, race-ethnicity, time of examination, and use of supplements.

[5]Adjusted for age, sex, race-ethnicity, BMI, and time of examination. Analysis was performed on natural log transformed values.

[6]Adjusted for age, sex, race-ethnicity, BMI, and PIR. Analysis was performed using on log transformed values. Significance for the interaction between sex and race-ethnicity was $P < 0.001$.

[7]Adjusted for race-ethnicity, BMI, and time of examination. Analysis was performed on natural log transformed values. Significance for the interaction between race-ethnicity and BMI was $P < 0.001$.

[8]Adjusted for age, sex, race-ethnicity, and BMI. Significance for the interaction between gender and race-ethnicity was $P < 0.001$.

[9]Adjusted for age and sex.

[10]Adjusted for age, race-ethnicity, BMI, and PIR. Analysis was performed on natural log transformed values.

[11]Adjusted for race-ethnicity, BMI, and use of supplements. Analysis was performed on natural log transformed values.

CHAPTER V

DISCUSSION AND CONCLUSION

To date, this is the most comprehensive study that investigated the association between serum vitamin D and MetS, components of MetS, CRP, and IR in children and adolescents in a nationally representative sample survey. We found the prevalence of MetS was 6.1% (7.0% boys, 4.3% girls). Previous studies using the modified NCEP-ATP III criteria for children found that the prevalence of MetS was between 4.2-12.7% [18, 21-23, 39]. We report the prevalence of vitamin D deficiency (<20 ng/mL) in children was 30.5%, where as others reported 22-55% using the same serum vitamin D cut-off concentrations [13, 44, 73]. Other reasons for differences in prevalence of vitamin D deficiency across studies are likely due to variability in defining vitamin D status and vitamin D assays [16], in characteristics of the subjects, geographical location [43, 51], season in which serum vitamin D measurement was collected, and in the measurement of dietary and supplemental vitamin D intakes. [16, 31]

In this study, vitamin D deficiency was significantly higher in non-Hispanic black (73.6%) as compared with other race-ethnicities [14, 43, 44] likely due to differences in skin pigmentation. Our findings also agree with previous findings by several investigators. [31, 43, 51] The variability in vitamin D concentration in race-ethnicity is because non-Hispanic black need 5-10 times longer sun exposure as compared non-Hispanic white to produce

the same amount of vitamin D.[52] Melanin in darker skin is known to interfere with UV light production, leading to diminished synthesis of vitamin D.[1]

In this large population-based study, we confirm the relationship between MetS and vitamin D status. The likelihood of having MetS in the highest tertile of serum vitamin D concentration was significantly higher relative to the lowest tertile of serum vitamin D concentration independent of age, sex, race-ethnicity, and BMI. These findings support the findings by several investigators. [14, 18, 19, 28, 30, 76] Additionally, serum vitamin D was significantly, inversely associated with several components of the MetS such as WC, SBP, and HOMA-IR score, and directly associated with low HDL-c in the multivariate regression analysis after adjusting the data for several confounding factors. In general, our results are in agreement with findings reported by Ford et al [19], Reis et al [76], and Kumar et al.[14] Ford et al [19] reported an inverse association between vitamin D and abdominal obesity, hyperglycemia, and hypertriglyceridemia in NHANES III. Reis et al [76] found a significant inverse association between serum vitamin D and MetS independent of BMI, parathyroid hormone concentration, and total calcium intake in adults (≥20 y) in NHANES 2003-2004. Using the NHANES 2001-2004 data, Kumar et al [14] found that serum vitamin D deficiency (<15 ng/mL) was significantly inversely associated with elevated BP, and directly associated with HDL-c in 1-21y old children. Reis et al [18] found low serum vitamin D was significantly associated with MetS and components of the MetS such as elevated BP and fasting glucose independent of adiposity in 3577 adolescents in NHANES 2001-2004.

We found low serum vitamin D significantly associated with increased WC, one of the measures of central adiposity. An inverse association between low serum vitamin D concentrations and body weight or BMI [11, 74] and obesity [31, 32, 43, 73] is documented in several epidemiological studies. The exact reasons for low serum vitamin D in overweight and obese individuals is not clear, but several mechanisms have been proposed. First, adiposity might influence the skin's capacity to produce vitamin D. However, there were no significant differences in the amount of vitamin D precursors between obese and non-obese people.[74] Wortsman et al [74] reported a 57% less increase in serum vitamin D concentrations in obese compared to non-obese individuals after 24 hr sunlight exposure. Hence, the release of vitamin D from skin into circulation may be affected in persons with obesity, but the capacity of the skin to produce vitamin D_3 is not affected in the obese state [74]. Second, sequestering of the fat soluble vitamin D within the adipose tissue has been hypothesized for low vitamin D concentrations in obesity.[74] Low vitamin D concentrations in obese individuals may cause secondary hyperparathyroidism leading to an increased influx of intracellular calcium, which in turn may lead to increased lipogenesis.[103] In addition, overweight and obese children are spending longer time indoors due to limited mobility and sedentary lifestyle and habits. This lesser sun exposure may explain low vitamin D synthesis and circulating concentrations. Thus, poor vitamin D status in obese individuals may accentuate obesity.

Our study is the first to investigate the association between vitamin D and IR on US children using the data reported in the recent NHANES 2001-2006. In our study,

children in the lowest vitamin D tertile had significantly increased IR compared with those in the highest vitamin D tertile. Although the mechanism and role of vitamin D in IR is not clearly elucidated, it is thought that vitamin D and vitamin-D calcium binding protein alter insulin action either at the adipocyte level or affect the release of insulin from the pancreatic β-cell, thereby mediating insulin sensitivity.[20, 27] A few findings in adults suggest an inverse association between serum vitamin D and IR and a direct association between vitamin D and insulin sensitivity.[20, 28, 30] In contrast, Reihner et al [32] and Tangorra et al [73] did not find an association between serum vitamin D and IR in obese children. The lack of association may be due to a small sample size or high prevalence of normal concentrations of $1,25(OH)_2D$ in obese subjects. The association between vitamin D and IR is further corroborated by the discovery of polymorphisms in VDR gene. These have been found to modify insulin secretion leading to IR and dysregulation of glucose homeostasis. [104, 105] Stunff et al [89] identified a single nucleotide polymorphism (SNP) on *p110 β* gene that was significantly linked with elevated fasting glucose, insulin, and HOMA-IR index in obese (BMI > 99.6th percentile) children (*n* =580). Although a relation between serum vitamin D and IR exists, but the biological mechanism through which vitamin D influences the pathophysiology associated with T2DM remains unclear.

Another newly recognized role of vitamin D is its ability to regulate BP by suppressing renin gene expression [67, 77]. The RAS regulates BP and blood volume homeostasis [67, 68]. The RAS is upregulated in obesity [66] and excessive RAS stimulation can lead to hypertension. The fact that adipose tissue is the site for vitamin D storage and is capable of producing all components of the RAS may explain the complex relationship

between vitamin D, adipocyte, and RAS.[78] A derangement in this complex system may potentially be the cause for elevated BP in overweight and obese individuals with low serum vitamin D concentrations.[66, 80] Findings from epidemiological studies shed light on the association between serum vitamin D and elevated BP.[7, 14, 18, 73] As in our study, Judd et al [7] also found serum vitamin D (<20 ng/mL) associated with SBP in children and adolescents in NHANES III. In the Framingham Offspring longitudinal study, Wang et al [48] reported vitamin D deficiency (<15 ng/mL) in 28% of individuals and found twice the higher CVD risk in vitamin D deficient individuals that had hypertension. Studies suggest reducing elevated BP through vitamin D supplementation[7] and weight loss.[84]

The anti-inflammatory role of $1,25(OH)_2D$ has been observed by numerous investigators. [40, 47, 95] The location of VDRs on inflammatory cells such as the peripheral blood mononuclear cells, T-lymphocyte, and macrophages is reason to believe that vitamin D also mediates the immune system response and inflammatory process. [40, 47, 95] The mechanism through which vitamin D deficiency affects the inflammatory marker, CRP is not clear. [40, 68] Sun et al [40] confirmed that the expression of various cytokines such as as interleukin (IL)-1, IL-6, IL-8, and TNF are down-regulated by $1,25(OH)_2D$ and that VDRs are involved in the inflammatory response pathway through its regulation of the nuclear factor kappa-light-enhancer of the activated B cells ($NF\text{-}_kB$). Evidence suggests that CRP is significantly associated with obesity [33, 34, 39] and MetS and its components [33, 38, 39] in children. In addition, low-grade inflammation is associated with CVD and hypertension as well.[36] Therefore, we hypothesized there would be an inverse relation between serum vitamin D and CRP. However, in this study we found no association between serum vitamin D concentrations and CRP in children. Lack of a relation between

serum vitamin D and CRP in this study may be due to relatively modest sample size and fewer overweight and obese children (BMI ≥85th percentile). Michos et al [15] found a weak inverse correlation between CRP and serum vitamin D, but this association was null after adjustment was made for BMI in the multivariate model. The use of a small sample size (*n* =650) could have deterred detection of an association.

This study has several strengths. The use of a nationally representative survey with large sample size of children and adolescents (*n*= 3700) allowed us to examine the relationship of vitamin D with MetS after taking into account several confounding variables. This study investigated not only the association between vitamin D and MetS, but also included the association between vitamin D and IR and inflammation. Previous studies that investigated the association between vitamin D and MetS in children had not investigated IR and inflammation.[14, 18] In addition, the use of HOMA-IR method allowed for less expensive and less invasive measurement of IR [89]. Although HOMA-IR is not the gold standard for the measurement of IR, it is considered a valid index for measurement of IR in epidemiological studies.[89]

The measurement of cause and effect relation is not possible because of the cross-sectional design of the study. Lack of uniform guidelines to define vitamin D deficiency or insufficiency make interpretation and comparison of results with published results challenging. [43, 106] The possibility of variability in the vitamin D assay could influence results from the combined NHANES data, since the serum samples were collected in north (>35 °N) in summer and in south (<35 °N) during winter. This variability is unlikely because we adjusted the serum vitamin D data to account for changes in assay between NHANES cycles using the NCHS guidelines. Not all potential confounding

factors influencing vitamin D concentrations could be taken into account in our analysis, because we were limited by a lack of information on geographical location, participant's use of sunscreen and nature of clothing. While the study looked at the generalized used of supplements, it would have been more useful to address only vitamin D supplement users.

In conclusion, children with poor vitamin D status are at increased risk for MetS and IR. Coexistence of poor vitamin D status with several of components of MetS such as increased WC, SBP, IR and low HDL-c is a concern because children with MetS are at high risk for future CVD. The risk is further accentuated with concomitant presence of vitamin D deficiency. Studies are warranted to define appropriate cuts off values for concentrations of vitamin D deficiency or insufficiency. Because of negative health outcomes associated with MetS and poor vitamin D status when existed individually or in combination, early detection and intervention of these conditions are paramount in children. Interventions to improve the vitamin D status of children should be targeted at improving the availability of vitamin D fortified foods in school nutrition programs and at the Women Infants and Children program

REFERENCES

1. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr.* Dec 2004;80(6 Suppl):1678S-1688S.

2. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet.* Nov 3 2001;358(9292):1500-1503.

3. Arnson Y, Amital H, Shoenfeld Y. Vitamin D and autoimmunity: new aetiological and therapeutic considerations. *Ann Rheum Dis.* Sep 2007;66(9):1137-1142.

4. Maki KC, Rubin MR, Wong LG, et al. Serum 23-hydroxyvitamin D is independently associated with high-density lipoprotein cholesterol and the metabolic syndrome in men and women. *J Clin Lipidology.* 2009;3:289-296.

5. Hypponen E, Boucher BJ, Berry DJ, Power C. 25-hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. *Diabetes.* Feb 2008;57(2):298-305.

6. Baz-Hecht M, Goldfine AB. The impact of vitamin D deficiency on diabetes and cardiovascular risk. *Curr Opin Endocrinol Diabetes Obes.* Apr 2010;17(2):113-119.

7. Judd SE, Nanes MS, Ziegler TR, Wilson PW, Tangpricha V. Optimal vitamin D status attenuates the age-associated increase in systolic blood pressure in white Americans: results from the third National Health and Nutrition Examination Survey. *Am J Clin Nutr.* Jan 2008;87(1):136-141

.**8.** Ganji V, Milone C, Cody MM, McCarthy F, Wang YT. Serum vitamin D concentrations are related to depression in young adult US population: the Third National Health and Nutrition Examination Survey. *Int Arch Med.* Nov 11 2010;3(1):29-30.

