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The Association Between Vitamin D and NAFLD Modified by Race

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Emory University,
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Abstract

The association between vitamin D and NAFLD modified by race

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Non-alcoholic fatty liver disease (NAFLD) is a spectrum of metabolic irregularities that through characteristic steatosis of the liver can result in non-alcoholic steatohepatitis and cirrhosis. NAFLD has a prevalence of between 20 - 30% worldwide and is considered the most common cause of liver disease globally. Current estimates approximate the prevalence of NAFLD to be as high as between 20-34% of the U.S. population. Along with strong association with general metabolic dysfunction, evidence is mounting to suggest vitamin D deficiency (VDD) is also associated, potentially causally, with NAFLD. One of the stark gaps in the observational literature on the association between vitamin-D status and NAFLD is that few studies included racially diverse populations in spite of evidence that suggests that African-Americans may experience VDD at higher rates than their Caucasian-American counterparts. To address this research gap, we evaluated the association between vitamin D status and NAFLD and considered variation by race in a nationally representative sample of the US population. A total of 9,538 adult participants were included from the NHANES III dataset. Baseline characteristics across contingency groups of interest were compared using the chi-square tests for categorical variables and two-sample t-tests or ANOVA for continuous variables. Two-sided P-values ≤ 0.05 were considered statistically significant. Stratified and un-stratified logistic regression models were used to assess the association between serum vitamin D level and NAFLD by race. All data were analyzed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R software version 4.0.1. Overall, compared to those in the lowest quintile of vitamin D, those in the highest quintile were approximately 36% less at risk for NAFLD (OR=0.641; 95% CI 0.463-0.888). However, in multi-variable models, that effect was attenuated to marginal significance in African-Americans (p-value =0.0447), but not in Mexican-Americans (p-value<0.001) and white-Americans (p-value<0.01). Our study indicates that overall, serum vitamin D level was weakly inversely associated with odds of NAFLD; however, differences in the strength of association were observed by racial group. It may not be effective to use vitamin D alone as a therapy for NAFLD. Clinicians might consider focusing on BMI and other metabolic irregularities.

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Chapter I. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of metabolic irregularities that through the characteristic steatosis of the liver, can eventually result in non-alcoholic steatohepatitis and cirrhosis. NAFLD has a prevalence of between 20 - 30% worldwide and is considered the most common cause of liver disease globally (1, 2). The prevalence of NAFLD increased from 15% to 25% between 2005 and 2010 (3), and current estimates approximate the prevalence of NAFLD to be as high as between 20-34% of the U.S. population (2-4). NAFLD is positively associated with conditions wherein chronic inflammation plays a key role, including obesity, insulin resistance, type 2 diabetes, and cardiovascular disease (5,6).

Along with the strong association with metabolic dysfunction, evidence is mounting to suggest vitamin D deficiency (VDD) is also associated, potentially causally, with NAFLD (7-9). Similarly, vitamin D deficiency is linked with many metabolic disorders characterized by chronic inflammation and dyslipidemia that have been positively associated with NAFLD (e.g., obesity, metabolic syndrome, type 2 diabetes) (10-18). Serum 25-hydroxyvitamin D concentrations tend to be lower in Black compared to White Americans on average and indeed African Americans tend to be at higher risk for deficiency (19, 20, 21).

One of the most stark gaps in the observational literature looking at the association between vitamin D status and NAFLD are that few studies included racially diverse populations in spite of evidence that suggests that African Americans may experience vitamin D deficiency at higher rates than their Caucasian American counterparts. Research in populations with a significant number of African Americans would contribute to our understanding of the potential differences in the relationship between NAFLD and vitamin D by race. To address this research gap, we evaluated the association between vitamin D status and NAFLD and considered variation by race in a nationally representative sample of the US population.

Chapter II. Methods

Study Population

The Third National Health and Nutrition Examination Survey (NHANES III) was a complex, multistage, stratified, clustered, probability sampling-designed cross-sectional population-based survey conducted between 1988 - 1994 with the aim to assess the civilian non-institutionalized population of the United States**.** The survey obtained information on the demographic, clinical, and laboratory data of the participants through standardized interviews, physical examinations, and testing of biological samples. Further detail about the design and sampling of the survey is available (22).

A total of 16,115 adults were interviewed for NHANES III, but only the participants that met these following criteria were included in this particular study: those between the age of 20–74 years old, and with available social, demographic, clinical and laboratory variables— liver ultrasound data, serum vitamin D levels, hepatitis antibody and antigen test results, and serum transferrin saturation levels. Those that consumed alcohol at amounts ≥21 drinks/week for men and ≥14 drinks/week for women, who had positive viral hepatitis test results (serum hepatitis B surface antigen or serum hepatitis C antibody), or transferrin saturation ≥50%, indicative of iron overload, were excluded. After the selection process, 9,538 participants were included in this analysis.

