Original Article

Insulin Resistance Mediates the Association Between Vitamin D and Non-Alcoholic Fatty Liver Disease

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Abstract

Background: Vitamin D (VD) deficiency and insulin resistance (IR) increase the risk of non-alcoholic fatty liver disease (NAFLD), but few studies have explored the potential mechanisms by which IR mediates the association between VD and the pathogenesis of NAFLD at the genetic level using publicly available databases. **Methods:** This is a cross-sectional study, and we utilized the National Health and Nutrition Examination Survey (NHANES) dataset, as well as data from GSE200765 obtained from the Gene Expression Omnibus (GEO) website. A total of 723 individuals who had completed liver ultrasound examination and the detection of VD levels were included in the final analysis. A gene expression dataset, GSE200765, was also downloaded from the GEO website, to explore the potential mechanism of VD and NAFLD. **Results:** In the NHANES data, covariates significantly differed in four VD categories, and the controlled attenuation parameter (CAP), vibration-controlled transient elastography-liver stiffness measurement (VCTE-LSM), and IR were reduced with an increase in VD levels. Mediation analysis revealed that IR mediated the association between VD and both CAP and LSM, and the estimated mediation effects were 29.0% and 39.8%, respectively. Bioinformatics analysis showed that seven differentially expressed genes (DEGs) (solute carrier family 2 member 2 [SLC2A2], protein phosphatase 1 regulatory subunit 3E [PPP1R3E], CAMP responsive element binding protein 3-like 3 [CREB3L3], Interleukin-6 [IL-6], peroxisome proliferator-activated receptor gamma coactivator 1-alpha [PPARGC1A], nuclear factor kappa B inhibitor alpha [NFKBIA], and phosphoenolpyruvate carboxykinase 2 [PCK2]) were enriched in the IR pathway in comparison groups (VD group vs. lipid group), suggesting that VD improved NAFLD via changed IR. **Conclusions:** VD deficiency and IR were the risk factors for NAFLD, and increased VD levels improved the status of NAFLD. The underlying mechanism may be that elevated VD levels reduced IR, which improved the expression of DEGs involved in the IR pathway.

Keywords: *Insulin resistance, mediation analysis, national health and nutrition examination survey, non-alcoholic fatty liver disease, vitamin D*

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a prevalent chronic liver disease affecting a significant proportion of the world population, including ~25.2% worldwide and 27.4% in Asia.[1] This condition is characterized by an accumulation of excessive fat in the liver, present in more than 5% of hepatocytes, according to histological analysis.[2,3] NAFLD can progress from simple steatosis to more severe forms, including non-alcoholic steatohepatitis (NASH), liver cirrhosis, and ultimately hepatocellular carcinoma (HCC), even in the absence of excessive alcohol consumption.[4] Left untreated, NAFLD can lead to increased morbidity and mortality, resulting in significant healthcare expenditures, impaired quality of life, and massive economic losses.[5,6]

The pathogenesis of NAFLD is primarily related to metabolic impairment and glucose-insulin homeostasis.[7] As a result, NAFLD is often considered the hepatic component of metabolic syndrome (MS).[8] The "two-hit hypothesis" is the most widespread model, which proposes that insulin resistance (IR) — associated with metabolic disorders, including obesity, type 2 diabetes, and hyperlipidemia — leads to excessive lipid deposition in liver cells in the "first hit" process. Once NAFLD is established, it increases hepatic IR, which represents the "second hit."[9] NAFLD also contributes to systemic inflammation and impairs insulin sensitivity in extra-hepatic tissues, exacerbating hepatic injury

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development.[10,11] Therefore, reversing IR may be a promising strategy for treating NAFLD. Although several studies have explored the possibility of drugs that can improve IR in NAFLD patients, the key to breaking the vicious cycle of metabolically associated fatty liver disease spectrum is yet to be found.[12,13]