9. Hoogendijk WJ, Lips P, Dik MG, Deeg DJ, Beekman AT, Penninx BW. Depression is associated with decreased 25-hydroxyvitamin D and increased parathyroid hormone levels in older adults. *Arch Gen Psychiatry.* May 2008;65(5):508-512.

10. Spina CS, Tangpricha V, Uskokovic M, Adorinic L, Maehr H, Holick MF. Vitamin D and cancer. *Anticancer Res.* Jul-Aug 2006;26(4A):2515-2524.

11. Saintonge S, Bang H, Gerber LM. Implications of a new definition of vitamin D deficiency in a multiracial us adolescent population: the National Health and Nutrition Examination Survey III. *Pediatrics.* Mar 2009;123(3):797-803.

12. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes Food and Nutrition Board. *Dietary Reference Intakes for Calcium,Phosphorus, Magnesium, Vitamin D, and Fluoride.* : Institute of Medicine;1997.

13. Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med.* Jun 2004;158(6):531-537.

14. Kumar J, Muntner P, Kaskel FJ, Hailpern SM, Melamed ML. Prevalence and Associations of 25-Hydroxyvitamin D Deficiency in US Children: NHANES 2001-2004. *Pediatrics.* 2009:e362-e370.

15. Michos ED, Streeten EA, Ryan KA, et al. Serum 25-hydroxyvitamin d levels are not associated with subclinical vascular disease or C-reactive protein in the old order amish. *Calcif Tissue Int.* Mar 2009;84(3):195-202.

16. Costello RB. Vitamin D and health in the 21st century: federal initiatives to advance research. *Am J Med Sci.* Jul 2009;338(1):34-39.

17. Cole CR, Grant FK, Tangpricha V, et al. 25-hydroxyvitamin D status of healthy, low-income, minority children in Atlanta, Georgia. *Pediatrics.* Apr;125(4):633-639.

18. Reis JP, von Muhlen D, Miller ER, 3rd, Michos ED, Appel LJ. Vitamin D Status and Cardiometabolic Risk Factors in the United States Adolescent Population. *Pediatrics.* Aug 3 2009:e371-e379.

19. Ford ES, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. *Diabetes Care.* May 2005;28(5):1228-1230.

20. Alvarez JA, Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. *Int J Endocrinol.* 2010;2010:Article ID 351385.

21. Duncan GE, Li SM, Zhou XH. Prevalence and trends of a metabolic syndrome phenotype among u.s. Adolescents, 1999-2000. *Diabetes Care.* Oct 2004;27(10):2438-2443.

22. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National

Health and Nutrition Examination Survey, 1988-1994. *Arch Pediatr Adolesc Med.* Aug 2003;157(8):821-827.

23. Johnson WD, Kroon JJ, Greenway FL, Bouchard C, Ryan D, Katzmarzyk PT. Prevalence of risk factors for metabolic syndrome in adolescents: National Health and Nutrition Examination Survey (NHANES), 2001-2006. *Arch Pediatr Adolesc Med.* Apr 2009;163(4):371-377.

24. Potenza MV, Mechanick JI. The metabolic syndrome: definition, global impact, and pathophysiology. *Nutr Clin Pract.* Oct-Nov 2009;24(5):560-577.

25. Thivel D, Malina RM, Isacco L, Aucouturier J, Meyer M, Duche P. Metabolic syndrome in obese children and adolescents: dichotomous or continuous? *Metab Syndr Relat Disord.* Dec 2009;7(6):549-555.

26. Lee S, Bacha F, Gungor N, Arslanian S. Comparison of different definitions of pediatric metabolic syndrome: relation to abdominal adiposity, insulin resistance, adiponectin, and inflammatory biomarkers. *J Pediatr.* Feb 2008;152(2):177-184.

27. Teegarden D, Donkin SS. Vitamin D: emerging new roles in insulin sensitivity. *Nutr Res Rev.* Jun 2009;22(1):82-92.

28. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr.* May 2004;79(5):820-825.

29. Huh SY, Gordon CM. Vitamin D deficiency in children and adolescents: epidemiology, impact and treatment. *Rev Endocr Metab Disord.* Jun 2008;9(2):161-170.

30. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance:

the Medical Research Council Ely Prospective Study 1990-2000. *Diabetes.* Oct 2008;57(10):2619-2625.

31. Alemzadeh R, Kichler J, Babar G, Calhoun M. Hypovitaminosis D in obese children and adolescents: relationship with adiposity, insulin sensitivity, ethnicity, and season. *Metabolism.* Feb 2008;57(2):183-191.

32. Reinehr T, de Sousa G, Alexy U, Kersting M, Andler W. Vitamin D status and parathyroid hormone in obese children before and after weight loss. *Eur J Endocrinol.* Aug 2007;157(2):225-232.

33. Oliveira AC, Oliveira AM, Adan LF, Oliveira NF, Silva AM, Ladeia AM. C-reactive protein and metabolic syndrome in youth: a strong relationship? *Obesity (Silver Spring).* May 2008;16(5):1094-1098.

34. Gillum RF. Association of serum C-reactive protein and indices of body fat distribution and overweight in Mexican American children. *J Natl Med Assoc.* Jul 2003;95(7):545-552.

35. de Ferranti SD, Rifai N. C-reactive protein: a nontraditional serum marker of cardiovascular risk. *Cardiovasc Pathol.* Jan-Feb 2007;16(1):14-21.

36. Szmitko PE, Verma S. C-reactive protein and the metabolic syndrome: useful addition to the cardiovascular risk profile? *J Cardiometab Syndr.* Winter 2006;1(1):66-69; quiz 70-61.

37. Skinner AC, Steiner MJ, Henderson FW, Perrin EM. Multiple markers of inflammation and weight status: cross-sectional analyses throughout childhood. *Pediatrics.* Apr 2010;125(4):e801-809.

38. Ford ES, Ajani UA, Mokdad AH. The metabolic syndrome and concentrations of C-reactive protein among U.S. youth. *Diabetes Care.* Apr 2005;28(4):878-881.

39. de Ferranti SD, Gauvreau K, Ludwig DS, Newburger JW, Rifai N. Inflammation and changes in metabolic syndrome abnormalities in US adolescents: findings from the 1988-1994 and 1999-2000 National Health and Nutrition Examination Surveys. *Clin Chem.* Jul 2006;52(7):1325-1330.

40. Sun J, Kong J, Duan Y, et al. Increased NF-kappaB activity in fibroblasts lacking the vitamin D receptor. *Am J Physiol Endocrinol Metab.* Aug 2006;291(2):E315-322.

41. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. *Pediatrics.* Aug 2008;122(2):398-417.

42. Tangpricha V, Koutkia P, Rieke SM, Chen TC, Perez AA, Holick MF. Fortification of orange juice with vitamin D: a novel approach for enhancing vitamin D nutritional health. *Am J Clin Nutr.* Jun 2003;77(6):1478-1483.

43. Rovner AJ, O'Brien KO. Hypovitaminosis D among healthy children in the United States: a review of the current evidence. *Arch Pediatr Adolesc Med.* Jun 2008;162(6):513-519.

44. Cole CR, Grant FK, Tangpricha V, et al. 25-hydroxyvitamin D status of healthy, low-income, minority children in Atlanta, Georgia. *Pediatrics.* Apr 2010;125(4):633-639.

45. Norris JM. Can the sunshine vitamin shed light on type 1 diabetes? *Lancet.* Nov 3 2001;358(9292):1476-1478.

46. Tsoukas CD, Provvedini DM, Manolagas SC. 1,25-dihydroxyvitamin D3: a novel immunoregulatory hormone. *Science.* Jun 29 1984;224(4656):1438-1440.

47. Bhalla AK, Amento EP, Clemens TL, Holick MF, Krane SM. Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. *J Clin Endocrinol Metab.* Dec 1983;57(6):1308-1310.

48. Wang TJ, Pencina MJ, Booth SL, et al. Vitamin D deficiency and risk of cardiovascular disease. *Circulation.* Jan 29 2008;117(4):503-511.

49. Holick MF. Vitamin D deficiency. *N Engl J Med.* Jul 19 2007;357(3):266-281.

50. Holick MF. The vitamin D epidemic and its health consequences. *J Nutr.* Nov 2005;135(11):2739S-2748S.

51. Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone.* May 2002;30(5):771-777.

52. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. *Lancet.* Jan 9 1982;1(8263):74-76.

53. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab.* Aug 1988;67(2):373-378.

54. Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics.* Nov 2008;122(5):1142-1152.

55. Ross AC, Manson JE, Abrams SA, et al. The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. *J Clin Endocrinol Metab.* Nov 29 2010.

56. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr.* Mar 2004;79(3):362-371.

57. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes.* Dec 1988;37(12):1595-1607.

58. DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care.* Mar 1991;14(3):173-194.

59. Steinberger J, Daniels SR, Eckel RH, et al. Progress and Challenges in Metabolic Syndrome in Children and Adolescents. A Scientific Statement From the American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular Disease in the Young; Council on Cardiovascular Nursing; and Council on Nutrition, Physical Activity, and Metabolism. *Circulation.* Jan 12 2009.

60. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med.* Jul 1998;15(7):539-553.

61. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet Med.* May 1999;16(5):442-443.

62. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA.* May 16 2001;285(19):2486-2497.

63. Zimmet P, Alberti KG, Kaufman F, et al. The metabolic syndrome in children and adolescents - an IDF consensus report. *Pediatr Diabetes.* Oct 2007;8(5):299-306.

64. de Ferranti SD, Gauvreau K, Ludwig DS, Neufeld EJ, Newburger JW, Rifai N. Prevalence of the metabolic syndrome in American adolescents: findings from the Third National Health and Nutrition Examination Survey. *Circulation.* Oct 19 2004;110(16):2494-2497.

65. Ford ES, Li C, Cook S, Choi HK. Serum concentrations of uric acid and the metabolic syndrome among US children and adolescents. *Circulation.* May 15 2007;115(19):2526-2532.

66. Morse SA, Zhang R, Thakur V, Reisin E. Hypertension and the metabolic syndrome. *Am J Med Sci.* Dec 2005;330(6):303-310.

67. Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J. Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. *J Steroid Biochem Mol Biol.* May 2004;89-90(1-5):387-392.

68. Zittermann A, Schleithoff SS, Koerfer R. Putting cardiovascular disease and vitamin D insufficiency into perspective. *Br J Nutr.* Oct 2005;94(4):483-492.

69. Hatanaka K, Li XA, Masuda K, Yutani C, Yamamoto A. Immunohistochemical localization of C-reactive protein-binding sites in human atherosclerotic aortic

lesions by a modified streptavidin-biotin-staining method. *Pathol Int.* Sep 1995;45(9):635-641.

70. Lagrand WK, Niessen HW, Wolbink GJ, et al. C-reactive protein colocalizes with complement in human hearts during acute myocardial infarction. *Circulation.* Jan 7 1997;95(1):97-103.

71. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation.* Oct 20 2009;120(16):1640-1645.

72. Caceres M, Teran CG, Rodriguez S, Medina M. Prevalence of insulin resistance and its association with metabolic syndrome criteria among Bolivian children and adolescents with obesity. *BMC Pediatr.* 2008;8:31.

73. Smotkin-Tangorra M, Purushothaman R, Gupta A, Nejati G, Anhalt H, Ten S. Prevalence of vitamin D insufficiency in obese children and adolescents. *J Pediatr Endocrinol Metab.* Jul 2007;20(7):817-823.

74. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr.* Sep 2000;72(3):690-693.

75. Brambilla P, Bedogni G, Moreno LA, et al. Crossvalidation of anthropometry against magnetic resonance imaging for the assessment of visceral and subcutaneous adipose tissue in children. *Int J Obes (Lond).* Jan 2006;30(1):23-30.

76. Reis JP, von Muhlen D, Miller ER, 3rd. Relation of 25-hydroxyvitamin D and parathyroid hormone levels with metabolic syndrome among US adults. *Eur J Endocrinol.* Jul 2008;159(1):41-48.

77. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest.* Jul 2002;110(2):229-238.

78. Goossens GH, Blaak EE, van Baak MA. Possible involvement of the adipose tissue renin-angiotensin system in the pathophysiology of obesity and obesity-related disorders. *Obes Rev.* Feb 2003;4(1):43-55.

79. Engeli S, Negrel R, Sharma AM. Physiology and pathophysiology of the adipose tissue renin-angiotensin system. *Hypertension.* Jun 2000;35(6):1270-1277.

80. Resnick LM, Muller FB, Laragh JH. Calcium-regulating hormones in essential hypertension. Relation to plasma renin activity and sodium metabolism. *Ann Intern Med.* Nov 1986;105(5):649-654.

