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

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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 $\geq$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 ($\leq$ 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 ($\leq$15 ng/mL) and 42% ($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 $\geq$40 inches in men or $\geq$35 inches in women, serum triglycerides $\geq$150 mg/dL, serum high-density lipoprotein cholesterol (HDL-c) <40 mg/dL for men or <50 mg/dL for women, high BP $\geq$130/85 mm Hg, and fasting plasma glucose $\geq$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 $\geq$90th percentile for age and sex, (2) triglycerides $\geq$110mg/dL (1.24 mmol/L), (3) HDL-c $\leq$40 mg/dL (1.03 mmol/L), (4) either systolic blood pressure (SBP) or diastolic blood pressure (DBP) $\geq$90th percentile for age, sex, and height, and (5) fasting glucose concentration $\geq$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 $\geq$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 ≥95th 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 and 1α-

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 ($NF_{-k}B$). [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 > 99.6[th] 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 $\pm$ 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)$_2$D 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 $1,25(OH)_2D$ and that VDR is involved in the inflammatory response pathway through its regulation of the $NF-_kB$. 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) $\times$ 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 $\geq$90th percentile for age and sex, (2) triglycerides $\geq$110 mg/dL (1.24 mmol/L), (3) HDL-C $\leq$40 mg/dL (1.03 mmol/L), (4) either SBP or DBP $\geq$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 ≥85th 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 ($\approx$ 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 $\geq$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] $59. \pm 0.4$

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	Insufficiency	Sufficiency	
	(<20 ng/mL)	(20-30 ng/mL)	(≥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 ≥85th 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 > 99.6[th] 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-$_k$B). 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

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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
```

66

```
   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
                    Cumulative
age     Frequency    Frequency
  1        1859         1859
  2        1841         3700
              Gender
                        Cumulative
RIAGENDR     Frequency   Frequency
      1        1820         1820
      2        1880         3700
                    Cumulative
race     Frequency    Frequency
  1         985          985
  2        1226         2211
  3        1333         3544
  4         156         3700
                    Cumulative
pir     Frequency    Frequency
  1         989          989
  2        1259         2248
  3        1280         3528
  4         172         3700

Six month time period
                        Cumulative
RIDEXMON     Frequency   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 Variable One	Sample 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

				Row	Column
age	metsyn	Frequency	Percent	Percent	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

				Row	Column
pir	metsyn	Frequency	Percent	Percent	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

```
                                                         Row      Column
            RIDEXMON    metsyn    Frequency    Percent   Percent   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
```

		Frequency	Percent	Row Percent	Column Percent
	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       VD
Frequency|      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)     VD
Frequency|      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      VD
Frequency|      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       VD
Frequency|      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)      VD
Frequency|      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?)
          VD
Frequency|      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     VD
Frequency|      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		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	Sample size	3700		1748		1541		411	
period: 1	Percent		41.14		57.36		38.59		22.36	
Any dietar	Sample size	3700		1748		1541		411	
supplements Percent		24.13		17.84		26.45		28.05	
taken?: 1										
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	Sample size	3700		1748		1541		411	
period: 2	Percent	58.86		42.64		61.41		77.64	
Any dietary	Sample size	3700		1748		1541		411	
supplements	Percent	75.81		81.96		73.55		71.95	
taken?: 2										
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	Sample size	3700		1748		1541		411	
supplements	Percent	0.06		0.20		0.00		0.00	
taken?: 3										
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
```

				Row	Column
RIAGENDR	vtert	Frequency	Percent	Percent	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

				Row	Column
race	vtert	Frequency	Percent	Percent	Percent
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

age	vtert	Frequency	Percent	Row Percent	Column 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

RIDEXMON	vtert	Frequency	Percent	Row Percent	Column 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       vstatus2
Frequency|        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)     vstatus2
Frequency|        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      vstatus2
Frequency|        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       vstatus2
Frequency|        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)     vstatus2
Frequency|        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?)
         vstatus2
Frequency|        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     vstatus2
Frequency|        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 period: 1	Sample size	3700	1748	1133	819
	Percent	41.14	57.36	41.69	26.53
Any dietary supplements taken?: 1	Sample size	3700	1748	1133	819
	Percent	24.13	17.84	25.27	28.48
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 period: 2	Sample size	3700	1748	1133	819
	Percent	58.86	42.64	58.31	73.47
Any dietary supplements taken?: 2	Sample size	3700	1748	1133	819
	Percent	75.81	81.96	74.73	71.52
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 supplements taken?: 3	Sample size	3700	1748	1133	819
	Percent	0.06	0.20	0.00	0.00
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 Effects B=0	Beta Coeff.	SE Beta	Lower 95% Limit Beta	Upper 95% Limit Beta	T-Test B=0	T-Test 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 Freedom	Wald F	P-value 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 #1	Conditional 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 Freedom	Wald F	P-value 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 #1	Conditional 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 Marginal #1	CONDMARG 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 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	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
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            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                          P-value
                    Freedom      Wald F    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  Condition-
   #1                 al
                      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        CONDMARG
   Marginal #1        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 T-Test Effects B=0	Beta Coeff.	SE Beta	Lower 95% Limit Beta	Upper 95% Limit Beta	T-Test B=0	P-value T-Test 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 Freedom	Wald F	P-value 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          CONDMARG
    Marginal #1          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
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            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                      P-value
                      Freedom      Wald F     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  Condition-
   #1                 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 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	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 Freedom	Wald F	P-value 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 #1	Conditional 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 Marginal #1	CONDMARG 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

```
-------------------------------------------------------------
```