Clinical and Laboratory Variables

A wide variety of clinical, laboratory, demographic and lifestyle variables were available in the data (22). Age, sex, and race as well as other demographic information were collected through standardized questionnaires, including data on education, income, smoking, alcohol consumption, physical activity, medical conditions and drug use. Type 2 diabetes mellitus was designated in patients with a history of diabetes diagnosis and/or treatment with a hypoglycemic agent or insulin. Insulin resistance was assessed using the Homeostasis Model Assessment of insulin resistance (HOMA-IR); HOMA-IR was equal to the fasting serum glucose x fasting serum insulin/22.5 (23). Individuals with HOMA-IR scores greater than

3.5 were defined as insulin resistant (23). Smoking status was categorized as never, former, or current smoker. Current smokers were identified as those who had smoked more than 100 cigarettes in their lifetime and who currently smoked at the time of the interview. Former smokers were those who had smoked greater than 100 cigarettes in their life but did not currently smoke. Never smokers were those who had smoked less than 100 cigarettes in their lifetime (1). Years of education ranged from 0 (never-attended) - 17 years. For our analysis, years of education were categorized additionally into less than a high school education, high school graduate and college graduate corresponding to 0 - 11, 12 - 15, and greater than 15 years of education, respectively. Hypertension was defined as systolic blood pressure over 140 mmHg or diastolic blood pressure over 90 mmHg and/or self-reported physician diagnosis of hypertension, or previous or current use of anti-hypertension medication. Systolic and diastolic blood pressure were the averages of multiple blood pressure measurements taken during data collection phases in the mobile examination centers. Household annual income was categorized for income levels 0 - 27 corresponding to income brackets from $0 – 50,000 or greater. Metabolic syndrome was defined: waist circumference greater than 102 cm in men or 88 cm, amongst women; triglyceride levels greater than 149 mg/dl; high-density lipoprotein (HDL) cholesterol level lower than 40 mg/dl in men and in women lower than 50 mg/dl; systolic or diastolic blood pressure of 130 mmHg or greater or 85 mmHg or greater, respectively; and fasting plasma glucose level 110 mg/dl or greater. Where three or more of these criteria were met metabolic syndrome was identified, in alignment with the National Cholesterol Education Program Adult Treatment Panel definition (24). Race and ethnicity were categorized as non-Hispanic white, non-Hispanic black, Mexican American, and other. 'Other' included Aleut, Eskimo, American Indian, Asian or Pacific Islander. Standard height and weight measurements were collected and used to calculate body mass index. During analysis, BMI was categorized into categories of < 25, between 25 - <30, and ≥ 30 kg/m^2. Physical activity was collected as reported participation in the activities: walking, jogging or running, bicycle riding, swimming, lifting weights, or doing aerobics or aerobic dancing, other dancing, calisthenics, or garden/yard work, over the preceding month. A metabolic intensity score was created– calculated from the ratio of the resting metabolic rate to the working rate (22). Vitamin D level

was assessed via Diasorin 25-OH-D assay after samples were collected through the mobile examination center phlebotomy procedures (22, 25).

NAFLD

Assessments for digestive diseases were conducted as part of the standard NHANES III examinations via ultrasonography of the liver and gallbladder among 20 to 74-year-old adults in the mobile examination centers. The information that was recorded included the extent of the liver parenchyma brightness, presence of liver to kidney contrast, deep beam attenuation, the definition of the gall-bladder walls, and echogenic walls in the small intrahepatic vessel. The extent of steatosis was divided into four categories: none, mild, moderate or severe. This categorical ordering was based on a standardized algorithm and the results from the liver and gallbladder ultrasonography. Given the lack of a consensus definition for NAFLD across the literature, and to have consistency with some previous publications on NAFLD from NHANES: the definition of NAFLD for our study was given as the presence of moderate to severe hepatic steatosis without any indication of other causes of chronic liver disease such as hepatitis-B, excessive alcohol consumption, hepatitis-C, or iron overload (26).

Statistical Analysis

All data were analyzed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) or R software version 4.0.1 using the survey procedures in SAS and using frequency count analyses and mean and standard error calculations for the categorical and continuous variables, respectively. Sample weights, Taylor series linearization, and strata and sampling cluster units were used together to account for complex survey design, nonresponse and unequal probability of selection existent in the data. Baseline characteristics across contingency groups of interest were compared using the chi-square tests for categorical variables and two-sample t-tests or ANOVA for continuous variables. Two-sided P-values ≤ 0.05were considered statistically significant. In analysis of the association between vitamin D and presence of NAFLD, simple and multiple logistic regression models were used: a crude model 1, a limited-adjustment model 2, and a more extensively adjusted model 3. Model 2 was adjusted for age, sex, and race. Model 3 was adjusted

for age, sex, race, as well as for annual household income, smoking-status, season of blood draw, BMI, insulin resistance as Homeostasis Model Assessment for insulin resistance (HOMA-IR) score, serum HDL, hypertension, and serum gamma glutamyl transferase. We conducted analyses to assess for effect modification of the association by race. We included factors and their cross-product terms with the continuous serum vitamin D factor in the models, then likelihood ratio tests were conducted to test for significance of interaction. Stratified analyses were conducted and reported according to race categories. We also evaluated the relationship between vitamin D status and NAFLD, overall and in race-stratified models, by categorizing values into quintiles of vitamin D concentration and including four indicator variables for the four highest quintiles in the models and using the lowest quintiles as the referent and by calculating p-trend in a separate model with a continuous variable representing category of vitamin D status (e.g., 1, 2, 3, 4, 5).