81. Lind L, Wengle B, Wide L, Sorensen OH, Ljunghall S. Hypertension in primary hyperparathyroidism--reduction of blood pressure by long-term treatment with vitamin D (alphacalcidol). A double-blind, placebo-controlled study. *Am J Hypertens.* Oct 1988;1(4 Pt 1):397-402.

82. Sanchez M, de la Sierra A, Coca A, Poch E, Giner V, Urbano-Marquez A. Oral calcium supplementation reduces intraplatelet free calcium concentration and insulin resistance in essential hypertensive patients. *Hypertension.* Jan 1997;29(1 Pt 2):531-536.

83. Xiang W, Kong J, Chen S, et al. Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. *Am J Physiol Endocrinol Metab.* Jan 2005;288(1):E125-132.

84. Miller ER, 3rd, Erlinger TP, Young DR, et al. Results of the Diet, Exercise, and Weight Loss Intervention Trial (DEW-IT). *Hypertension.* Nov 2002;40(5):612-618.

85. Bland R, Markovic D, Hills CE, et al. Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. *J Steroid Biochem Mol Biol.* May 2004;89-90(1-5):121-125.

86. Ferrannini E, Mari A. How to measure insulin sensitivity. *J Hypertens.* Jul 1998;16(7):895-906.

87. Katsuki A, Sumida Y, Gabazza EC, et al. Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes. *Diabetes Care.* Feb 2001;24(2):362-365.

88. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* Jul 1985;28(7):412-419.

89. Le Stunff C, Dechartres A, Miraglia Del Giudice E, Froguel P, Bougneres P. A single-nucleotide polymorphism in the p110beta gene promoter is associated with partial protection from insulin resistance in severely obese adolescents. *J Clin Endocrinol Metab.* Jan 2008;93(1):212-215.

90. Rammos G, Tseke P, Ziakka S. Vitamin D, the renin-angiotensin system, and insulin resistance. *Int Urol Nephrol.* 2008;40(2):419-426.

91. Leiter LA, Lewanczuk RZ. Of the renin-angiotensin system and reactive oxygen species Type 2 diabetes and angiotensin II inhibition. *Am J Hypertens.* Jan 2005;18(1):121-128.

92. Wei Y, Sowers JR, Clark SE, Li W, Ferrario CM, Stump CS. Angiotensin II-induced skeletal muscle insulin resistance mediated by NF-kappaB activation via NADPH oxidase. *Am J Physiol Endocrinol Metab.* Feb 2008;294(2):E345-351.

93. Evliyaoglu O, Berberoglu M, Adiyaman P, Aycan Z, Ocal G. Evaluation of insulin resistance in Turkish girls with premature pubarche using the homeostasis assessment (HOMA) model. *Turk J Pediatr.* Apr-Jun 2007;49(2):165-170.

94. Sesso HD, Wang L, Buring JE, Ridker PM, Gaziano JM. Comparison of interleukin-6 and C-reactive protein for the risk of developing hypertension in women. *Hypertension.* Feb 2007;49(2):304-310.

95. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1,25-dihydroxyvitamin D3 receptors in human leukocytes. *Science.* Sep 16 1983;221(4616):1181-1183.

96. Lyon CJ, Law RE, Hsueh WA. Minireview: adiposity, inflammation, and atherogenesis. *Endocrinology.* Jun 2003;144(6):2195-2200.

97. Centers for Disease Control and Prevention (CDC) National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Analytic and reporting guidelines. 2010; http://www.cdc.gov/nchs/data/nhanes/nhanes_general_guidelines_june_04.pdf. Accessed March 1, 2010.

98. Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Laboratory Protocol 2003-2004; http://www.cdc.gov/nchs/nhanes/nhanes2003-2004/lab03_04.htm. Accessed march 1, 2010.

99. Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Laboratory Protocol 2005-2006; http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/lab05_06.htm. Accessed March 1 2010.

100. Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Demographic Files. . 2001-2002; http://www.cdc.gov/nchs/nhanes/nhanes2001-2002/DEMO_B.htm. Accessed March 1, 2010.

101. Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Examination Protocol. 2001-2002; http://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/bpx_b_doc.pdf. Accessed March 1, 2010.

102. Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Laboratory Protocol. 2001-2002; http://www.cdc.gov/nchs/nhanes/nhanes2001-2002/lab01_02.htm. Accessed March 1, 2010.

103. McCarty MF, Thomas CA. PTH excess may promote weight gain by impeding catecholamine-induced lipolysis-implications for the impact of calcium, vitamin

D, and alcohol on body weight. *Med Hypotheses.* Nov-Dec 2003;61(5-6):535-542.

104. Oh JY, Barrett-Connor E. Association between vitamin D receptor polymorphism and type 2 diabetes or metabolic syndrome in community-dwelling older adults: the Rancho Bernardo Study. *Metabolism.* Mar 2002;51(3):356-359.

105. Chiu KC, Chuang LM, Yoon C. The vitamin D receptor polymorphism in the translation initiation codon is a risk factor for insulin resistance in glucose tolerant Caucasians. *BMC Med Genet.* 2001;2:2.

106. Greer FR. Defining Vitamin D Deficiency in Children: Beyond 25-OH Vitamin D Serum Concentrations. *Pediatrics.* Nov 2009;124(5):1471-1473

APPENDICES

APPENDIX A

SAMPLE SIZE AND HEALTH CHARACTERISCTICS OF 12-17 y OLD CHILDREN AND ADOLESCENTS IN NHANES 2001-2006

```
                                S U D A A N
            Software for the Statistical Analysis of Correlated Data
          Copyright      Research Triangle Institute      October 2009
                                Release 10.0.1
DESIGN SUMMARY: Variances will be computed using the Taylor Linearization Method,
Assuming a With
Replacement (WR) Design
    Sample Weight: MEC6YR
    Stratification Variables(s): SDMVSTRA
    Primary Sampling Unit: SDMVPSU
Number of observations read    :  30070    Weighted count :285937478
Observations in subpopulation  :   3700    Weighted count : 17681659
Denominator degrees of freedom :     45
Variance Estimation Method: Taylor Series (WR)
For Subpopulation: METSYNPREV = 1  by: Variable, SUDAAN Reserved Variable One.
-----------------------------------------------------
Variable
   Gender                  size       mean         SE
-----------------------------------------------------
Waist Circumference  (cm)
   Total                    3648     78.414     0.3737
   1                        1799     78.631     0.4355
   2                        1849     78.204     0.5057

Variable
   SUDAAN Reserved                                        Geometric
     Variable One          size       mean         SE   mean          SE
-----------------------------------------------------------------------------
Age at Screening
  Adjudicated -
  Recode
   Total                    3700     14.499     0.0454   14.397     0.0452
   1                        3700     14.499     0.0454   14.397     0.0452
average diastolic 1
  reading (mm/Hg)
   Total                    3652     59.013     0.3498   58.230     0.3275
   1                        3652     59.013     0.3498   58.230     0.3275
average systolic 1
  reading (mm/Hg)
   Total                    3652    107.112     0.2766  106.668     0.2809
   1                        3652    107.112     0.2766  106.668     0.2809
Waist Circumference
  (cm)
   Total                    3648     78.414     0.3737  77.470     0.3515
```

1	3648	78.414	0.3737	77.470	0.3515
Body Mass Index (kg/m**2)					
Total	3672	22.339	0.1411	21.859	0.1284
1	3672	22.339	0.1411	21.859	0.1284
VITD					
Total	3700	23.936	0.3936	22.208	0.3870
1	3700	23.936	0.3936	22.208	0.3870
Direct HDL-Cholesterol (mg/dL)					
Total	3692	52.699	0.2865	51.417	0.2773
1	3692	52.699	0.2865	51.417	0.2773
Triglyceride (mg/dL)					
Total	2203	88.869	2.4247	77.828	1.3717
1	2203	88.869	2.4247	77.828	1.3717
GLUCOSE					
Total	2216	93.865	0.5328	92.993	0.3578
1	2216	93.865	0.5328	92.993	0.3578
INSULIN					
Total	2201	13.129	0.4185	10.511	0.2453
1	2201	13.129	0.4185	10.511	0.2453
HOMAIR					
Total	2198	3.145	0.1247	2.415	0.0587
1	2198	3.145	0.1247	2.415	0.0587
C-reactive protein(mg/dL)					
Total	3700	0.146	0.0108	0.043	0.0012
1	3700	0.146	0.0108	0.043	0.0012

The FREQ Procedure

age	Frequency	Cumulative Frequency
1	1859	1859
2	1841	3700

Gender

RIAGENDR	Frequency	Cumulative Frequency
1	1820	1820
2	1880	3700

race	Frequency	Cumulative Frequency
1	985	985
2	1226	2211
3	1333	3544
4	156	3700

pir	Frequency	Cumulative Frequency
1	989	989
2	1259	2248
3	1280	3528
4	172	3700

Six month time period

RIDEXMON	Frequency	Cumulative Frequency

```
       1        1951          1951
       2        1749          3700

  Any dietary supplements taken?
                        Cumulative
DSD010    Frequency     Frequency
     1         714           714
     2        2984          3698
     3           2          3700

                          Cumulative
bmigroup    Frequency     Frequency
       1        3181          3181
       2         491          3672
       3          28          3700
Variable
   SUDAAN Reserved     Sample
     Variable One      size          Percent
-------------------------------------------
AGE: 1
   Total                   3700        50.18
   1                       3700        50.18
Gender: 1
   Total                   3700        49.14
   1                       3700        49.14
RACE: 1
   Total                 3700        61.77
   1                       3700        61.77
PIR: 1
   Total                 3700        17.40
   1                       3700        17.40
Six month time
  period:
   Total                 3700        41.14
   1                       3700        41.14
Any dietary
  supplements
  taken?: 1
   Total                 3700        24.13
   1                       3700        24.13
BMIGROUP: 1
   Total                   3700        87.89
   1                       3700        87.89
AGE: 2
   Total                   3700        49.82
   1                       3700        49.82
Gender: 2
   Total                   3700        50.86
   1                       3700        50.86
RACE: 2
   Total                   3700        14.57
   1                       3700        14.57
PIR: 2
   Total                   3700        28.79
   1                       3700        28.79
Six month time
  period: 2
```

```
   Total                    3700        58.86
   1                        3700        58.86
Any dietary  supplements  taken?: 2
   Total                    3700        75.81
   1                        3700        75.81
BMIGROUP: 2
   Total                    3700        11.47
   1                        3700        11.47
-------------------------------------------------------------
|                |                  | SUDAAN Reserved         |
| Variable        |                  | Variable One            |
|                 |                  |-------------------------|
|                 |                  | Total      | 1          |
-------------------------------------------------------------
| RACE: 3         | Sample size      |       3700 |       3700 |
|                 | Percent          |      17.60 |      17.60 |
| PIR: 3          | Sample size      |       3700 |       3700 |
|                 | Percent          |      49.98 |      49.98 |
|                 |                  |            |            |
| Any dietary     | Sample size      |       3700 |       3700 |
| supplements     | Percent          |       0.06 |       0.06 |
| taken?: 3       |                  |            |            |
| BMIGROUP: 3     | Sample size      |       3700 |       3700 |
|                 | Percent          |       0.64 |       0.64 |
| RACE: 4         | Sample size      |       3700 |       3700 |
|                 | Percent          |       6.07 |       6.07 |
| PIR: 4          | Sample size      |       3700 |       3700 |
|                 | Percent          |       3.84 |       3.84 |
-------------------------------------------------------------
The FREQ Procedure
                                Cumulative    Cumulative
VD    Frequency     Percent     Frequency      Percent
 1        1748       47.24          1748        47.24
 2        1541       41.65          3289        88.89
 3         411       11.11          3700       100.00
  -------------------------------------------
    Variable One      size          Percent
-------------------------------------------
VSTATUS2: 1
   Total                    3700        30.57
   1                        3700        30.57
VD: 1
   Total                    3700        30.57
   1                        3700        30.57
VSTATUS2: 2
   Total                    3700        34.20
   1                        3700        34.20
VD: 2
   Total                    3700        49.77
   1                        3700        49.77
VSTATUS2: 3
   Total                    3700        35.23
   1                        3700        35.23
VD: 3
   Total                    3700        19.66
   1                        3700        19.66
```

