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Serum Vitamin D Concentrations and Unexplained Elevation in ALT Among US Adults

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Abstract

Background—Low serum levels of vitamin D are associated with metabolic syndrome.

Participants in NHANES III with unexplained elevation in ALT levels have high prevalence of metabolic syndrome. We hypothesized that the serum concentrations of vitamin D were inversely associated with unexplained elevation in ALT.

Methods—A total of 6,826 fasting subjects underwent morning physical examination and met the inclusion and exclusion criteria. From these participants, we have constructed cases with unexplained elevation in ALT ($n = 308$) and compared their serum vitamin D concentrations to matched controls with normal ALT ($N = 979$). We examined the prevalence of unexplained elevation in ALT level across different quartiles of vitamin D levels.

Results—Participants with unexplained elevation in ALT had significantly lower serum vitamin D levels compared to controls (61.8 ± 26.0 nmol/l vs. 66.8 ± 27.1 nmol/l, $P < 0.01$). The unadjusted prevalence of unexplained elevation in ALT in patients with highest to lowest quartiles of serum vitamin D levels were 21.4, 21.4, 25.6, and 31.5%, respectively. Compared to lowest quartile, patients with top two quartiles of serum vitamin D levels had significantly lower prevalence of unexplained elevation in ALT (OR, 95% CI for highest quartile 0.62 [0.43–0.89] and for third quartile 0.61 [0.42–0.86]). This relationship persisted even after controlling for metabolic syndrome, insulin resistance, and serum triglycerides.

Conclusions—This study suggests a significant inverse relationship between serum vitamin D levels and unexplained elevation in ALT. Further studies are needed to confirm this observation and to understand the basis for and implications of this observation.

Keywords

Serum vitamin D; Metabolic syndrome; Unexplained ALT elevation

Introduction

Vitamin D, a fat-soluble vitamin, mediates its biological effects by binding to the vitamin D receptor and acting as a transcription factor modulating gene expression of several transport proteins [1]. Serum levels of 25-Hydroxyvitamin D are the measure of body stores of vitamin D [2, 3], and its levels are not influenced by underlying kidney function. A reduction of serum 25-Hydroxyvitamin D levels, the substrate for the kidney and other tissues for the generation of calcitriol (1, 25(OH)₂D₃), produces secondary hyperparathyroidism leading to reducing bone mineral density and interrupting calcium metabolism [3]. Recently, vitamin D has been recognized to have numerous non-calcemic functions [1, 4–6]. Specifically, there is increasing evidence that its low serum levels increase the risk of insulin resistance, diabetes, cardiovascular disease, and metabolic syndrome [5, 6], although the underlying molecular mechanism of this association remains to be explicated.

Nonalcoholic fatty liver disease (NAFLD) is one of the most common forms of chronic liver disease and in some patients it can progress to cirrhosis, liver failure, and hepatocellular carcinoma [7]. Studies have shown a high prevalence of the metabolic syndrome and insulin resistance in patients with NAFLD [8]. We have previously reported a strong relationship between metabolic syndrome and the prevalence of unexplained elevation in ALT in the participants of the third National Health and Examination Survey (NHANES III) [9]. Because low serum vitamin D concentrations are associated with various measures of insulin resistance and metabolic syndrome [3, 5], it is plausible that subjects with unexplained elevation in ALT levels may have reduced levels of 25-Hydroxyvitamin D. To test this hypothesis, we analyzed the relationship between serum vitamin D levels and unexplained elevation in ALT using the NHANESIII dataset.

Research Design and Methods

The NHANES III was conducted in the United States from 1988 through 1994 by the National Center for Health Statistics of the Centers for Disease Control and Prevention. The NHANES III used complex, multi-stage, stratified, clustered samples of civilian, non-institutionalized populations 2 months of age and older to collect information about the health and diet of people in the United States. A detailed description of the survey and its sampling procedures are available on its website [10]. The NHANES III study was approved by the Centers for Disease Control and Prevention's Institutional Review Board and all participants provided written informed consent.

Subject Selections

We focused our analyses on fasting participants who had the morning physical examination, non-pregnant women and 20 years old. After excluding participants with missing ALT, serum glucose, serum insulin, serum vitamin D values, the remaining 6,826 subjects constituted our study cohort.

Subjects were considered to have unexplained elevation in ALT if their ALT levels were above the upper limit of normal of the NHANES III reference laboratory (>40 µl in men and >31 µl in women) in the absence of a positive hepatitis B surface antigen or hepatitis C antibody, serum transferrin saturation greater than 50%, or significant alcohol consumption (*N* = 308). The control group without liver disease consisted of age (±5 years)-, gender-, ethnicity-, and body mass index (BMI)-matched (±1 kg/m²) participants with normal ALT (< 40 µl in men and < 31 µl in women), serum transferrin saturation < 50%, undetectable hepatitis B surface antigen and hepatitis C antibodies in the serum, and no significant

alcohol consumption. Depending upon the availability, one to four controls were randomly selected for each participant with unexplained elevation in ALT ($N = 979$).