Chapter III. Results

Among all NHANES III participants, 9,538 met our inclusion criteria for this study. Of these, 2,157 were classified as diagnosed with NAFLD, a weighted prevalence of 19.86% (95% confidence interval: 19.07 - 20.65) (table 1). The NAFLD population tended to be older and more likely to be male than those without NAFLD. There were minor differences in the racial distributions by disease status. NAFLD cases were slightly poorer on average than NAFLD non-cases and were comprised of a greater proportion of former smokers. As expected, cases on average were more likely to be overweight and obese than NAFLD non-cases with a higher prevalence of insulin resistance, metabolic syndrome, diabetes, and hypertension. Serum Vitamin D levels were on average lower amongst NAFLD cases than NAFLD non-cases.

Serum vitamin D level was positively associated with income level and being male. Vitamin D level also showed a positive association with insulin resistance, diabetes, the metabolic syndrome, hypertension, and NAFLD in the descriptive analyses (Table 2).

The distributions of key baseline characteristics by racial categories in our study population are also provided, given the nature of our research question, and are included in the appendix section. We found race was highly associated with serum vitamin D concentration. NAFLD was highest in Mexican Americans, and then non-Hispanic whites, followed by non-Hispanic black Americans (Table 3). Metabolic disorders—specifically insulin resistance and diabetes—and hypertension also varied highly by race. Socio-economic indicators such as income and education level disparately were lower in African- and Mexican American participants. For smokers, prevalence of former smokers was highest in white Americans and current smoking was most common in black Americans. BMI and physical activity score also varied by race, with black Americans more likely to have higher BMIs and Mexican Americans more likely to have a low activity index score (Table 3).

Vitamin D modeled as a continuous variable was inversely associated with NAFLD in unadjusted analyses (OR=0.990; 95% CI 0.986-0.995). This association changed little when adjusted for age, sex, and race (OR=0.991; 95% CI 0.986-0.995); however, in model 3, when adjusted for the battery of

metabolic syndrome-related risk factors, the association was attenuated and null (OR=1.002; 95% CI 0.996-1.009). In stratified analyses, the crude association between serum Vitamin D and NAFLD was strongest amongst the non-Hispanic white American race— increasing vitamin D was associated with decreasing odds of NAFLD (OR=0.990; 95% CI 0.983-0.994). This relationship was similar in the Mexican American group; however, (OR=0.990; 95% CI 0.985-0.995) the association was null for the African American group (OR=1; 95% CI 0.987-1.003). The variations by race that occurred in the crude model also held in model 2 with a similar pattern— white Americans demonstrated the strongest protective effect (OR=0.989; 95% CI 0.983-0.995), Mexican Americans demonstrated an association that was similar (OR=0.989; 95% CI 0.984-0.995), and in African Americans the association was in the same direction but not statistically significant (OR=0.992; 95% CI 0.984-1.001). The association was non-significant for all races after the adjustments made for model 3(white Americans: OR=0.999; 95% CI 0.992-1.007; Mexican Americans: OR=1.002; 95% CI 0.995-1.008; black Americans: OR=0.994, 95% CI 0.986-1.002). The likelihood ratio test assessing statistical significance of interaction by race indicated highly significant results ($p < 0.0001$) (Table 4) despite the observed stratified values not being meaningfully different.

This pattern held when vitamin D was modeled as a categorical variable. Compared to those in the lowest quintile of vitamin D, those in the highest quintile were approximately 36% less at risk for NAFLD (OR=0.645; 95% CI 0.478-0.870) in unadjusted analyses. Analyses in model 2 showed similar results (OR=0.641; 95% CI 0.463-0.888) (Table 5). In model 3the results were attenuated (OR=0.984; 95% CI 0.696-1.390) (Table 5). In analyses stratified by race, the racial groups that showed the strongest associations were non-Hispanic white Americans and Mexican Americans, consistently across models (Table 5). But black American's associations demonstrated across models were consistently non-significant (Table 5).

Chapter IV. Discussion

We conducted secondary analyses in a nationally representative population of US adults to further understand this association between NAFLD and serum vitamin D, and the variation in this association by race. Our results suggest serum Vitamin D may be weakly associated with NAFLD status. The association disappears after adjustment for BMI, insulin resistance, and other metabolic disorder factors. Serum Vitamin D level's relationship with NAFLD is mildly effect modified by race.