APPENDIX B

CHARACTERISTICS OF 12-17y OLD CHILDREN AND ADOLESCENTS WITH METABOLIC SYNDROME IN NHANES 2001-2006

```
The FREQ Procedure
                        Cumulative
metsyn    Frequency     Frequency
     0        3487          3487
     1         213          3700

Table of age by metsyn
      age       metsyn

Frequency│       0│       1│  Total
       1 │    1753 │     106 │   1859
       2 │    1734 │     107 │   1841
Total        3487      213     3700

Table of RIAGENDR by metsyn
RIAGENDR(Gender)     metsyn
Frequency│       0│       1│  Total
       1 │    1693 │     127 │   1820
       2 │    1794 │      86 │   1880
Total        3487      213     3700

Table of race by metsyn
    Race   metsyn
Frequency│       0│       1│  Total
       1 │     920 │      65 │    985
       2 │    1177 │      49 │   1226
       3 │    1241 │      92 │   1333
       4 │     149 │       7 │    156
Total        3487      213     3700

Table of pir by metsyn
pir       metsyn
Frequency│       0│       1│  Total
       1 │     933 │      56 │    989
       2 │    1185 │      74 │   1259
       3 │    1208 │      72 │   1280
       4 │     161 │      11 │    172
Total        3487      213     3700

Table of RIDEXMON by metsyn
RIDEXMON(Six month time period)
          metsyn
Frequency│       0│       1│  Total
       1 │    1829 │     122 │   1951
       2 │    1658 │      91 │   1749
Total        3487      213     3700

Table of DSD010 by metsyn
DSD010(Any dietary supplements taken?)
```

```
          metsyn
Frequency|       0|       1|  Total
       1|     685|      29|     714
       2|    2801|     183|    2984
       3|       1|       1|       2
Total        3487     213     3700

Table of bmigroup by metsyn
bmigroup     metsyn
Frequency|       0|       1|  Total
       1|    3140|      41|    3181
       2|     319|     172|     491
       3|      28|       0|      28
Total        3487     213     3700

Table of vstatus2 by metsyn
vstatus2     metsyn
Frequency|       0|       1|  Total
       1|    1617|     131|    1748
       2|    1077|      56|    1133
       3|     793|      26|     819
Total        3487     213     3700

Table of vstatus2 by metsyn
vstatus2     metsyn
Frequency|       0|       1|  Total
       1|     201|     118|     319
       2|      81|      37|     118
       3|      37|      17|      54
Total         319     172      491
|                 |                  | AGE                                 |
| Variable        |                  |-------------------------------------|
|                 |                  | Total      | 1          | 2          |
|   TSYN: 1       | Sample size      |       3700 |       1859 |       1841 |
|                 | Percent          |       6.07 |       5.90 |       6.25 |
|                 |                  | Gender                              |
METSYN: 1       | Sample size      |       3700 |       1820 |       1880 |
|                 | Percent          |       6.07 |       7.91 |       4.29 |
|                 |                  | RACE
|                 |                  | Total      | 1          | 2          | 3     | 4
| METSYN: 1       | Sample size      |  3700 |        985 |       1226 |       1333 | 156
||            | Percent          |  6.07 |       6.58 |       4.24 |       6.62 |3.73
|                 |                  | PIR
|                 | Total            | 1          | 2          | 3          | 4
|| METSYN: 1       | Sample size      | 3700 |     989 |       1259 |       1280 |    172
||                 | Percent          | 6.07 |     6.54 |       6.39 |      5.57 |  8.01
||                 |                  | Six month time period               |
| |                 |                  | Total      | 1          | 2          |
| METSYN: 1       | Sample size      |       3700 |       1951 |       1749 |
|                 | Percent          |       6.07 |       5.19 |       6.68 |
|                 |                  | Any dietary supplements taken?
|| METSYN: 1       | Sample size      |       3700 |        714 |       2984 |          2
||                 | Percent          |       6.07 |       4.18 |       6.61 |      81.92
|
|                 |                  | BMIGROUP
|
```

		Total	1	2	3
METSYN: 1	Sample size	3700	3181	491	28
	Percent	6.07	1.81	39.07	0.00
		VSTATUS2			
METSYN: 1	Sample size	3700	1748	1133	819
	Percent	6.07	8.71	6.36	3.50
METSYN: 1	Sample size	491	319	118	54
BMI ≥85th percentile	Percent	39.07	41.96	38.58	32.85

DIFFERENCE IN PREVALENCE OF METS USING RAO-SCOTT CHI-SQUARE TEST

Table of RIAGENDR by metsyn

RIAGENDR	metsyn	Frequency	Percent	Row Percent	Column Percent
Male	No	1693	45.2568	92.0919	48.1814
	Yes	127	3.8863	7.9081	64.0239
	Total	1820	49.1430	100.000	
Female	No	1794	48.6732	95.7061	51.8186
	Yes	86	2.1838	4.2939	35.9761
	Total	1880	50.8570	100.000	
Total	No	3487	93.9299		100.000
	Yes	213	6.0701		100.000
	Total	3700	100.000		

Rao-Scott Modified Chi-Square Test

Pearson Chi-Square	21.1853
Design Correction	1.2883
Rao-Scott Chi-Square	16.4442
DF	1
Pr > ChiSq	<.0001
F Value	16.4442
Num DF	1
Den DF	45
Pr > F	0.0002

Table of race by metsyn

race	metsyn	Frequency	Percent	Row Percent	Column Percent
Non-hispanic white	No	920	57.7061	93.4244	61.4353
	Yes	65	4.0616	6.5756	66.9123
	Total	985	61.7677	100.000	
Non-hispanic black	No	1177	13.9476	95.7597	14.8489
	Yes	49	0.6176	4.2403	10.1746
	Total	1226	14.5652	100.000	
Hispnic/Mexican American	No	1241	16.4357	93.3826	17.4979
	Yes	92	1.1647	6.6174	19.1875
	Total	1333	17.6004	100.000	
Other	No	149	5.8405	96.2722	6.2179
	Yes	7	0.2262	3.7278	3.7257
	Total	156	6.0666	100.000	
Total	No	3487	93.9299		100.000
	Yes	213	6.0701		100.000
	Total	3700	100.000		

Table of race by metsyn

Rao-Scott Modified Chi-Square Test

Pearson Chi-Square	6.6912
Design Correction	1.1393
Rao-Scott Chi-Square	5.8733
DF	3
Pr > ChiSq	0.1179
F Value	1.9578
Num DF	3
Den DF	135
Pr > F	0.1233

Table of age by metsyn

age	metsyn	Frequency	Percent	Row Percent	Column Percent
12-14y	No	1753	47.2201	94.1043	50.2716
	Yes	106	2.9583	5.8957	48.7366
	Total	1859	50.1784	100.000	
15-17y	No	1734	46.7099	93.7543	49.7284
	Yes	107	3.1117	6.2457	51.2634
	Total	1841	49.8216	100.000	
Total	No	3487	93.9299		100.000
	Yes	213	6.0701		100.000
	Total	3700	100.000		

Rao-Scott Modified Chi-Square Test

Pearson Chi-Square	0.1988
Design Correction	1.9271
Rao-Scott Chi-Square	0.1032
DF	1
Pr > ChiSq	0.7481
F Value	0.1032
Num DF	1
Den DF	45
Pr > F	0.7495

Table of age by metsyn

age	metsyn	Frequency	Percent	Row Percent	Column Percent
12-14y	No	1753	47.2201	94.1043	50.2716
	Yes	106	2.9583	5.8957	48.7366
	Total	1859	50.1784	100.000	
15-17y	No	1734	46.7099	93.7543	49.7284
	Yes	107	3.1117	6.2457	51.2634
	Total	1841	49.8216	100.000	
Total	No	3487	93.9299		100.000
	Yes	213	6.0701		100.000
	Total	3700	100.000		

Rao-Scott Modified Chi-Square Test

Pearson Chi-Square	0.1988
Design Correction	1.9271
Rao-Scott Chi-Square	0.1032
DF	1
Pr > ChiSq	0.7481
F Value	0.1032
Num DF	1
Den DF	45
Pr > F	0.7495

Table of age by metsyn

age	metsyn	Frequency	Percent	Row Percent	Column Percent
12-14y	No	1753	47.2201	94.1043	50.2716
	Yes	106	2.9583	5.8957	48.7366
	Total	1859	50.1784	100.000	
15-17y	No	1734	46.7099	93.7543	49.7284
	Yes	107	3.1117	6.2457	51.2634
	Total	1841	49.8216	100.000	
Total	No	3487	93.9299		100.000
	Yes	213	6.0701		100.000
	Total	3700	100.000		

Rao-Scott Modified Chi-Square Test

Pearson Chi-Square	0.1988
Design Correction	1.9271
Rao-Scott Chi-Square	0.1032
DF	1
Pr > ChiSq	0.7481
F Value	0.1032
Num DF	1
Den DF	45
Pr > F	0.7495

Table of pir by metsyn

pir	metsyn	Frequency	Percent	Row Percent	Column Percent
low income: <1.0	No	933	16.9108	93.4610	17.9888
	Yes	56	1.1832	6.5390	19.7436
	Total	989	18.0939	100.000	

moderate income: 1.0-2.5		No	1185	28.0230	93.6068	29.8093
		Yes	74	1.9139	6.3932	31.9382
		Total	1259	29.9369	100.000	
high income:=2.5		No	1208	49.0736	94.4284	52.2019
		Yes	72	2.8955	5.5716	48.3182
Total	1280	51.9692	100.000			
Total		No	3326	94.0074		100.000
		Yes	202	5.9926		100.000
		Total	3528	100.000		

Frequency Missing = 172

Table of pir by metsyn

Rao-Scott Modified Chi-Square Test

Pearson Chi-Square	1.2160
Design Correction	1.5717
Rao-Scott Chi-Square	0.7737
DF	2
Pr > ChiSq	0.6792
F Value	0.3868
Num DF	2
Den DF	90
Pr > F	0.6803

Sample Size = 3528

Table of RIDEXMON by metsyn

RIDEXMON	metsyn	Frequency	Percent	Row Percent	Column Percent
winter	No	1829	39.0040	94.8095	41.5246
	Yes	122	2.1353	5.1905	35.1781
	Total	1951	41.1393	100.000	
summer	No	1658	54.9259	93.3152	58.4754
	Yes	91	3.9347	6.6848	64.8219
	Total	1749	58.8607	100.000	
Total	No	3487	93.9299		100.000
	Yes	213	6.0701		100.000
	Total	3700	100.000		

Rao-Scott Modified Chi-Square Test

Pearson Chi-Square	3.5090
Design Correction	1.9537
Rao-Scott Chi-Square	1.7960
DF	1

```
                                Pr > ChiSq           0.1802

                                F Value              1.7960
                                Num DF                    1
                                Den DF                   45
                                Pr > F               0.1869
Table of bmigroup by metsyn

                                                                                Row
Column
                            bmigroup    metsyn     Frequency    Percent    Percent
Percent
_____________________________________________________________________________________
______
          Normal wt: <85th percentile        No          3140    86.8576    98.1907
92.5094
                                            Yes            41     1.6005     1.8093
26.1975

                                          Total          3181    88.4581    100.000
-----------------------------------------------------------------------------------------
------
Overweight and obese:>=85th percentile        No           319     7.0330    60.9346
7.4906
                                            Yes           172     4.5089    39.0654
73.8025

                                          Total           491    11.5419    100.000
                                Total        No          3459    93.8906    100.000
                                            Yes           213     6.1094    100.000
                                          Total          3672    100.000
_____________________________________________________________________________________
                                     Frequency Missing = 28
                              Rao-Scott Modified Chi-Square Test

                                Pearson Chi-Square    907.1730
                                Design Correction       9.1762

                                Rao-Scott Chi-Square   98.8612
                                DF                          1
                                Pr > ChiSq              <.0001

                                F Value                98.8612
                                Num DF                      1
                                Den DF                     45
                                Pr > F                  <.0001
                                      Sample Size = 3672

Table of VITAMIND by metsyn
                                                                  Row     Column
                 VITAMIND    metsyn     Frequency    Percent    Percent    Percent
              ____________________________________________________________________
                deficiency        No          1781    32.2832    91.1831    34.3694
                                 Yes           138     3.1216     8.8169    51.4259

                               Total          1919    35.4048    100.000
             insufficiency        No          1309    42.7540    95.1412    45.5169
```

	Yes	61	2.1834	4.8588	35.9705
	Total	1370	44.9375	100.000	
sufficiency	No	397	18.8927	96.1082	20.1136
	Yes	14	0.7650	3.8918	12.6036
	Total	411	19.6578	100.000	
Total	No	3487	93.9299		100.000
	Yes	213	6.0701		100.000
	Total	3700	100.000		

Rao-Scott Modified Chi-Square Test

Pearson Chi-Square	27.6657
Design Correction	1.4262
Rao-Scott Chi-Square	19.3982
DF	2
Pr > ChiSq	<.0001
F Value	9.6991
Num DF	2
Den DF	90
Pr > F	0.0002