Vitamin D (VD) plays a crucial role in regulating calcium and phosphorus metabolism, immune function, and gene expression in the body. There are two major forms of VD, 25-hydroxyvitamin D3 (25-OH-D3) and 25-hydroxyvitamin D2 (25-OH-D2), which can be measured to determine the amount of VD present in the body. Multiple studies,[14,15] have shown that low levels of VD can lead to liver inflammation and fibrogenesis, increasing the risk of NAFLD, while VD supplementation can have therapeutic effects in these patients.[16-18] In the last few decades, experimental research has shown that VD deficiency may have adverse effects on insulin sensitivity and glucose homeostasis, ultimately leading to IR in hepatic cells; however, the mechanisms underlying the relationship between VD and IR are not fully understood. One possible mechanism is that VD can activate the VD receptor (VDR),[7,19] which interacts with other transcription factors and regulates the expression of various genes involved in the insulin signaling pathway.[19,20] Very low levels of VD may impair VDR function and affect the normal operation of the insulin signaling pathway; however, present studies are still in the initial stages.[19,21]

Currently, the role of IR in VD deficiency and NAFLD remains unclear, which limits the use of VD in NAFLD patients. By combining epidemiological surveys and biological analyses, we can shed light on the role of IR in the association between VD and NAFLD from a macro-micro perspective, providing evidence for the prevention and control of NAFLD. In this study, we utilized publicly available databases to investigate the potential mechanisms through which IR mediates the relationship between VD and the pathogenesis of NAFLD at the genetic level, aiming to estimate the impact of IR on the VD-NAFLD relationship.

Methods

National Health and Nutrition Examination Survey (NHANES) Dataset

The 2017–2018 NHANES dataset was downloaded from the NHANES website to investigate the role of IR in the association between VD and NAFLD. This dataset included 16,211 individuals, who were from 30 different survey locations, of which 9,254 completed the interview and 8,704 were examined. The inclusion criteria are as follows: (1) the individual's age ≥18 years old; (2) participants who completed the liver examination and VD test. The exclusion criteria are as follows: (1) Individuals who had consumed alcohol more than three times in the last year and those who did not complete the liver examination by ultrasound transient elastography were excluded based on the diagnostic criteria of NAFLD; (2) individuals who were pregnant, those who had hepatitis A, hepatitis B, and hepatitis C, and those who missed the value of fasting plasma glucose (FPG), fasting insulin (Fins), and VD levels were also excluded. Finally, 723 individuals were included in the present study. NHANES were approved by the National Center for Health Statistics (NCHS) Ethics Review Board; the approvals can be downloaded from https://www.cdc.gov/nchs/nhanes/irba98.htm. Written informed consent was obtained from all participants. This study was approved by the Xuzhou Central Hospital Review Board.

VD, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), the Controlled Attenuation Parameter (CAP), and Liver Stiffness Measurements (LSM)

The role of HOMA-IR in the association between VD and NAFLD was explored. High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was used for the quantitative detection of 25-OH-D3 and 25-OH-D2 in human serum. To better evaluate exposure, the NHANES provides a new index to reflect the VD status, the LBXVIDMS, which combines 25-OH-D3 levels and 25-OH-D2 levels. The sample was divided into four groups based on LBXVIDMS quartiles (≤47.6, 47.7–66.3, 66.4–85.4, and >85.5). HOMA-IR was evaluated by FPG and Fins using the following formula: HOMA-IR = FPG (mmol/L) * Fins (μU/ml)/22.5. The sample was divided into three groups based on HOMA-IR tertiles (lower HOMA-IR, median HOMA-IR, and higher HOMA-IR). CAP and vibration-controlled transient elastography (VCTE)-LSM were used to assess the liver status of individuals, and they were measured by the NHANES mobile examination center (MEC) using a FibroScan1 model 502 V2 Touch equipped with a medium (M) or extra-large (XL) wand (probe). The details of CAP and VCTE-LSM quality controls can

be obtained from https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/LUX_J.htm.

Bioinformatics Analysis

To investigate the potential pathological mechanism of VD and HOMA-IR in the development of NAFLD, a gene expression dataset, GSE200765, was selected from the Gene Expression Omnibus (GEO) website (https://www.ncbi.nlm.nih.gov/geo/). GSE200765 included 14 hepatoma-derived human liver cell line (HepaRG) cell samples. Six samples were selected for this analysis, of which three samples, GSM6043350, GSM6043351, and GSM6043352, were pretreated with a mixture of oleic acid (200 μM) and palmitic acid (200 μM), and as the lipid group. The other three samples, GSM6043358, GSM6043359, and GSM6043360, were treated with a mixture of oleic acid (200 μM) and palmitic acid (200 μM) + synthetic vitamin D3 (10 nM), and as the VD group.