Serum vitamin D levels weakly, but significantly, predicted NAFLD status in our study. In accordance with our findings, animal models indicated an association in which vitamin D is protective against NAFLD through a variety of mechanisms— primarily though, through anti-inflammatory & antioxidant pathways (47-50). Anti-fibrotic properties of vitamin D are also indicated among animal models and show an inverse association with lipid excesses in the liver, lipid excesses generally, and with insulin resistance (51). Kim et al. also showed vitamin D serum levels were inversely associated with the severity of liver steatosis and fibrosis in a population-based cross-sectional study (52). Other cross-sectional analyses indicate inverse correlations between serum vitamin D concentration and severity and progression of NAFLD hepatic steatosis and with the likelihood of NALFD presence— Zhai, Manco, as well as Targher all conducted studies that indicated increased progression of steatosis towards hepatitis with decreased vitamin D in the serum (37-39), while a meta-analysis of cross-sectional and case-control studies found that there was a 26% increase in the likelihood of vitamin D deficiency amongst patients with NAFLD compared to controls (40).

The association between Vitamin D and NAFLD declines to non-significance when adjusted for metabolic syndrome disorder risk factor variables. A likely reason is that Vitamin D may be strongly associated with some metabolic risk factors that mediate its relationship to NAFLD and progression in NAFLD (e.g., insulin resistance, hyperlipidemia). Kim et al and Liangpunsakul et al conducted studies which found similar results and suggested similar conclusions (52, 23). Liangpunsakul et al found Vitamin D level wasn't associated with ALT, an enzyme strongly indicative of NAFLD liver damage.

This may suggest limited direct hepato-protective effects of vitamin D. Indeed, Barchetta, Sharifi, and Foroughi all found no association of Vitamin D with reductions in liver enzymes suggesting liver damage in randomized control trials (33, 43, 45, 46). Kim et al. also found the association between vitamin D and NAFLD progression and severity disappeared after adjustment for metabolic factors in cross-sectional analysis (52).

Limitations for our study included the fact that disease status for disease states of diabetes and hypertension was self-reported in part; diabetes status, in data collection, was also limited in the delineation between type I and type II diabetes: this could result in some degree of misinformation bias. In addition, racial categories for this data were restricted to just white, black, and Mexican American— therefore our ability to evaluate the relationship between vitamin D and NAFLD amongst Asian-Americans or other racial-ethnic groups was limited. In addition, our study design was cross-sectional, so the associations found could not be used to imply causality. However, this study also had several strengths: This study was from a nationally representative, population-based sample; this sample was large enough that we were able to conduct sub-group analyses with a fair amount of statistical power and acquire estimates with reasonable degree of precision.

In conclusion, serum vitamin D level was marginally associated with odds of NAFLD— however, the association did not hold when adjusted for metabolic risk factors. These factors are likely on the causal pathway mediating vitamin D's relationship to NAFLD. Racial groups affected the strength of these associations, marginally: white Americans had the strongest protective effects, Mexican Americans had similar results though slightly less, and black Americans displayed largely null associations. Our study indicates that vitamin D may not be effective as a therapy for NAFLD, alone, and clinicians should focus on managing BMI and metabolic irregularities such as insulin resistance, metabolic syndrome, and diabetes.

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Tables

Table 1. Distribution of Select Variables among non-NAFLD vs. NAFLD-diagnosed subjects in Cross Section of U.S. Populatic