Table of supp by metsyn

supp	metsyn	Frequency	Percent	Row Percent	Column Percent	
yes	No	685	23.1343	95.8201	24.6173	
	Yes	29	1.0092	4.1799	16.7519	
	Total	714	24.1435	100.000		
no	No	2801	70.8414	93.3888	75.3827	
	Yes	183	5.0151	6.6112	83.2481	
	Total	2984	75.8565	100.000		
Total	No	3486	93.9758			100.000
	Yes	212	6.0242		100.000	
	Total	3698	100.000			

Frequency Missing = 2

Rao-Scott Modified Chi-Square Test

Pearson Chi-Square	7.0719
Design Correction	1.2584
Rao-Scott Chi-Square	5.6197
DF	1
Pr > ChiSq	0.0178
F Value	5.6197
Num DF	1
Den DF	45
Pr > F	0.0221

Sample Size = 3698

APPENDIX C

DESCRIPTIVE STATISTICS FOR PREVALENCE OF VITAMIN D DEFICIENCY IN 12-17 y OLD CHLDREN AND ADOLESCENTS IN NHANES 2001-2006

The FREQ Procedure

Table of age by VD

age Frequency	VD 1	2	3	Total
1	869	790	200	1859
2	879	751	211	1841
Total	1748	1541	411	3700

Table of RIAGENDR by VD

RIAGENDR(Gender) Frequency	VD 1	2	3	Total
1	773	821	226	1820
2	975	720	185	1880
Total	1748	1541	411	3700

Table of race by VD

race Frequency	VD 1	2	3	Total
1	141	550	294	985
2	902	296	28	1226
3	624	628	81	1333
4	81	67	8	156
Total	1748	1541	411	3700

Table of pir by VD

pir Frequency	VD 1	2	3	Total
1	566	367	56	989
2	641	498	120	1259
3	452	620	208	1280
4	89	56	27	172
Total	1748	1541	411	3700

Table of RIDEXMON by VD

RIDEXMON(Six month time period) Frequency	VD 1	2	3	Total
1	1094	743	114	1951
2	654	798	297	1749
Total	1748	1541	411	3700

Table of DSD010 by VD

DSD010(Any dietary supplements taken?) Frequency	VD 1	2	3	Total
1	247	360	107	714
2	1499	1181	304	2984
3	2	0	0	2
Total	1748	1541	411	3700

Table of bmigroup by VD

bmigroup Frequency	VD 1	2	3	Total
1	1408	1391	382	3181
2	319	143	29	491
3	21	7	0	28
Total	1748	1541	411	3700

Variable		Total	VD 1	2	3
AGE: 1	Sample size	3700	1748	1541	411
	Percent	50.18	50.05	50.37	49.89
Gender: 1	Sample siz	3700	1748	1541	411
	Percent	49.14	43.60	50.79	53.58
RACE: 1	Sample si	3700	1748	1541	411
Percent		61.77	29.46	70.09	90.96
PIR: 1	Sample size	3700	1748	1541	411
	Percent	17.40	25.72	15.24	9.93
Six month time period: 1	Sample size	3700	1748	1541	411
	Percent	41.14	57.36	38.59	22.36
Any dietary supplements taken?: 1	Sample size	3700	1748	1541	411
	Percent	24.13	17.84	26.45	28.05
BMIGROUP: 1	Sample size	3700	1748	1541	411
Percent		87.89	80.13	90.69	92.86
- AGE: 2	Sample size	3700	1748	1541	411
	Percent	49.82	49.95	49.63	50.11
Gender: 2	Sample size	3700	1748	1541	411
	Percent	50.86	56.40	49.21	46.42
RACE: 2	Sample size	3700	1748	1541	411
	Percent	14.57	35.23	7.00	1.60
PIR: 2	Sample size	3700	1748	1541	411
	Percent	28.79	33.08	27.74	24.76
Six month time period: 2	Sample size	3700	1748	1541	411
	Percent	58.86	42.64	61.41	77.64
Any dietary supplements taken?: 2	Sample size	3700	1748	1541	411
	Percent	75.81	81.96	73.55	71.95
BMIGROUP: 2	Sample size	3700	1748	1541	411
	Percent	11.47	18.45	8.89	7.14
RACE: 3	Sample size	3700	1748	1541	411
	Percent	17.60	24.69	17.81	6.04
PIR: 3	Sample size	3700	1748	1541	411
	Percent	49.98	36.83	54.76	58.31
Any dietary supplements taken?: 3	Sample size	3700	1748	1541	411
	Percent	0.06	0.20	0.00	0.00
BMIGROUP: 3	Sample size	3700	1748	1541	411
	Percent	0.64	1.42	0.42	0.00
RACE: 4	Sample size	3700	1748	1541	411
	Percent	6.07	10.63	5.11	1.40
PIR: 4	Sample size	3700	1748	1541	411
	Percent	3.84	4.37	2.26	6.99

DIFFERENCE IN PREVALENCE OF VITAMIN D USING RAO-SCOTT CHI-SQUARE TEST

Data Summary

Number of Strata	45
Number of Clusters	90
Number of Observations	3700
Sum of Weights	17681659

Table of RIAGENDR by vtert

RIAGENDR	vtert	Frequency	Percent	Row Percent	Column Percent
Male	Tertile 1: <19.5 ng/mL	773	13.3312	27.1273	3.6035
	Tertile 2: 19.5-26.2 ng/mL	588	16.7110	34.0048	48.8686
	Tertile 3: >26.2 ng/mL	459	19.1009	38.8679	54.2167
	Total	1820	49.1430	100.000	
Female	Tertile 1: <19.5 ng/mL	975	17.2424	33.9038	56.3965
	Tertile 2: 19.5-26.2 ng/mL	545	17.4848	34.3804	51.1314
	Tertile 3: >26.2 ng/mL	360	16.1297	31.7159	45.7833
	Total	1880	50.8570	100.000	
Total	Tertile 1: <19.5 ng/mL	1748	30.5736		100.000
	Tertile 2: 19.5-26.2 ng/mL	1133	34.1958		100.000
	Tertile 3: >26.2 ng/mL	819	35.2306		100.000
	Total	3700	100.000		

Rao-Scott Modified Chi-Square Test

Pearson Chi-Square	27.3535
Design Correction	1.4178
Rao-Scott Chi-Square	19.2935
DF	2
Pr > ChiSq	<.0001
F Value	9.6468
Num DF	2
Den DF	90
Pr > F	0.0002

Table of race by vtert

race	vtert	Frequency	Row Percent	Column Percent	ercent
Non-hispanic white	Tertile 1: <19.5 ng/mL	141	9.0061	14.5805	9.4570
	Tertile 2: 19.5-26.2 ng/m	353	22.6946	36.7418	66.3666
	Tertile 3: >26.2 ng/mL	491	30.0671	48.6776	85.3437
Total	985	61.7677	100.000		
Non-hispanic black	Tertile 1: <19.5 ng/mL	902	10.7697	73.9413	35.2255
	Tertile 2: 19.5-26.2 ng/mL	239	2.8262	19.4037	8.2647
	Tertile 3: >26.2 ng/mL	85	0.9693	6.6550	2.7514
Total	1226	14.5652	100.000		
Hispnic/Mexican American	Tertile 1: <19.5 ng/mL	624	7.5480	42.8855	24.6880
	Tertile 2: 19.5-26.2 ng/mL	492	6.8963	39.1825	20.1671
	Tertile 3: >26.2 n	217	3.1561	17.9320	8.9585
	Total	1333	17.6004	100.000	
Other	Tertile 1: <19.5 ng/mL	81	3.2498	53.5688	10.6295
	Tertile 2: 19.5-26.2 ng/mL	49	1.7787	29.3202	5.2016
	Tertile 3: >26.2 ng/mL	26	1.0381	17.1111	2.9465
Total	156	6.0666	100.000		
Total	Tertile 1: <19.5 ng/mL	1748	30.5736		100.000

```
                          Tertile 2: 19.5-26.2 ng/mL  1133   34.1958            100.000
                              Tertile 3: >26.2 ng/m   819   35.2306            100.000
          Total         3700   100.000
```

```
──────── Table of race by vtert
Rao-Scott Modified Chi-Square Test              Pearson Chi-Square    957.3708
                              Design Correction       2.5486
                               Rao-Scott Chi-Square  375.6431
                               DF                           6
                               Pr > ChiSq              <.0001

                               F Value                62.6072
                               Num DF                       6
                               Den DF                     270
                               Pr > F                  <.0001
Table of age by vtert
                                                                          Row     Column
         age                          vtert     Frequency    Percent    Percent    Percent
      ──────────────────────────────────────────────────────────────────────────────────
      12-14y        Tertile 1: <19.5 ng/mL           869    15.3010    30.4932    50.0464
                 Tertile 2: 19.5-26.2 ng/mL          589    17.1889    34.2555    50.2660
                     Tertile 3: >26.2 ng/mL          401    17.6886    35.2513    50.2080

                                   Total          1859    50.1784    100.000
      ----------------------------------------------------------------------------------
      15-17y        Tertile 1: <19.5 ng/mL           879    15.2726    30.6546    49.9536
                 Tertile 2: 19.5-26.2 ng/mL          544    17.0070    34.1357    49.7340
                     Tertile 3: >26.2 ng/mL          418    17.5420    35.2097    49.7920
Total          1841    49.8216    100.000
      ----------------------------------------------------------------------------------
       Total        Tertile 1: <19.5 ng/mL          1748    30.5736               100.000
                 Tertile 2: 19.5-26.2 ng/mL         1133    34.1958               100.000
                     Tertile 3: >26.2 ng/mL          819    35.2306               100.000
Total          3700    100.000
      ──────────────────────────────────────────────────────────────────────────────────
                              Rao-Scott Modified Chi-Square Test
                                 Pearson Chi-Square    0.0122
                                 Design Correction     2.0214
                                 Rao-Scott Chi-Square  0.0060
                                 DF                         2
                                 Pr > ChiSq            0.9970
                                 F Value               0.0030
                                 Num DF                     2
                                 Den DF                    90
                                 Pr > F                0.9970
       Sample Size = 3700
Table of RIDEXMON by vtert
                                                                          Row     Column
     RIDEXMON                           vtert     Frequency    Percent    Percent
Percent
──────────────────────────────────────────────────────────────────────────────────────
       winter        Tertile 1: <19.5 ng/mL    1094    17.5381    42.6311    57.3637
                 Tertile 2: 19.5-26.2 ng/mL    577    14.2560    34.6529    41.6892
                     Tertile 3: >26.2 ng/mL    280     9.3452    22.7160    26.5259
 Total          1951    41.1393    100.000
       summer        Tertile 1: <19.5 ng/mL    654    13.0355    22.1463    42.6363
                 Tertile 2: 19.5-26.2 ng/mL    556    19.9399    33.8764    58.3108
```

```
                    Tertile 3: >26.2 ng/mL   539    25.8853    43.9773    73.4741
 Total          1749    58.8607    100.000
        Total        Tertile 1: <19.5 ng/mL   1748    30.5736               100.000
                  Tertile 2: 19.5-26.2 ng/mL   1133    34.1958               100.000
                    Tertile 3: >26.2 ng/mL   819    35.2306               100.000
 Total          3700    100.000
```

```
                              Rao-Scott Modified Chi-Square Test

                                Pearson Chi-Square    238.0872
                                Design Correction       4.4249

                                Rao-Scott Chi-Square   53.8059
                                DF                          2
                                Pr > ChiSq              <.0001

                                F Value                26.9029
                                Num DF                      2
                                Den DF                     90
                                Pr > F                  <.0001

Table of bmigroup by vtert
                                                                          Row     Column
                       bmigroup              vtert   Frequency  Percent  Percent Percent
Normal wt: <85th percentile   Tertile 1: <19.5 ng/mL   1408  24.6569  27.8741  81.2827
                              Tertile 2: 19.5-26.2 ng/mL  1011  30.8415  34.8657 89.8285
                             Tertile 3: >26.2 ng/mL        762   32.9596  37.2602 93.2871
     Total        3181  88.4581  100.000
Overweight and obese:>=85th percentileTertile 1: <19.5 ng/mL 319  5.6778  49.1934 18.7173
                                Tertile 2: 19.5-26.2 ng/mL 118  3.4923  30.2574 10.1715
                                Tertile 3: >26.2 ng/mL      54  2.3718  20.5492  6.7129
  Total       491 11.5419  100.000 100.000
                                Total  Tertile 1: <19.5 ng/mL    1727  30.3348
                                       Tertile 2: 19.5-26.2 ng/mL    1129  34.3338
                                       Tertile 3: >26.2 ng/mL    816  35.3314
                                                          Total    3672  100.000  100.000
                                    Frequency Missing = 28
                                Rao-Scott Modified Chi-Square Test

                                Pearson Chi-Square    88.1228
                                Design Correction      1.7194

                                Rao-Scott Chi-Square  51.2518
                                DF                          2
                                Pr > ChiSq              <.0001

                                F Value                25.6259
                                Num DF                      2
                                Den DF                     90
                                Pr > F                  <.0001
```