Statistical analysis

The statistical software R 4.1.3 was used to analyze epidemiological and bioinformatics data. R packages, "tidyverse" and "Hmisc," were utilized to explore the association between VD levels and NAFLD. The individuals were classified into four groups (≤47.6 nmol/L, 47.7-66.3 nmol/L, 66.4-85.4 nmol/L, ≥85.5 nmol/L) based on the quartile of VD. To examine the differences in covariates among these groups, we employed the one-way ANOVA or Chi-square test. In addition, we analyzed the prevalence of NAFLD within various VD and IR categories. The IR categories were determined by dividing the levels of HOMA-IR, and we used the Chi-square test to investigate the prevalence of NAFLD across these groups. Furthermore, we conducted multivariate logistic regression to assess the relationship between VD categories and the risk of NAFLD. Using the "mediation" and "bda" R packages, we analyzed the mediation effect of HOMA-IR in the relationship between VD and CAP and LSM after adjusting for sex, age, race, BMI, high-density lipoprotein (HDL), and gamma-glutamyltransferase (GGT). For bioinformatics analysis, the R package "limma" was used to identify DEGs in the GSE200765 dataset, and the cutoff threshold of $|\log2FC| > 2$ and $P.adj < 0.05$ were used to select the DEGs. Functional enrichment analysis was performed using the database for annotation, visualization, and integrated discovery (DAVID; http://www.david.abcc.ncifcrf.gov/). Heatmaps were used to visualize DEGs in different groups, namely, those exposed to VD or not. $P < 0.05$ was considered statistically significant.

Results

Baseline Clinical Characteristics of Participants Based on VD Categories

A total of 723 individuals were included in the final analysis. Age, sex, race, body mass index (BMI), HDL, Fins, HOMA-IR, GGT, LSM, and CAP significantly differed among the four VD groups, and LSM, CAP, and HOMA-IR levels reduced with increasing VD [Table 1]. The prevalence of NAFLD was 80.7% in the VD ≤47.6 nmol/L group and 58.9% in the VD ≥85.4 nmol/L group. The prevalence of hepatic fibrosis was 14.9%, 14.4%, 9.4%, and 8.9% in VD ≤47.6 nmol/L, 47.7 nmol/L ~ 66.3 nmol/L, 66.4 nmol/L ~ 85.4 nmol/L, and ≥85.5 nmol/L groups, respectively. The covariates were categorized into two groups based on the status of NAFLD. The findings revealed significant differences in BMI, triglyceride, HDL, FPG, Fins, HOMA-IR, glutamic-pyruvic transaminase (ALT), GGT, LSM, and CAP between the two groups.

Multifactor Analysis of the Association Between VD and Liver Indices, the Risk Developing of NAFLD and HOMA-IR

After adjusting for sex, age, race, BMI, HDL, and GGT, multifactor linear regression was performed to explore the association between VD and CAP, LSM, HOMA-IR, and Fins, respectively [Supplement Figure 1a-d]. The results showed that VD was negatively associated with these indices (all $P < 0.05$). Using a multivariate logistic regression model, we have explored the association between VD categories and the risk of developing NAFLD. Table 2 shows that with reducing VD level, the risk of developing NAFLD increased, and compared with the individuals with VD level ≥85.5 nmol/L, the participants with VD ≤47.6 nmol/L have a higher risk of developing NAFLD (OR 95% confidence interval [C.I] =3.429 [2.081 ~ 5.683]), after adjustment for sex, age, race, BMI, FPG, smoking status, ALT, AST, TC, TG, and GGT, the OR and 95% C.I was 2.332 (1.316 ~ 4.133).