	No-NAFLD (n= 7,382) (80.14 + - 0.79)	NAFLD (n= 2,157) (19.86 + - 0.79)	P- Value
Proportion No-NAFLD vs. NAFLD (N= 9,539)	80.14 + - 0.79	19.86 + - 0.79	
Age (years)	42.44 + - 0.18	48.16 + - 0.33	< 0.0001
Sex (% male)	45.2 + - 0.80	53.37 + - 1.59	0.0003
Race %			
Non-Hispanic White	40.31 + - 1.4	38.1 + - 1.69	0.0021
Non-Hispanic Black	29.56 + - 0.65	21.54 + - 0.90	
Mexican-American	25.84 + - 0.40	36.53 + - 0.77	
Other	4.29 + - 0.97	3.83 + - 1.26	
Total Household Annual Income category (%)			
< 15000	17.33 + - 1.11	22.8 + - 2.02	0.025
15000-25000	27.7 + - 1.07	27.3 + - 1.86	
>25000	54.9 + - 1.50	49.89 + - 3.01	
Education (Years)	11.55 + - 0.04	10.49 + - 0.09	< 0.0001
Smoking Status (%)			
Never smoker	51.7 + - 1.08	48.94 + - 1.64	< 0.0001
Current smoker	26.23 + - 1.00	20.89 + - 1.34	
Former smoker	22.09 + - 0.82	30.17 + - 1.87	
Census Region (%)			
Northeast	20.60 + - 1.57	19.94 + - 2.34	0.986
Midwest	23.93 + - 1.59	24.10 + - 3.15	
South	35.03 + - 2.82	34.88 + - 3.16	
West	20.44 + - 3.14	21.10 + - 3.86	
Season of Screening (%)			
Winter	16.78 + - 3.02	14.93 + - 3.17	0.619
Spring	19.43 + - 2.87	19.95 + - 3.78	
Summer	32.84 + - 5.00	33.80 + - 5.74	
Fall	30.96 + - 4.84	31.32 + - 5.63	
Body Mass Index (Kg/m^2)	26.44 + - 0.06	30.4 + - 0.14	< 0.0001
Body Mass Index (%)			
< 25 kg/m^2	60.26 + - 1.16	23.3 + - 1.68	< 0.0001
25-30 kg/m^2	23.00 + - 0.84	29.4 + - 1.14	
≥ 30 kg/m^2	16.7 + - 0.80	47.2 + - 2.12	
Physical Activity Score	9.0 + - 0.09	7.1 + - 0.14	< 0.0001
Insulin Resistance Porportion (%)	11.25 + - 0.80	44.43 + - 2.00	< 0.0001
Homeostasis Model Assesment for Insulin Resistance Score	2.81 + - 0.07	5.80 + - 0.23	< 0.0001
Met_S (%)	13.61 + - 0.51	50.25 + - 1.66	< 0.0001
Serum HDL cholesterol (mg/dL)	51.72 + - 0.17	45.87 + - 0.32	< 0.0001
Serum LDL cholesterol (mg/dL)	127.22 + - 0.66	129.91 + - 1.29	0.09
Total Serum cholesterol (mg/dL)	202.54 + - 0.50	212.12 + - 0.96	< 0.0001
Serum triglycerides (mg/dL)	122.5 + - 1.11	188.37 + - 3.23	< 0.0001
Systolic BP (mmHg)	120.62 + - 0.23	128.44 + - 0.43	< 0.0001
Diastolic BP (mmHg)	71.62 + - 0.14	75.61 + - 0.27	< 0.0001
Hypertension (%)	24.39 + - 1.03	45.7 + - 1.7	< 0.0001
Diabetes (%)	3.35 + - 0.338	10.78 + - 0.904	< 0.0001
Glycated Hemoglobin (a1c) (%)	5.39 + - 0.01	5.93 + - 0.03	< 0.0001
Serum alanine aminotransferase (U/L)	15.71 + - 0.13	23.69 + - 0.46	< 0.0001
Serum aspartate aminotransferase (U/L)	20.17 + - 0.13	24.73 + - 0.39	< 0.0001
Serum gamma glutamyltransferase (U/L)	27.67 + - 0.46	42.27 + - 1.36	< 0.0001
Albumin (g/dL)	4.15 + - 0.004	4.13 + - 0.008	0.27
Vitamin D Serum Level (nmol/L)	55.45 + - 0.25	52.5 + - 0.42	< 0.0001
Fasting plasma glucose (mg/dL)	96.76 + - 0.32	113.98 + - 1.16	< 0.0001

Table 2. Distribution of Select Variables among Serum Vitamin D Tertiles in Cross Section of U.S. Population NHANES III 1988-1