APPENDIX D

SAMPLE SIZE OF VARIABLES ACCORDING TO SERUM VITAMIN D TERTILE CATEGORIES

Table of age by vstatus2

age Frequency	vstatus2 1	2	3	Total
1	869	589	401	1859
2	879	544	418	1841
Total	1748	1133	819	3700

Table of RIAGENDR by vstatus2

RIAGENDR(Gender) Frequency	vstatus2 1	2	3	Total
1	773	588	459	1820
2	975	545	360	1880
Total	1748	1133	819	3700

Table of race by vstatus2

race Frequency	vstatus2 1	2	3	Total
1	141	353	491	985
2	902	239	85	1226
3	624	492	217	1333
4	81	49	26	156
Total	1748	1133	819	3700

Table of pir by vstatus2

pir Frequency	vstatus2 1	2	3	Total
1	566	291	132	989
2	641	366	252	1259
3	452	433	395	1280
4	89	43	40	172
Total	1748	1133	819	3700

Table of RIDEXMON by vstatus2

RIDEXMON(Six month time period) Frequency	vstatus2 1	2	3	Total
1	1094	577	280	1951
2	654	556	539	1749
Total	1748	1133	819	3700

Table of DSD010 by vstatus2

DSD010(Any dietary supplements taken?) Frequency	vstatus2 1	2	3	Total
1	247	255	212	714
2	1499	878	607	2984
3	2	0	0	2
Total	1748	1133	819	3700

Table of bmigroup by vstatus2

bmigroup Frequency	vstatus2 1	2	3	Total

1	1408	1011	762	3181
2	319	118	54	491
3	21	4	3	28
Total	1748	1133	819	3700

Variable		VSTATUS2			
		Total	1	2	3
AGE: 1	Sample size	3700	1748	1133	819
	Percent	50.18	50.05	50.27	50.21
Gender: 1	Sample size	3700	1748	1133	819
	Percent	49.14	43.60	48.87	54.22
RACE: 1	Sample size	3700	1748	1133	819
	Percent	61.77	29.46	66.37	85.34
PIR: 1	Sample size	3700	1748	1133	819
	Percent	17.40	25.72	16.87	10.70
Six month time	Sample size	3700	1748	1133	819
period: 1	Percent	41.14	57.36	41.69	26.53
Any dietary	Sample size	3700	1748	1133	819
supplements	Percent	24.13	17.84	25.27	28.48
taken?: 1					
BMIGROUP: 1	Sample size	3700	1748	1133	819
	Percent	87.89	80.13	89.61	92.95
AGE: 2	Sample size	3700	1748	1133	819
	Percent	49.82	49.95	49.73	49.79
Gender: 2	Sample size	3700	1748	1133	819
	Percent	50.86	56.40	51.13	45.78
RACE: 2	Sample size	3700	1748	1133	819
	Percent	14.57	35.23	8.26	2.75
PIR: 2	Sample size	3700	1748	1133	819
	Percent	28.79	33.08	28.09	25.74
Six month time	Sample size	3700	1748	1133	819
period: 2	Percent	58.86	42.64	58.31	73.47
Any dietary	Sample size	3700	1748	1133	819
supplements	Percent	75.81	81.96	74.73	71.52
taken?: 2					
BMIGROUP: 2	Sample size	3700	1748	1133	819
	Percent	11.47	18.45	10.15	6.69
RACE: 3	Sample size	3700	1748	1133	819
	Percent	17.60	24.69	20.17	8.96
PIR: 3	Sample size	3700	1748	1133	819
	Percent	49.98	36.83	52.84	58.61
Any dietary	Sample size	3700	1748	1133	819
supplements	Percent	0.06	0.20	0.00	0.00
taken?: 3					
BMIGROUP: 3	Sample size	3700	1748	1133	819
	Percent	0.64	1.42	0.24	0.36
RACE: 4	Sample size	3700	1748	1133	819
	Percent	6.07	10.63	5.20	2.95
PIR: 4	Sample size	3700	1748	1133	819
	Percent	3.84	4.37	2.21	4.95

vstatus2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1	1748	47.24	1748	47.24
2	1133	30.62	2881	77.86
3	819	22.14	3700	100.00

APPENDIX E

ASSOCIATION BETWEEEN SERUM VITAMIN D AND CONCENTRATIONS AND PREVALENCE OF METABOLIC SYNDROME

```
Table of vstatus2 by metsyn
vstatus2     metsyn
Frequency│       0│       1│  Total
       1 │    1617 │     131 │   1748
       2 │    1077 │      56 │   1133
       3 │     793 │      26 │    819
Total        3487      213     3700

Table of vstatus2 by metsyn
vstatus2     metsyn
Frequency│       0│       1│  Total
       1 │     201 │     118 │    319
       2 │      81 │      37 │    118
       3 │      37 │      17 │     54
Total         319      172      491
--
| Variable                        |                   | VSTATUS2
|                 |                   | Total      | 1          | 2          | 3
| METSYN: 1       | Sample size       |       3700 |       1748 |       1133 |        819
||                 | Percent           |       6.07 |       8.71 |       6.36 |       3.50
|| METSYN: 1       | Sample size       |        491 |        319 |        118 |         54
||for BMI≥85th percentile | Percent    |      39.07 |      41.96 |      38.58 |      32.85
|-----------------------------------------------------------------------------------------
```

LOGISTIC REGRESSION

```
Number of observations read       :  30070    Weighted count:285937478
Observations in subpopulation     :   3444    Weighted count: 16630443
Observations used in the analysis :   3444    Weighted count: 16630443
R-Square for dependent variable METSYN (Cox & Snell, 1989): 0.146248
-2 * Normalized Log-Likelihood with Intercepts Only :  1511.06
-2 * Normalized Log-Likelihood Full Model           :   966.51
Approximate Chi-Square (-2 * Log-L Ratio)           :   544.55
Degrees of Freedom                                  :       11
Response variable METSYN: METSYN
For Subpopulation: SUBGROUP = 1 by: Independent Variables and Effects.
-------------------------------------------------------------------------
Independent
P-value
  Variables and        Beta                      Lower 95%    Upper 95%
T-Test
  Effects              Coeff.          SE Beta   Limit Beta   Limit Beta   T-Test B=0
B=0
-------------------------------------------------------------------------
Intercept           -0.99         1.33        -3.67         1.69        -0.75     0.4596
VSTATUS
  1                  0.82         0.35         0.10         1.53         2.31     0.0258
  2                  0.77         0.31         0.13         1.40         2.43     0.0190
  3                  0.00         0.00         0.00         0.00
Gender
```

1	0.79	0.21	0.37	1.22	3.74	0.0005
2	0.00	0.00	0.00	0.00		
RACE						
1	0.00	0.00	0.00	0.00		
2	-2.35	0.67	-3.70	-1.01	-3.52	0.0010
3	-1.66	0.40	-2.47	-0.85	-4.13	0.0002
4	-1.06	0.93	-2.93	0.81	-1.14	0.2611
Age at Screening Adjudicated - Recode	0.03	0.07	-0.11	0.18	0.48	0.6339
BMIGROUP						
1	3.29	0.36	-4.01	-2.56	-9.15	0.0000
2	0.00	0.00	0.00	0.00		
BMIRACE						
0	-0.86	1.26	-3.39	1.68	-0.68	0.4990
1	0.42	1.28	-2.16	2.99	0.33	0.7462
2	0.81	1.31	-1.84	3.46	0.62	0.5402
3	0.00	0.00	0.00	0.00		

Contrast	Degrees of Freedom	Wald F	P-value Wald F
OVERALL MODEL	12	75.29	0.0000
MODEL MINUS INTERCEPT	11	30.30	0.0000
INTERCEPT	.	.	.
VSTATUS	**2**	**3.45**	**0.0404**
RIAGENDR	1	13.97	0.0005
RACE	3	8.00	0.0002
RIDAGEYR	1	0.23	0.6339
BMIGROUP	1	83.73	0.0000
BMIRACE	3	3.40	0.0257

Independent Variables and Effects	Odds Ratio	Lower 95% Limit OR	Upper 95% Limit OR
Intercept	0.37	0.03	5.39
VSTATUS			
1	2.26	1.11	4.61
2	2.15	1.14	4.05
3	1.00	1.00	1.00
Gender			
1	2.21	1.44	3.40
2	1.00	1.00	1.00
RACE			
1	1.00	1.00	1.00
2	0.09	0.02	0.36
3	0.19	0.08	0.43
4	0.35	0.05	2.26
Age at Screening Adjudicated - Recode	1.03	0.90	1.19
BMIGROUP			
1	0.04	0.02	0.08
2	1.00	1.00	1.00

```
BMIRACE
  0                          0.42         0.03         5.35
  1                          1.52         0.12        19.88
  2                          2.25         0.16        31.81
  3                          1.00         1.00         1.00
-----------------------------------------------------------
```

LOGISTIC REGRESSION FOR SUBJECTS WITH ≥85th PERCENTILE

```
Number of observations read       :  30070    Weighted count:285937478
Observations in subpopulation     :    441    Weighted count:  1868574
Observations used in the analysis :    441    Weighted count:  1868574
R-Square for dependent variable METSYN (Cox & Snell, 1989): 0.082810
Response variable METSYN: METSYN
For Subpopulation: SUBGROUPBMI = 1 by: Independent Variables and Effects.
---------------------------------------------------------------------------
Independent
P-value
  Variables and        Beta                       Lower 95%    Upper 95%
T-Test
  Effects              Coeff.          SE Beta   Limit Beta   Limit Beta   T-Test B=0
B=0
---------------------------------------------------------------------------
Intercept           -2.61         1.50        -5.64         0.42        -1.74     0.0892
VSTATUS
  1                  0.72         0.49        -0.27         1.70         1.47     0.1483
  2                  0.40         0.48        -0.57         1.38         0.84     0.4072
  3                  0.00         0.00         0.00         0.00          .        .
Gender
  1                 0.84         0.27         0.30         1.39         3.10     0.0033
  2                 0.00         0.00         0.00         0.00          RACE
  1                 0.00         0.00         0.00         0.00          .        .
  2                 1.15         0.35        -1.86        -0.44        -3.27     0.0021
  3                 0.02         0.34        -0.67         0.71         0.05     0.9607
  4                 0.31         0.79        -1.89         1.28        -0.39     0.6994
Age at Screening
  Adjudicated -
  Recode            0.10         0.11        -0.12         0.31         0.92     0.3638
---------------------------------------------------------------------------
Contrast               Degrees
                       of                       P-value
                       Freedom        Wald F   Wald F
-------------------------------------------------------
OVERALL MODEL                8         8.19     0.0000
MODEL MINUS
  INTERCEPT                  7         4.34     0.0010
INTERCEPT                    .          .        .
VSTATUS                      2         1.12     0.3347
RIAGENDR                     1         9.60     0.0033
RACE                         3         5.86     0.0018
RIDAGEYR                     1         0.84     0.3638
-------------------------------------------------------
Independent
  Variables and                      Lower 95%    Upper 95%
  Effects              Odds Ratio   Limit OR     Limit OR
-----------------------------------------------------------
Intercept                   0.07         0.00         1.52
VSTATUS
```

1	2.05	0.77	5.49
2	1.50	0.57	3.97
3	1.00	1.00	1.00
Gender			
1	2.33	1.34	4.03
2	1.00	1.00	1.00
RACE			
1	1.00	1.00	1.00
2	0.32	0.15	0.64
3	1.02	0.51	2.03
4	0.74	0.15	3.59
Age at Screening Adjudicated - Recode	1.10	0.89	1.36