Mediated effect of IR in the association between VD and CAP and LSM

Mediation analysis revealed that IR plays a mediation effect in the association between VD and CAP and LSM. The mediation effect of IR in the relationship between VD and CAP and VD and LSM was 0.290 and 0.398, respectively [Figure 1], and the P values of the Sobel test were all <0.001. The prevalence of NAFLD and the distribution of HOMA-IR categories in different VD categories were also evaluated, and the results showed that the prevalence and proportion of a higher IR category were reduced with increasing VD [Figure 2a and b]. Further analysis revealed that the prevalence of NAFLD in different IR categories was significantly different, and the group with higher IR had a higher prevalence of NAFLD. However, individuals with higher IR had a lower prevalence of NAFLD in the VD ≥85.5 nmol/L group compared with the VD ≤47.6 nmol/L group [Figure 2c-f]. We conducted an estimation of the association between HOMA-IR categories and the likelihood of developing NAFLD. The

Table 1: Baseline clinical characteristics of participants based on VD categories

	VD levels (nmol/L)				*P* value
	≤47.6	47.7~66.3	66.4~85.4	≥85.5	
n	181	181	181	180	
Age (years)	49.83±17.62	51.55±16.71	56.55±16.25	63.68±14.52	<0.001
Sex (Male)	86 (47.5%)	110 (60.8%)	89 (49.2%)	85 (47.2%)	0.028
BMI (kg/m2)	32.42±8.28	30.16±6.29	29.94±6.92	28.29±6.05	<0.001
TC (mmol/L)	4.82±1.13	4.90±1.01	4.92±1.18	4.76±1.10	0.493
TG (mmol/L)	1.39±1.32	1.48±1.29	1.33±0.98	1.20±0.64	0.095
HDL (mmol/L)	1.31±0.42	1.31±0.34	1.37±0.39	1.45±0.42	0.003
FPG (mmol/L)	6.58±2.39	6.64±2.63	6.46±1.79	6.51±1.77	0.879
Fins (IU/mL)*	2.59±0.81	2.45±0.93	2.34±0.62	2.22±0.66	<0.001
HOMA-IR*	1.32±0.91	1.18±1.05	1.07±0.71	0.95±0.78	<0.001
ALT (U/L)*	3.00±0.64	3.05±0.61	2.95±0.48	2.93±0.54	0.212
AST (U/L)*	3.07±0.54	3.07±0.45	3.00±0.33	3.04±0.35	0.292
GGT (U/L)*	3.35±0.80	3.37±0.70	3.18±0.69	3.14±0.66	0.002
Median stiffness (E/kPa)*	1.77±0.56	1.76±0.49	1.64±0.36	1.61±0.40	0.002
Median CAP (db/m)	297.18±49.44	289.34±54.74	283.85±49.88	258.52±49.08	<0.001
Race					
Mexican American	37 (20.4%)	34 (18.8%)	15 (8.3%)	7 (3.9%)	<0.001
Other Hispanic	12 (6.6%)	16 (8.8%)	25 (13.8%)	11 (6.1%)	
Non-Hispanic White	38 (21.0%)	58 (32.0%)	76 (42.0%)	101 (56.1%)	
Non-Hispanic Black	64 (35.4%)	38 (21.0%)	28 (15.5%)	32 (17.8%)	
Other Race	30 (16.6%)	35 (19.3%)	37 (20.4%)	29 (16.1%)	
Smoking					
Yes	70 (38.7%)	79 (43.6%)	82 (45.3%)	86 (47.8%)	0.354
No	111 (61.3%)	102 (56.4%)	99 (54.7%)	94 (52.2%)	
NAFLD					
Yes	146 (80.7%)	131 (72.4%)	135 (74.6%)	106 (58.9%)	<0.001#
No	35 (19.3%)	50 (27.6%)	46 (25.4%)	74 (41.1%)	
Hepatic fibrosis					
Yes	27 (14.9%)	26 (14.4%)	17 (9.4%)	16 (8.9%)	0.032#
No	154 (85.1%)	155 (85.6%)	164 (90.6%)	164 (91.1%)	