The average alcohol consumption was calculated based on the responses to two survey questions that asked about the number of days of drinking over the past 12 months and the number of drinks on a given drinking day. Average alcohol consumption of more than 2 drinks/day in men or more than 1 drink/day in women was considered as significant alcohol consumption.

The presence of the metabolic syndrome was determined based on the Third Report of the National Cholesterol Education Program Adult Treatment Panel III [11]. The Adult Treatment Panel III clinical definition of the metabolic syndrome requires the presence of three or more of the following features: (1) waist circumference greater than 102 cm in men or greater than 88 cm in women; (2) triglyceride level 150 mg/dl or greater; (3) high-density lipoprotein (HDL) cholesterol level less than 40 mg/dl in men and less than 50 mg/dl in women; (4) systolic blood pressure 130 mm Hg or greater or diastolic pressure 85 mm Hg or greater; and (5) fasting plasma glucose level 110 mg/dl or greater [9].

Subjects were considered to have diabetes mellitus if they reported ongoing use of oral hypoglycemic drugs or insulin injection, or if their fasting plasma glucose level was 126 mg/dl or greater. Subjects were considered to have hypertension if they were receiving antihypertensive medications, if they were told by their physicians that they had high blood pressure, if their systolic pressure was 140 mm Hg or greater, or if their diastolic pressure was 90 mm Hg or greater, as defined by the recent Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure guidelines [12].

The presence of insulin resistance was estimated by the homeostasis model assessment method (HOMA), a measure of insulin resistance, which was calculated by the following equation: $[\text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mmol/l)}] / 22.5$. Subjects with score of 3.5 or greater by this equation were considered to have insulin resistance [9].

Laboratory Measurements

All venous blood samples were immediately centrifuged and shipped weekly at -20°C to a central laboratory. Plasma glucose level was measured by the modified hexokinase method. Serum transaminases were determined using the alpha-ketoglutarate reactions. Serum insulin was measured by a radioimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden). Serum triglycerides were measured quantitatively by a peroxidase-catalyzed reaction and HDL cholesterol was measured after precipitation of the other lipoproteins (Boehringer Mannheim, Indianapolis, Indiana). Low-density lipoprotein (LDL) cholesterol was calculated by using the following formula: $\text{LDL} = \text{total cholesterol} - (\text{TG}/5) - \text{HDL}$.

Serum levels of 25-Hydroxyvitamin D were measured using a radioimmunoassay (RIA) and employed ^{125}I as a tracer. In brief, the assay consisted of a two-step procedure. The first procedure involved a rapid extraction of 25-Hydroxyvitamin D and other hydroxylated metabolites from serum or plasma with acetonitrile. Following extraction, the treated sample was assayed by using an equilibrium RIA procedure, which was based on an antibody with specificity to 25-Hydroxyvitamin D. The sample, antibody, and tracer were incubated for 90 min at $20-25^{\circ}\text{C}$. Phase separation was accomplished after a 20 min incubation at $20-25^{\circ}\text{C}$ with a second antibody-precipitating complex. The measurement methods and procedures are described in detail elsewhere in the NHANES laboratory manual [10].

Statistical Analyses

Quality control procedures, database management, and statistical analyses were performed using SAS-callable SUDAAN software for Windows (SAS Institute, Cary, NC). Basic descriptive statistics, including means, standard deviations (SD), and percentages, were used to characterize the study participants. Comparisons between groups were made using the Student *t* test for the continuous and the χ^2 test for the categorical variables.

The levels of serum vitamin D concentrations were divided into quartiles based on its distribution. The proportions of unexplained elevation in ALT cases across quartiles were examined. The strength of association between unexplained elevation in ALT and serum vitamin D concentrations was further analyzed by multivariate logistic regression analysis after adjusting for other covariates such as the presence of metabolic syndrome and insulin resistance. The results were reported as odds ratio (OR) with 95% confidence intervals and *P*-values. Linear regression analyses were used when analyzing the association between index of insulin resistance (HOMA), serum ALT, and serum vitamin D concentrations; all variables were log-transformed to account for the asymmetric distribution of data. The strength of association for linear regression analyses was reported as 'correlation coefficient (*r*)'. In all analyses, a *P* value < 0.05 was considered to be statistically significant.

Results

Characteristics of the Study Participants

Not unexpectedly, subjects with unexplained elevation in ALT had higher prevalence of metabolic syndrome (42% vs. 27%, *P* < 0.01), diabetes (13.6% vs. 7.3%, *P* < 0.01), hypertension (29% vs. 26%, *P* < 0.01), insulin resistance (41.5% vs. 25.2%, *P* < 0.01), homeostasis model assessment method scores (4.7 ± 4.1 vs. 2.8 ± 2.2 , *P* < 0.01), and serum triglycerides (174.2 ± 127.8 vs. 141.9 ± 97.8 , *P* < 0.01) (Table 1).