APPENDIX F

ADJUSTED CONCENTRATIONS OF INDICATORS OF METABOLIC SYNDROME ACCORDING TO TERTILES OF SERUM VITAMIN D IN 12-17y OLD CHOLDREN AND ADOLESCENTS IN NHANES 2001-2006

```
Number of observations read       :  30070    Weighted count:285937478
Observations in subpopulation     :    773    Weighted count:  3322590
Observations used in the analysis :    773    Weighted count:  3322590
Weighted mean response is -1.789071
Multiple R-Square for the dependent variable LOGCRP: 0.027673
Response variable LOGCRP: LOGCRP
For Subpopulation: CRPBMI = 1 by: Independent Variables and Effects.
Independent
P-value
  Variables and        Beta                      Lower 95%    Upper 95%
T-Test  Effect Coeff SE Beta   Limit Beta   Limit Beta   T-Test B=0   B=0
Intercept
 -1.91         0.23        -2.37        -1.46        -8.41     0.0000
VSTATUS
  1            -0.02         0.19        -0.41         0.37        -0.10     0.9178
  2            -0.22         0.17        -0.56         0.13        -1.24     0.2205
  3            0.00         0.00         0.00         0.00          .         .
RACE
  1            0.15         0.23        -0.32         0.62         0.63     0.5338
  2            0.30         0.19        -0.09         0.68         1.54     0.1312
  3            0.34         0.23        -0.12         0.80         1.49     0.1428
  4            0.00         0.00         0.00         0.00          .         .
NEWAGE         0.09         0.03         0.02         0.15         2.74     0.0088
---------------------------------------------------------------------------
Contrast               Degrees
                       of                      P-value
                       Freedom        Wald F   Wald F
-------------------------------------------------------
OVERALL MODEL                 7       180.71     0.0000
MODEL MINUS
  INTERCEPT                   6         2.64     0.0282
INTERCEPT                     .          .        .
VSTATUS                       2         1.45     0.2460
RACE                          3         1.19     0.3237
NEWAGE                        1         7.51     0.0088
-------------------------------------------------------
Conditional Marginal   Condition-
  #1                   al
                       Marginal             SE     T:Marg=0    P-value
-----------------------------------------------------------------------
VSTATUS
  1                         -1.73         0.11       -15.87     0.0000
  2                         -1.92         0.10       -18.73     0.0000
  3                         -1.71         0.14       -12.60     0.0000
-----------------------------------------------------------------------
Contrasted
  Conditional          CONDMARG
  Marginal #1          Contrast             SE       T-Stat    P-value
```

```
-------------------------------------------------------------------
COND_EFF Contrast #  1  0.19         0.14         1.42     0.1622
COND_EFF Contrast #  2 -0.02         0.19        -0.10     0.9178
COND_EFF Contrast #  3  0.22         0.17        -1.24     0.2205
-------------------------------------------------------------------
```

LOGCRP LINEAR REGRESSION

```
Number of observations read       :  30070    Weighted count:285937478
Observations in subpopulation     :   4414    Weighted count: 21553668
Observations used in the analysis :   4414    Weighted count: 21553668
Weighted mean response is -3.059159
Multiple R-Square for the dependent variable LOGCRP: 0.168923
Response variable LOGCRP: LOGCRP
For Subpopulation: CRPVITD = 1 by: Independent Variables and Effects.
-----------------------------------------------------------------------Independent
P-value
  Variables and        Beta                      Lower 95%    Upper 95%
T-Test
  Effects              Coeff.          SE Beta   Limit Beta   Limit Beta   T-Test B=0
B=0
-------------------------------------------------------------------------------------
Intercept          -1.89         0.13        -2.15        -1.63       -14.66     0.0000
VSTATUS
  1                -0.14         0.07        -0.28         0.01        -1.84     0.0719
  2                -0.03         0.06        -0.16         0.09        -0.59     0.5609
  3                 0.00         0.00         0.00         0.00          .        .
RACE
  1                 0.07         0.11        -0.15         0.30         0.63     0.5291
  2                 0.14         0.10        -0.06         0.33         1.40     0.1676
  3                 0.30         0.10         0.10         0.50         3.06     0.0038
  4                 0.00         0.00         0.00         0.00          .        .
BMIGROUP
  1                -1.50         0.07        -1.65        -1.36       -20.88     0.0000
  2                 0.00         0.00         0.00         0.00          .        .
PIR
  1                 0.16         0.06         0.03         0.29         2.54     0.0144
  2                 0.04         0.06        -0.09         0.16         0.58     0.5629
  3                 0.00         0.00         0.00         0.00          .        .
NEWAGE              0.09         0.02         0.06         0.12         6.17     0.0000
-----------------------------------------------------------------------
Contrast               Degrees
                       of                      P-value
                       Freedom        Wald F   Wald F
-------------------------------------------------------
OVERALL MODEL                10      2020.33     0.0000
MODEL MINUS
  INTERCEPT                   9        84.63     0.0000
INTERCEPT                     .          .         .
VSTATUS                       2         1.78     0.1801
RACE                          3         7.94     0.0002
BMIGROUP                      1       436.17     0.0000
PIR                           2         3.27     0.0473
NEWAGE                        1        38.04     0.0000
-------------------------------------------------------------------
Conditional Marginal   Condition-
  #1                   al
                       Marginal             SE     T:Marg=0    P-value
```

```
-----------------------------------------------------------------------
VSTATUS
  1                          -3.14         0.05       -58.78     0.0000
  2                          -3.04         0.04       -69.74     0.0000
  3                          -3.01         0.04       -70.77     0.0000
-----------------------------------------------------------------------
COND_EFF Contrast #  1     -0.10         0.07        -1.50     0.1415
COND_EFF Contrast #  2     -0.14         0.07        -1.84     0.0719
COND_EFF Contrast #  3     -0.03         0.06        -0.59     0.5609
-----------------------------------------------------------------------
```

LOGHOMA LINEAR REGRESSION

```
Number of observations read       :  30070    Weighted count:285937478
Observations in subpopulation     :   2184    Weighted count: 10355404
Observations used in the analysis :   2184    Weighted count: 10355404
Weighted mean response is 0.880053
Multiple R-Square for the dependent variable LOGHOMA: 0.229754
Response variable LOGHOMA: LOGHOMA
For Subpopulation: HOMAVITD = 1 by: Independent Variables and Effects.
-------------------------------------------------------------------------------------------
----
Independent
P-value
  Variables and        Beta                      Lower 95%    Upper 95%
T-Test
  Effects              Coeff.          SE Beta   Limit Beta   Limit Beta   T-Test B=0
B=0
-------------------------------------------------------------------------------------------
Intercep1.61        0.12     1.37         1.84        13.79     0.0000
VSTATUS
  1                 0.14     0.04         0.06         0.22         3.43     0.0013
  2                 0.06     0.04        -0.03         0.15         1.39     0.1715
  3                 0.00     0.00         0.00         0.00         .
RACE
  1                 0.05         0.08        -0.21         0.11        -0.65     0.5167
  2                -0.11         0.08        -0.27         0.06        -1.33     0.1916
  3                -0.02         0.08        -0.19         0.14        -0.28     0.7790
  4                 0.00         0.00         0.00         0.00          .        .
BMIGROUP
  1                -0.84         0.06        -0.96        -0.73       -14.59     0.0000
  2                 0.00         0.00         0.00         0.00          .        .
Any dietary
  supplements taken?
  1                -0.09         0.04        -0.18        -0.01        -2.13     0.0389
  2                 0.00         0.00         0.00         0.00          .        .
-------------------------------------------------------------------------------------------
-------
Contrast               Degrees
                       of                       P-value
                       Freedom        Wald F   Wald F
-------------------------------------------------------
OVERALL MODEL                 8       508.75     0.0000
MODEL MINUS
  INTERCEPT                   7        36.61     0.0000
INTERCEPT                     .          .        .
VSTATUS                       2         6.28     0.0039
RACE                          3         2.11     0.1124
```

```
BMIGROUP                      1       212.92     0.0000
DSD010                        1         4.52     0.0389
-----------------------------------------------------------------------
Conditional Marginal   Condition-
  #1                   al
                       Marginal             SE     T:Marg=0    P-value
-----------------------------------------------------------------------
VSTATUS
  1                          0.95         0.03        35.95     0.0000
  2                          0.87         0.03        29.01     0.0000
  3                          0.81         0.03        23.57     0.0000
-----------------------------------------------------------------------
Contrasted
  Conditional          CONDMARG
  Marginal #1          Contrast             SE       T-Stat    P-value
-----------------------------------------------------------------------
COND_EFF Contrast #  1  0.08         0.04         2.17     0.0350
COND_EFF Contrast #  2  0.14         0.04         3.43     0.0013
COND_EFF Contrast #  3  0.06         0.04         1.39     0.1715
-----------------------------------------------------------------------
```

LOGGLU LINEAR REGRESSSION

```
Number of observations read       :  30070    Weighted count:285937478
Observations in subpopulation     :   2206    Weighted count: 10446523
Observations used in the analysis :   2206    Weighted count: 10446523
Weighted mean response is 4.532903
Multiple R-Square for the dependent variable LOGGLU: 0.093170
Response variable LOGGLU: LOGGLU
For Subpopulation: GLUVITD = 1 by: Independent Variables and Effects.
-------------------------------------------------------------------------------------------
-------
Independent
P-value
  Variables and        Beta                      Lower 95%    Upper 95%
T-Test
  Effects              Coeff.          SE Beta   Limit Beta   Limit Beta   T-Test B=0
B=0
-------------------------------------------------------------------------------------------
-------
Intercept          4.54         0.02         4.49         4.58       204.55     0.0000
VSTATUS
  1               0.01         0.01        -0.01         0.03         1.39     0.1721
  2               0.01         0.01        -0.01         0.03         1.07     0.2918
  3               0.00         0.00         0.00         0.00          .        .
Gender
  1               0.06         0.01         0.04         0.07         7.43     0.0000
  2               0.00         0.00         0.00         0.00          .        .
RACE
  1              -0.01         0.02        -0.04         0.03        -0.38     0.7078
  2              -0.05         0.01        -0.08        -0.02        -3.94     0.0003
  3              -0.01         0.01        -0.04         0.01        -0.98     0.3315
  4               0.00         0.00         0.00         0.00          .        .
BMIGROUP
  1              -0.03         0.01        -0.05        -0.01        -2.48     0.0171
  2               0.00         0.00         0.00         0.00          .        .
Six month time
```

```
  period
  1              -0.01         0.01        -0.02         0.00        -1.87     0.0680
  2               0.00         0.00         0.00         0.00          .         .
NEWAGE            0.01         0.00        -0.01        -0.00        -3.77     0.0005
-------------------------------------------------------------------------------------------
Contrast               Degrees
                       of                      P-value
                       Freedom        Wald F   Wald F
-------------------------------------------------------
OVERALL MODEL                10    300552.16     0.0000
MODEL MINUS
  INTERCEPT                   9        14.86     0.0000
INTERCEPT                     .          .        .
VSTATUS                       2         0.96     0.3895
RIAGENDR                      1        55.27     0.0000
RACE                          3        11.75     0.0000
BMIGROUP                      1         6.14     0.0171
RIDEXMON                      1         3.50     0.0680
NEWAGE                        1        14.21     0.0005
-------------------------------------------------------
Conditional Marginal   Condition-
  #1                   al
                       Marginal             SE     T:Marg=0    P-value
-----------------------------------------------------------------------
VSTATUS
  1                         4.54         0.01       794.84     0.0000
  2                         4.54         0.01       727.66     0.0000
  3                         4.52         0.01       618.50     0.0000
-----------------------------------------------------------------------
Contrasted
  Conditional          CONDMARG
  Marginal #1          Contrast             SE       T-Stat    P-value
-----------------------------------------------------------------------
COND_EFF Contrast #   1      0.00         0.01         0.46     0.6511
COND_EFF Contrast #   2      0.01         0.01         1.39     0.1721
COND_EFF Contrast #   3      0.01         0.01         1.07     0.2918
-----------------------------------------------------------------------
```

BMXWAIST LINEAR REGRESSION

```
Number of observations read       :  30070    Weighted count:285937478
Observations in subpopulation     :   4591    Weighted count: 22277006
Observations used in the analysis :   4591    Weighted count: 22277006
Multiple R-Square for the dependent variable BMXWAIST: 0.091219
Response variable BMXWAIST: Waist Circumference (cm)
For Subpopulation: WCVITD = 1 by: Independent Variables and Effects.
-------------------------------------------------------------------------------------------
Independent
P-value
  Variables and        Beta                      Lower 95%    Upper 95%
T-Test
  Effects              Coeff.          SE Beta   Limit Beta   Limit Beta   T-Test B=0
B=0
-------------------------------------------------------------------------------------------
-------
Intercept           71.23         1.19        68.84        73.62        60.05     0.0000
VSTATUS
  1                 8.10         0.87         6.35         9.86         9.30     0.0000
```