Serum Vit D levels (tertiles)	< 43.6 nmol/L (n= 3309)	43.6 - 63.3 nmol/L (n = 3319)	> 63.3 nmol/L (n= 3335)	P - values
Age (years)	42.80 + - 0.26	44.59 + - 0.28	44.16 + - 0.29	< .0001
Sex (% male)	33.66 + - 1.60	47.27 + - 1.32	52.16 + - 1.10	< .0001
Race %				
Non-Hispanic White	51.99 + - 2.54	76.09 + - 1.71	90.35 + - 0.84	< .0001
Non-Hispanic Black	28.51 + - 1.85	8.82 + - 0.65	2.28 + - 0.25	
Mexican-American	7.23 + - 0.68	6.01 + - 0.63	2.88 + - 0.28	
Other	12.26 + - 1.43	9.08 + - 1.20	4.48 + - 0.76	
Total Household Annual Income category (%)				
< 15000	25.36 + - 1.73	18.86 + - 1.33	14.59 + - 1.00	< .0001
15000-25000	28.79 + - 1.58	27.56 + - 1.61	27.61 + - 1.44	
>25000	45.85 + - 2.45	53.58 + - 1.86	57.81 + - 1.66	
Education (Years)	11.26 + - 0.06	11.10 + - 0.07	11.65 + - 0.07	< .0001
Smoking Status (%)				
Never smoker	47.67 + - 1.41	48.53 + - 1.30	45.68 + - 1.41	0.0007
Current smoker	30.00 + - 1.32	25.24 + - 1.20	26.76 + - 1.29	
Former smoker	22.34 + - 1.06	22.24 + - 1.26	27.56 + - 1.01	
Census Region (%)				
Northeast	14.75 + - 1.56	18.65 + - 1.42	24.28 + - 2.17	< .0001
Midwest	18.12 + - 1.47	22.68 + - 1.71	27.75 + - 2.94	
South	43.04 + - 3.68	35.85 + - 2.91	31.22 + - 2.82	
West	24.09 + - 4.00	22.81 + -3.38	16.75 + - 3.44	
Season of Screening (%)				
Winter	24.92 + - 4.68	19.21 + - 3.41	11.92 + - 2.61	< .0001
Spring	24.94 + - 3.63	21.55 + - 3.25	14.85 + - 2.79	
Summer	23.23 + - 4.53	30.84 + -5.01	40.17 + - 5.97	
Fall	26.91 + - 4.59	28.4 + - 4.34	33.07 + - 5.90	
Body Mass Index (Kg/m^2)	28.61 + - 0.12	27.53 + - 0.1	26.04 +- 0.08	< .0001
Body Mass Index (%)				
< 25 kg/m^2	41.68 + - 1.63	48.64 + - 1.49	60.41 + - 1.22	< .0001
25-30 kg/m^2	24.67 + - 1.25	25.83 + - 1.41	23.24 + - 1.04	
≥ 30 kg/m^2	33.66 + - 1.60	25.53 + - 1.33	16.35 + - 0.99	
Physical Activity Score	7.03 + - 0.12	8.55 + - 0.13	10.26 + - 0.14	< .0001
Insulin Resistance Porportion (%)	26.71 + - 1.51	20.54 + - 1.04	12.60 + - 1.24	< .0001
Homeostasis Model Assesment for Insulin	4.10 + - 0.15	3.43 + - 0.09	3.01 + - 0.13	< .0001
Met_S (%)	27.44 + - 1.40	23.54 + - 0.95	16.72 + - 1.07	< .0001
Serum HDL cholesterol (mg/dL)	50.93 + - 0.27	49.80 + - 0.25	50.58 + - 0.26	0.38
Serum LDL cholesterol (mg/dL)	125.48 + - 1.03	127.80 + - 0.98	129.78 + - 0.99	0.31
Total Serum cholesterol (mg/dL)	203.02 + - 0.77	205.39 + - 0.74	206.01 + - 0.75	0.011
Serum triglycerides (mg/dL)	131.06 + - 1.92	142.54 + - 2.07	139.12 + - 1.94	0.61
Systolic BP (mmHg)	122.94 + - 0.35	122.92 + - 0.34	121.42 + - 0.34	< .0001
Diastolic BP (mmHg)	72.91 + - 0.23	72.66 + - 0.22	71.98 + - 0.21	0.007
Hypertension (%)	33.99 + - 1.24	30.00 + - 1.55	25.57 + - 1.21	< .0001
Diabetes (%)	7.49 + - 0.77	5.45 + - 0.63	3.29 + - 0.37	< .0001
Glycated Hemoglobin (a1c) (%)	5.64 + - 0.022	5.561 + - 0.02	5.37 + - 0.020	< .0001
Serum alanine aminotransferase (U/L)	16.90 + - 0.24	17.93 + - 0.27	17.65 + - 0.23	0.87
Serum aspartate aminotransferase (U/L)	20.85 + - 0.23	21.39 + - 0.27	21.26 + - 0.16	0.81
Serum gamma glutamyltransferase (U/L)	34.46 + - 1.02	30.86 + - 0.73	27.80 + - 0.60	< .0001
Albumin (g/dL)	4.06 + - 0.01	4.16 + - 0.01	4.21 + - 0.01	< .0001
Fasting plasma glucose (mg/dL)	103.19 + - 0.75	101.04 + - 0.60	97.94 + - 0.52	< .0001
NAFLD (%)	24.69 + - 1.52	20.9 + - 1.42	17.10 + - 1.04	0.0007

Table 4. Odds Ratios and 95% Confidence Intervals for Multiple and Simple Logistic Regression Models of the Relationship between Vitamin D and NAFLD in a Cross Section of U.S. Population Adults, Aged 20-74 years old; NHANES III 1988-1994 (N= 9,538)

		Serum Vitamin D continuous		
		OR	95 % Confidence Limits	
Model 1 (Unadjusted)		0.99	0.986	0.995
Model 2 Limited*		0.991	0.985	0.996
Model 3**		1.002	0.996	1.009
Model 1 stratified				
	Non-Hispanic White	0.99	0.983	0.994
	Non-Hispanic Black	1	0.987	1.003
	Mexican-American	0.99	0.985	0.995
Model 2 Limited* stratified				
	Non-Hispanic White	0.989	0.983	0.995
	Non-Hispanic Black	0.992	0.984	1.001
	Mexican-American	0.989	0.984	0.995
Model 3** stratified				
	Non-Hispanic White	0.999	0.992	1.007
	Non-Hispanic Black	0.994	0.986	1.002
	Mexican-American	1.002	0.995	1.008
	Likelihood Ratio Test Result P-value:			< 0.0001

* Adjusted for Age, Sex, and Race variables

**Adjusted for Age, sex, race, household income level, smoking status, season of blood draw, BMI, Insulin ResistanceHOMA-IR, hypertension, Serum HDL, Serum gamma glutamyl transferase