Association Between Serum Concentrations of Vitamin D and Unexplained Elevation in ALT

The concentrations of serum vitamin D ranged from 8.7 to 208.2 nmol/l (mean 65.6, median 61.4 nmol/l). Compared to controls, individuals with unexplained elevation in ALT had significantly lower vitamin D concentrations (61.8 ± 26.0 vs. 66.8 ± 27.1 nmol/l, *P* < 0.01) (Table 1). The unadjusted prevalence of unexplained elevation in ALT in patients with highest to lowest quartiles of serum vitamin D levels were 21.4, 21.4, 25.6, and 31.5%, respectively (Table 2). Compared to the lowest quartile, patients with the top two quartiles of serum vitamin D levels had significantly lower prevalence of unexplained elevation in ALT (OR, 95% CI for highest quartile 0.62 [0.43–0.89] and for third quartile 0.61 [0.42–0.86]) (Table 2). After adjustments using multiple logistic models, the *adjusted* odds ratios for having unexplained elevation in ALT for the third quartile relative to the lowest quartile were 0.57 (95% CI 0.39–0.82) for model 1 and 0.66 (95% CI 0.45–0.96) for model 2. The *adjusted* odds ratios for the fourth quartile relative to the lowest quartile were 0.68 (95% CI 0.47–0.97) for model 1 and 0.78 (95% CI 0.53–0.99) for model 2 (Table 2).

Historically, vitamin D concentrations >25 nmol/l were considered as normal [5]. However, epidemiological studies suggest higher levels are necessary to prevent osteoporosis and fractures; thus, the new cutoff level of 75 nmol/l is currently recommended to define vitamin D adequacy [4, 13]. According to this cut-off, the prevalence of vitamin D deficiency in our study population was very high at 68% and the prevalence of unexplained elevation in ALT was 70.4%.

We, however, did not find a linear association between serum levels of vitamin D and serum ALT in either univariate ($r = -0.03$, $P = 0.23$) or multivariate analyses when controlling for HOMA scores ($r = 0.02$, $P = 0.40$).

Association Between Serum Concentrations of Vitamin D, HOMA Scores, and Serum ALT

Since subjects with unexplained elevation in ALT had significantly higher prevalence of insulin resistance, we next explored the association between serum concentrations of vitamin D and HOMA scores. The mean concentrations of vitamin D among those with and without insulin resistance were 59.7 and 68.1 nmol/l, respectively ($P < 0.01$). We found significant inverse association between serum vitamin D concentration and HOMA scores ($r = -0.12$, $P < 0.01$).

Discussion

In this study, we examined the relationship between the serum levels of vitamin D and unexplained elevation in ALT in subjects participating in the NHANES III survey. There are several important findings derived from our analyses. The serum concentrations of vitamin D were significantly lower in subjects with unexplained elevation in ALT compared to well-matched controls. When splitting the levels of vitamin D into quartiles, subjects in the top two quartiles had significantly lower prevalence of unexplained elevation in ALT relative to that of the lowest quartile. After several model adjustments, the *adjusted* odds ratio for having unexplained elevation in ALT for the top two quartiles ranged from 0.57 to 0.78. In conjunction with previous reports [3], we found the inverse association between serum levels of vitamin D and HOMA scores, but not with serum ALT.

Our findings have several clinical implications. First, unexplained elevation in ALT signifies the presence of non-alcoholic fatty liver disease (NAFLD) in NHANES III participants [14]. Thus, our results suggest the inverse relationship between higher levels of serum vitamin D and the presence of NAFLD. Second, our findings suggest that screening for vitamin D deficiency in subjects with unexplained elevation in ALT/NAFLD in clinical practice is meritorious, given the high prevalence of vitamin D deficiency in US adults. Lastly, further investigation on whether vitamin D may play a role in the prevention or treatment of unexplained elevation in ALT appears warranted.

Epidemiological studies of the general population have indicated a close association between insulin sensitivity, diabetes, and the metabolic syndrome with serum levels of 25-Hydroxyvitamin D [3, 5]. We found the inverse association between serum concentrations of vitamin D and HOMA scores, but not with serum ALT, suggesting that vitamin D has no direct hepatoprotective effects. The decrease in prevalence of unexplained elevation in ALT is likely mediated through the improvement in insulin resistance in parallel with the increase in serum vitamin D concentrations. Vitamin D is shown to improve pancreatic beta cell function and increase insulin sensitivity [6]. Its deficiency has been implicated in several chronic inflammatory cascades, notably increasing nuclear factor- κ B activity [15] and interrupting insulin signaling pathways [16]. These cellular and molecular mechanisms may underlie the potential association among unexplained elevation in ALT, insulin resistance and vitamin D deficiency.