2	3.51	0.47	2.57	4.45	7.52	0.0000
3	0.00	0.00	0.00	0.00	.	.
RACE 1	6.80	1.20	4.37	9.22	5.64	0.0000
2	1.55	1.01	-0.48	3.58	1.54	0.1303
3	6.45	1.08	4.27	8.63	5.96	0.0000
4	0.00	0.00	0.00	0.00	.	.
Gender						
1	1.45	0.56	0.31	2.58	2.57	0.0135
2	0.00	0.00	0.00	0.00	.	.
Six month time period						
1	-1.98	0.64	-3.27	-0.70	-3.11	0.0033
2	0.00	0.00	0.00	0.00	.	.
Any dietary supplements taken?						
1	-2.00	0.53	-3.06	-0.95	-3.81	0.0004
2	0.00	0.00	0.00	0.00	.	.
NEWAGE	1.54	0.18	1.17	1.90	8.41	0.0000

Contrast	Degrees of Freedom	Wald F	P-value Wald F
OVERALL MODEL	10	13713.86	0.0000
MODEL MINUS INTERCEPT	9	68.11	0.0000
INTERCEPT	.	.	.
VSTATUS	2	56.84	0.0000
RACE	3	32.16	0.0000
RIAGENDR	1	6.61	0.0135
RIDEXMON	1	9.64	0.0033
DSD010	1	14.54	0.0004
NEWAGE	1	70.81	0.0000

Conditional Marginal #1	Conditional Marginal	SE	T:Marg=0	P-value
VSTATUS				
1	84.41	0.71	118.40	0.0000
2	79.81	0.41	192.58	0.0000
3	76.30	0.48	157.92	0.0000

Contrasted Conditional Marginal #1	CONDMARG Contrast	SE	T-Stat	P-value
COND_EFF Contrast # 1	4.59	0.87	5.28	0.0000
COND_EFF Contrast # 2	8.10	0.87	9.30	0.0000
COND_EFF Contrast # 3	3.51	0.47	7.52	0.0000

LOGHDD LINEAR REGRESSION

Number of observations read : 30070 Weighted count:285937478
Observations in subpopulation : 4404 Weighted count: 21499913
Observations used in the analysis : 4404 Weighted count: 21499913
Response variable LOGHDD: LOGHDD
For Subpopulation: HDLVITD = 1 by: Independent Variables and Effects.

Independent Variables and Effects	Beta Coeff.	SE Beta	Lower 95% Limit Beta	Upper 95% Limit Beta	T-Test B=0	P-value T-Test B=0
Intercept	3.94	0.04	3.87	4.01	110.85	0.0000
VSTATUS 1	-0.06	0.01	-0.08	-0.03	-4.46	0.0001
2	-0.06	0.01	-0.08	-0.04	-5.45	0.0000
3	0.00	0.00	0.00	0.00	.	.
BMIGROUP						
1	0.17	0.01	0.15	0.20	15.45	0.0000
2	0.00	0.00	0.00	0.00	.	.
PIR						
1	-0.04	0.01	-0.07	-0.01	-2.88	0.0061
2	-0.02	0.01	-0.04	0.01	-1.34	0.1864
3	0.00	0.00	0.00	0.00	.	.
NEWAGE	-0.01	0.00	-0.01	-0.00	-2.43	0.0191
Gender, RACE						
1, 1	-0.22	0.03	-0.28	-0.15	-6.63	0.0000
1, 2	-0.04	0.03	-0.11	0.02	-1.27	0.2093
1, 3	-0.13	0.03	-0.19	-0.08	-4.59	0.0000
1, 4	-0.16	0.04	-0.24	-0.08	-4.07	0.0002
2, 1	-0.10	0.03	-0.17	-0.03	-2.93	0.0053
2, 2	-0.00	0.03	-0.07	0.06	-0.09	0.9286
2, 3	-0.07	0.03	-0.13	-0.01	-2.19	0.0335
2, 4	0.00	0.00	0.00	0.00	.	

Contrast	Degrees of Freedom	Wald F	P-value Wald F
OVERALL MODEL	14	88506.69	0.0000
MODEL MINUS INTERCEPT	13	113.79	0.0000
INTERCEPT	.	.	.
VSTATUS	2	16.01	0.0000
BMIGROUP	1	238.70	0.0000
PIR	2	4.44	0.0173
NEWAGE	1	5.91	0.0191
RIAGENDR * RACE	7	56.91	0.0000

Conditional Marginal #1	Conditional Marginal	SE	T:Marg=0	P-value
VSTATUS				
1	3.89	0.01	414.94	0.0000
2	3.89	0.01	554.59	0.0000
3	3.95	0.01	436.90	0.0000

Contrasted Conditional Marginal #1	CONDMARG Contrast	SE	T-Stat	P-value
COND_EFF Contrast # 1	0.00	0.01	0.04	0.9658
COND_EFF Contrast # 2	-0.06	0.01	-4.46	0.0001

```
COND_EFF Contrast #  3      -0.06         0.01        -5.45     0.0000
-----------------------------------------------------------------
LOGTR LINEAR REGRESSION
Number of observations read       :  30070    Weighted count:285937478
Observations in subpopulation     :   2191    Weighted count: 10376377
Observations used in the analysis :   2191    Weighted count: 10376377
Weighted mean response is 4.355361
Multiple R-Square for the dependent variable LOGTR: 0.130919
Response variable LOGTR: LOGTR
For Subpopulation: TRIVITD = 1by: Independent Variables and Effects.
-------------------------------------------------------------------------------------
Independent
P-value
  Variables and        Beta                      Lower 95%    Upper 95%
T-Test
  Effects              Coeff.          SE Beta   Limit Beta   Limit Beta   T-Test B=0
B=0
-------------------------------------------------------------------------------------
Intercept         5.30         0.53         4.23         6.38         9.93     0.0000
VSTATUS
  1              -0.00         0.04        -0.08         0.08        -0.04     0.9705
  2               0.03         0.03        -0.04         0.10         0.91     0.3659
  3               0.00         0.00         0.00         0.00          .        .Six
month time  period
  1              -0.07         0.03        -0.13        -0.01        -2.26     0.0287
  2               0.00         0.00         0.00         0.00          .        .
BMIGROUP, RACE
  1, 1           -0.94         0.53        -2.01         0.13        -1.77     0.0835
  1, 2       .   -1.20         0.53        -2.27        -0.13        -2.25     0.0291
  1, 3           -0.95         0.53        -2.02         0.13        -1.78     0.0822
  1, 4           -0.96         0.52        -2.01         0.08        -1.87     0.0686
  2, 1           -0.57         0.54        -1.67         0.52        -1.06     0.2953
  2, 2           -0.93         0.52        -1.98         0.12        -1.78     0.0824
  2, 3           -0.53         0.53        -1.59         0.53        -1.01     0.3195
  2, 4            0.00         0.00         0.00         0.00          .        .
-----------------------------------------------------------------------
Contrast               Degrees
                       of                      P-value
                       Freedom        Wald F   Wald F
-------------------------------------------------------
OVERALL MODEL                11     14864.72     0.0000
MODEL MINUS
  INTERCEPT                  10        44.33     0.0000
INTERCEPT                     .          .        .
VSTATUS                       2         0.59     0.5595
RIDEXMON                      1         5.11     0.0287
BMIGROUP * RACE               7        45.43     0.0000
-------------------------------------------------------
Conditional Marginal#1 Conditional
                       Marginal             SE     T:Marg=0    P-value
-----------------------------------------------------------------
VSTATUS
  1                          4.34         0.03       165.30     0.0000
  2                          4.38         0.02       175.73     0.0000
  3                          4.35         0.03       146.47     0.0000
-----------------------------------------------------------------
Contrasted
```

Conditional Marginal #1	CONDMARG Contrast	SE	T-Stat	P-value
COND_EFF Contrast # 1	-0.03	0.04	-0.88	0.3859
COND_EFF Contrast # 2	-0.00	0.04	-0.04	0.9705
COND_EFF Contrast # 3	0.03	0.03	0.91	0.3659

BPSY LINEAR REGRESSION

Number of observations read : 30070 Weighted count:285937478
Observations in subpopulation : 4513 Weighted count: 21903379
Observations used in the analysis : 4513 Weighted count: 21903379
Weighted mean response is 108.411819
Multiple R-Square for the dependent variable BPSY: 0.157109
Response variable BPSY: average systolic 1 reading (mm/Hg)
For Subpopulation: BPSYVITD = 1by: Independent Variables and Effects.

Independent Variables and Effects	Beta Coeff.	SE Beta	Lower 95% Limit Beta	Upper 95% Limit Beta	T-Test B=0	P-value T-Test B=0
Intercept	106.96	1.21	104.53	109.40	88.50	0.0000
VSTATUS 1	1.99	0.63	0.71	3.26	3.13	0.0031
2	0.70	0.54	-0.39	1.79	1.30	0.2011
3	0.00	0.00	0.00	0.00	.	.
BMIGROUP						
1	-6.64	0.51	-7.67	-5.61	-12.98	0.0000
2	0.00	0.00	0.00	0.00	.	.
NEWAGE	1.14	0.10	0.93	1.34	11.06	0.0000
Gender, RACE						
1, 1	8.56	1.12	6.30	10.82	7.63	0.0000
1, 2	9.66	1.10	7.44	11.87	8.79	0.0000
1, 3	7.60	1.21	5.15	10.04	6.26	0.0000
1, 4	7.85	1.68	4.47	11.24	4.67	0.0000
2, 1	4.09	1.16	1.75	6.43	3.52	0.0010
2, 2	4.72	1.12	2.47	6.97	4.23	0.0001
2, 3	3.59	1.16	1.25	5.93	3.09	0.0034
2, 4	0.00	0.00	0.00	0.00	.	

Contrast	Degrees of Freedom	Wald F	P-value Wald F
OVERALL MODEL	12	18780.14	0.0000
MODEL MINUS INTERCEPT	11	78.67	0.0000
INTERCEPT	.	.	.
VSTATUS	2	5.14	0.0098
BMIGROUP	1	168.36	0.0000
NEWAGE	1	122.36	0.0000
RIAGENDR * RACE	7	65.59	0.0000

Conditional Marginal #1	Condition-al Marginal	SE	T:Marg=0	P-value

```
-----------------------------------------------------------------
VSTATUS
  1                        109.54         0.53       208.47     0.0000
  2                        108.26         0.40       269.69     0.0000
  3                        107.56         0.39       274.75     0.0000
-----------------------------------------------------------------
COND_EFF Contrast #  1      1.28         0.54         2.39     0.0211
COND_EFF Contrast #  2      1.99         0.63         3.13     0.0031
COND_EFF Contrast #  3      0.70         0.54         1.30     0.2011
-----------------------------------------------------------------
```

BPDI LINEAR REGRESSION

```
Number of observations read       :  30070    Weighted count:285937478
Observations in subpopulation     :   4536    Weighted count: 21964438
Observations used in the analysis :   4536    Weighted count: 21964438
Multiple R-Square for the dependent variable BPDI: 0.044782
Response variable BPDI: average diastolic 1 reading (mm/Hg)
For Subpopulation: BPDIVITD = 1 by: Independent Variables and Effects.
Independent  P-value
  Variables and        Beta                      Lower 95%    Upper 95%
T-Test
  Effects              Coeff.          SE Beta   Limit Beta   Limit Beta   T-Test B=0
B=0
-------------------------------------------------------------------------------------
Intercept           61.79         0.58        60.61        62.96       106.34     0.0000
VSTATUS
  1                -0.55         0.67        -1.90         0.80        -0.82     0.4188
  2                -0.78         0.78        -2.35         0.79        -1.00     0.3232
  3                 0.00         0.00         0.00         0.00          .        .
Gender  1    .     -3.22         0.51        -4.24        -2.20        -6.38     0.0000
  2                 0.00         0.00         0.00         0.00          .        .
NEWAGE             1.10         0.12         0.85         1.35         9.01     0.0000
Contrast               Degrees
                       of                      P-value
                       Freedom        Wald F   Wald F

OVERALL MODEL                 5      8339.62     0.0000
MODEL MINUS
  INTERCEPT                   4        38.22     0.0000
INTERCEPT                     .          .        .
VSTATUS                       2         0.51     0.6013
RIAGENDR                      1        40.65     0.0000
NEWAGE                        1        81.23     0.0000

Conditional Marginal   Condition-
  #1                   al
                       Marginal             SE     T:Marg=0    P-value
VSTATUS
  1                        59.62         0.47       127.16     0.0000
  2                        59.39         0.56       106.35     0.0000
  3                        60.17         0.55       108.76     0.0000
Contrasted
  Conditional          CONDMARG
  Marginal #1          Contrast             SE       T-Stat    P-value
-----------------------------------------------------------------
COND_EFF Contrast #  1    0.23         0.58         0.40     0.6920
COND_EFF Contrast #  2   -0.55         0.67        -0.82     0.4188
COND_EFF Contrast #  3   -0.78         0.78        -1.00     0.3232
-----------------------------------------------------------------
```