Table 5. Odds Ratios and 95% Confidence Interval Estimates for Crude and Adjusted Logistic Regression Models of the Relationship between Vitamin D Quintile and NAFLD in a Cross Section of U.S. Population Adults, Aged 20-74 years old; NHANES III 1988-1994 (N= 9,538)

	Qunitle of Vitamin D <35.5 nmol/L (n=1893)	35.5 - 46.8 nmol/L (n=1940)	46.9-59 nmol/L (n=1904)	50 - 73.7 nmol/L (n= 1892)	> 73.7 nmol/L (n=1909)		
	OR (REF) (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)		
Model 1 (Unadjusted)	1 -	1.04 (0.785, 1.37)	0.875 (0.662, 1.157)	0.76 (0.579, 0.997)	0.645 (0.478, 0.87)	P trend	< 0.001
Model 2*	1 -	0.992 (0.746, 1.319)	0.826 (0.618, 1.103)	0.725 (0.541,0.972)	0.641 (0.463, 0.888)	P trend	< 0.01
Model 3**	1 -	1.001 (0.719, 1.393)	0.961 (.700, 1.320)	0.944 (0.673, 1.323)	0.984 (0.696, 1.390)	P trend	0.824
Model 1 (Unadjusted)							
Non-Hispanic White	1 -	0.941 (0.628, 1.409)	0.764 (0.505, 1.102)	0.646 (0.447, 0.935)	0.552 (0.371, 0.821)	P trend	< 0.001
Non-Hispanic Black	1 -	0.98 (.762, 1.260)	0.916 (0.635, 1.323)	0.796 (0.469, 1.350)	0.753 (0.462, 1.226)	P trend	0.1828
Mexican American	1 -	0.874 (0.686, 1.114)	0.868 (0.633, 1.189)	0.75 (0.546, 1.189)	0.551 (0.413, 0.736)	P trend	< 0.001
Model 2*							
Non-Hispanic White	1 -	0.911 (0.604, 1.375)	0.708 (0.470, 1.069)	0.622 (0.418, 0.927)	0.551 (0.360, 0.845)	P trend	< 0.01
Non-Hispanic Black	1 -	0.925 (0.720, 1.189)	0.856 (0.580, 1.264)	0.689 (0.410, 1.189)	0.639 (0.395, 1.033)	P trend	0.0447
Mexican American	1 -	0.861 (0.655, 1.133)	0.883 (0.650, 1.199)	0.742 (0.533,1.034)	0.532 (0.382, 0.742)	P trend	< 0.001
Model 3**							
Non-Hispanic White	1 -	0.905 (0.559, 1.465)	0.808 (0.517, 1.262)	0.793 (0.502, 1.252)	0.837 (0.526, 1.333)	P trend	0.9221
Non-Hispanic Black	1 -	0.891 (0.682, 1.165)	0.905 (0.596, 1.373)	0.765 (0.421, 1.389)	0.8 (0.515, 1.245)	P trend	0.1051
Mexican American	1 -	0.911 (0.652, 1.272)	1.024 (0.705, 1.485)	0.962 (0.644, 1.436)	0.792 (0.528, 1.187)	P trend	0.455

* Adjusted for age, sex, and race

**Adjusted for Age, sex, race, household income level, smoking status, season of blood draw, BMI, Insulin ResistanceHOMA-IR, hypertension, serum HDL, serum gamma-glutamyl transferase

Appendix-1:

Supplemental Table 3.

Distribution of Select Variables by Race in Cross Section of U.S. Population NHANES III 1988-1994 (N= 9,538)