The strength of our study is that NHANES III used uniform methods to collect data on 25-Hydroxyvitamin D, insulin levels, and serum ALT and the study design allows the results to be extrapolated to the entire US population [3]. Despite its strength, our study has several shortcomings. The cross sectional design of the NHANES III dataset prohibits us to examine the causative nature or temporal association between serum vitamin D concentrations and unexplained elevation in ALT. Moreover, the analyses were based on a single measurement

of serum vitamin D, of which levels are influenced by seasonal changes, dietary intake, and ultraviolet exposure [5]. For instance, the highest levels of vitamin D are found during the summer months, and the lowest levels during the winter [17, 18]. These factors are not accounted for in our analyses. Lastly, measurement of parathyroid hormone was not part of NHANESIII; thus, the potential effect of this hormone on vitamin D levels cannot be explored [5].

In summary, we have shown the inverse relationship between serum concentrations of vitamin D and the prevalence of unexplained elevation in ALT. When using the cutoff at 75 nmol/l, we found a high prevalence of vitamin D deficiency in US adults. Thus, screening for serum vitamin D levels is warranted especially in subjects with unexplained elevation in ALT. Further studies are needed to confirm this observation and to understand the basis for and implications of this observation.

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Table 1

Baseline characteristics and laboratory data of subjects with unexplained elevation in ALT and controls

Variables	Unexplained ALT elevation (<i>n</i> = 308)	Controls (<i>n</i> = 979)	<i>P</i> value
Age (years)	39.0 ± 13.5	40.0 ± 14.0	NS
Gender (female, %)	51	50	NS
Race (Caucasian, %)	82	82	NS
BMI (kg/m ²)	29.1 ± 5.6	28.5 ± 5.3	NS
Metabolic syndrome (%)	42	27	<0.01
DM (%)	13.6	7.3	<0.01
HTN (%)	29	26	<0.01
Insulin resistance ^a (%)	41.5	25.2	<0.01
Serum creatinine (mg/dl)	1.0 ± 0.2	1.0 ± 0.2	NS
ALT (I μ /l)	55.5 ± 24.2	16.6 ± 7.5	<0.01
AST (I μ /l)	43.1 ± 24.3	19.9 ± 7.2	<0.01
Serum calcium (md/dl)	9.2 ± 0.4	9.2 ± 0.4	NS
TG (mg/dl)	174.2 ± 127.8	141.9 ± 97.8	<0.01
LDL (mg/dl)	144.5 ± 115.6	136.4 ± 92.8	<0.01
HDL (mg/dl)	44.7 ± 12.4	49.2 ± 13.9	<0.01
Plasma glucose (mg/dl)	109.5 ± 40	100.4 ± 28.2	<0.01
Fasting insulin (μ U/ml)	16.5 ± 10.3	11.3 ± 6.6	<0.01
C-peptide (pmol/ml)	1.1 ± 0.4	0.7 ± 0.4	<0.01
HOMA	4.7 ± 4.1	2.8 ± 2.2	<0.01
Vitamin D (nmol/l)	61.8 ± 26.0	66.8 ± 27.1	<0.01

^aThe presence of insulin resistance was estimated by the homeostasis model assessment method (HOMA)

BMI body mass index, *DM* diabetes mellitus, *HTN* hypertension

Unadjusted prevalence and adjusted odds ratio with 95% confidence interval of having unexplained elevation in ALT stratified by quartiles of serum vitamin D concentration among US adults aged 20 years old

Table 2

Measurement	Quartiles of serum vitamin D (nmol/l)				P value
	45.4 (n = 325)	45.4–61.4 (n = 325)	61.4–81.9 (n = 324)	81.9–208.2 (n = 316)	
Unadjusted prevalence of unexplained elevation in ALT (%)	31.5	25.6	21.4	21.4	<0.001
Unadjusted odds ratios (95% CI)	1	0.75 (0.53–1.10)	0.61 (0.42–0.86)	0.62 (0.43–0.89)	<0.01
Model 1 ^a	1	0.77 (0.54–1.10)	0.57 (0.39–0.82)	0.68 (0.47–0.97)	<0.01
Model 2 ^b	1	0.79 (0.55–1.15)	0.66 (0.45–0.96)	0.78 (0.53–0.99)	<0.01
Model 3 ^c	1	0.80 (0.55–1.14)	0.66 (0.45–0.96)	0.77 (0.53–0.99)	<0.01

^a Adjusted for metabolic syndrome

^b Adjusted for metabolic syndrome, and insulin resistance

^c Adjusted for metabolic syndrome, insulin resistance, and serum triglycerides