	Non-Hispanic White (n= 3801)	Non-Hispanic Black (n= 2645)	Mexican-American (n= 2693)	Other (n= 399)	P - value
Age (years)	48.26 + - 0.24	41.65 + - 0.28	40.79 + - 0.29	41.80 + - 0.71	<0.0001
Sex (% male)	47.41 +- 0.64	42.12 + - 1.31	48.41 +- 0.93	45.15 +- 2.30	<.01
Total Household Annual Income category (%)					
< 15000	14.18 + - 0.97	36.95 + - 1.98	34.92 + - 1.19	26.44 + - 5.01	<0.0001
15000-25000	26.27 + - 1.20	30.81 + - 1.36	34.97 + - 1.17	34.97 + - 3.0	
>25000	59.54 + - 1.59	32.23 + - 1.77	30.11 + - 1.41	38.59 + - 5.69	
Education (Years)	12.63 + - 0.04	11.67 + - 0.05	9.03 + - 0.08	11.56 + - 0.19	<0.0001
Smoking Status (%)					
Never smoker	43.60 + - 0.99	53.63 + - 1.49	61.28 + - 1.03	63.87 + - 3.45	<0.0001
Current smoker	27.92 + - 1.04	29.98 + - 1.41	19.26 + - 0.87	19.94 + - 3.09	
Former smoker	28.60 + - 0.73	16.38 + - 0.84	19.46 + - 0.86	16.19 + - 2.27	
Census Region (%)					
Northeast	21.80 + -1.91	15.60 + - 1.55	1.48 + - 0.35	25.65 + - 3.65	<0.0001
Midwest	26.70 + - 2.14	19.52 + - 1.78	10.01 + - 2.76	12.23 + - 3.28	
South	33.53 + - 3.01	56.24 + - 2.79	27.85 + - 5.37	29.18 + - 6.97	
West	17.96 + - 3.63	8.63 + - 1.59	60.66 + - 6.11	32.94 + - 4.52	
Season of Screening (%)					
Winter	14.49 + - 3.31	27.66 + - 6.08	21.93 + - 3.55	25.08 + - 6.92	<0.0001
Spring	16.59 + - 3.05	15.17 + - 4.02	58.33 + - 4.84	26.53 + - 4.88	
Summer	37.59 + - 5.79	21.18 + - 3.74	14.69 + - 3.73	21.12 + - 6.07	
Fall	31.33 + - 5.51	35.99 + - 6.44	5.04 + - 1.09	27.27 + - 5.30	
Body Mass Index (Kg/m^2)	26.74 + - 0.09	28.30 + - 0.12	27.57 + - 0.10	26.50 + - 0.26	0.17
Body Mass Index (%)					
< 25 kg/m^2	54.21 + - 1.24	42.16 + - 1.40	43.55 + - 1.74	57.15 + - 3.44	<0.0001
25-30 kg/m^2	24.17 + - 0.79	26.33 + - 0.93	30.44 + - 1.26	20.41 + - 2.52	
≥ 30 kg/m^2	21.62 + - 1.05	31.5 + - 1.15	26.00 + - 1.38	22.43 + - 2.30	
Physical Activity Score	9.68 +- 0.10	8.46 +- 0.14	7.27 +- 0.14	7.09 + - 0.36	<0.0001
Insulin Resistance Porportion (%)	15.99 + - 1.15	27.47 + - 1.47	25.12 + - 1.23	22.24 + - 2.45	<0.0001
Homeostasis Model Assesment for Insulin Resistance Score	3.00 + - 0.08	3.95 + - 0.16	3.83 + - 0.16	3.36 + - 0.18	0.03
Met_S (%)	21.34 +- 0.66	19.11 + - 0.71	22.81 + - 1.43	20.30 + - 2.59	0.4
Serum HDL cholesterol (mg/dL)	49.76 + - 0.24	53.54 + - 0.30	48.61 + - 0.26	48.49 + -0.67	0.52
Serum LDL cholesterol (mg/dL)	131.48 + - 0.87	127.01 + - 1.19	122.43 + -1.08	130.53 + - 2.64	0.13
Total Serum cholesterol (mg/dL)	209.58 +- 0.67	201.39 +-0.84	202.19 + - 0.82	201.04 + - 2.10	<.01
Serum triglycerides (mg/dL)	145.69 +- 1.82	110.86 + - 1.72	155.27+-2.46	135.20 +- 5.24	0.3
Systolic BP (mmHg)	122.81+-0.31	124.49+-0.40	120.32+-0.37	118.88+-0.90	<.01
Diastolic BP (mmHg)	72.57+-0.19	73.97+-0.27	71.04+-0.23	71.96+-0.56	0.88
Diabetes (%)	4.58 + - 0.41	6.78 + - 0.537	6.44 + - 0.46	4.35 +- 1.05	0.02
Hypertension (%)	28.97 + - 1.13	35.66 + - 1.26	22.55 + - 1.33	22.03 + - 2.29	<0.0001
Glycated Hemoglobin (a1c) (%)	5.38 + - 0.014	5.69 + - 0.024	5.57 + - 0.023	5.53 + - 0.05	0.0001
Serum alanine aminotransferase (U/L)	16.28 + - 0.17	14.98 + - 0.18	21.46 + - 0.39	18.95 + - 0.77	<.01
Serum aspartate aminotransferase (U/L)	20.40 + - 0.14	20.48 + - 0.22	22.93 + - 0.34	21.31 + - 0.47	<.01
Serum gamma glutamyltransferase (U/L)	26.72 + - 0.58	36.35 + - 1.11	32.47 + - 0.88	28.59 + - 1.56	0.01
Albumin (g/dL)	4.19 + - 0.01	4.04 + - 0.007	4.19 + - 0.007	4.15 + - 0.02	0.021
Vitamin D Serum Level (nmol/L)	65.29 + - 0.31	42.09 + - 0.32	52.50 + - 0.35	52.26 + - 0.86	<0.0001
Fasting plasma glucose (mg/dL)	98.80 + - 0.46	101.49 + - 0.81	102.95 + - 0.75	99.14 + - 1.23	0.4
NAFLD (%)	19.65 + - 0.88	16.87 + - 1.39	27.87 +- 2.17	21.10 + - 2.80	<0.0001