BMI=body mass index; TC=total cholesterol, TG=triglyceride, HDL=high-density lipoprotein cholesterol, LDL=low density lipoprotein cholesterol, FPG=fasting plasma glucose, Fins=fasting insulin, HOMA-IR=homeostasis model assessment of insulin resistance, ALT=glutamic-pyruvic transaminase, AST=glutamic oxalacetic transaminase, GGT=gamma-glutamyl transpeptidase, CAP=controlled attenuation parameter. *: The variable was transferred by ln. #: The test was using trend analysis

Table 2: The association between VD and the risk of developing NAFLD

	Quartile	Non-NAFLD	NAFLD	Model1		Model2		Model3	
				OR	95% C.I	OR	95% C.I	OR	95% C.I
Vitamin D levels (nmol/L)	≤47.6	35 (19.3%)	146 (80.7%)	3.439	2.081~5.683	2.503	1.444~4.339	2.332	1.316~4.133
	47.7~66.3	50 (27.6%)	131 (72.4%)	2.070	1.299~3.298	1.719	1.025~2.882	1.604	0.940~2.737
	66.4~85.4	46 (25.4%)	135 (74.6%)	2.223	1.407~3.511	2.028	1.235~3.329	1.962	1.178~3.268
	≥85.5	74 (41.1%)	106 (58.9%)	1	1	1	1	1	1
P for trend		<0.001		<0.001		0.005		0.014	

Model 1: Adjusted for sex, age, and race. Model 2: Adjusted for sex, age, race, BMI, FPG, and smoking status. Model 3: Adjusted for sex, age, race, BMI, FPG, smoking status, ALT, AST, TC, TG, and GGT. C.I=confidence interval, NAFLD=non-alcoholic fatty liver disease

findings revealed that individuals with VD ≤47.6 nmol/L exhibited a 3.276-fold increase (2.176~6.379) in the risk of developing NAFLD when the IR categories were altered [Figure 2c]. This result suggested that higher VD levels improved the IR status and reduced the prevalence of NAFLD.

Exploring the Potential Mechanisms of the Mediation Effect of IR in the Association Between VD and CAP and LSM Using the GSE200765 Dataset

The GSE200765 dataset was used to explore VD cytoprotection function in human liver cell lipotoxicity. This dataset was re-analyzed to explore changes in hepatic genes under the treatment of VD. Compared with HepaRG

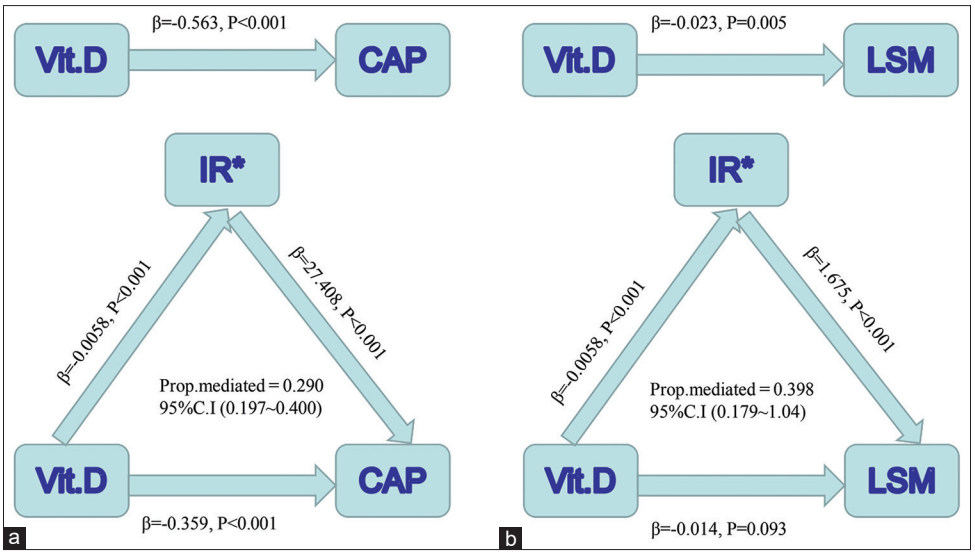

Figure 1: The mediation effect of HOMA-IR in the association of (a) VD with median CAP and (b) median stiffness. CAP = controlled attenuation parameter, HOMA-IR = homeostatic model assessment of insulin resistance, VD = vitamin D

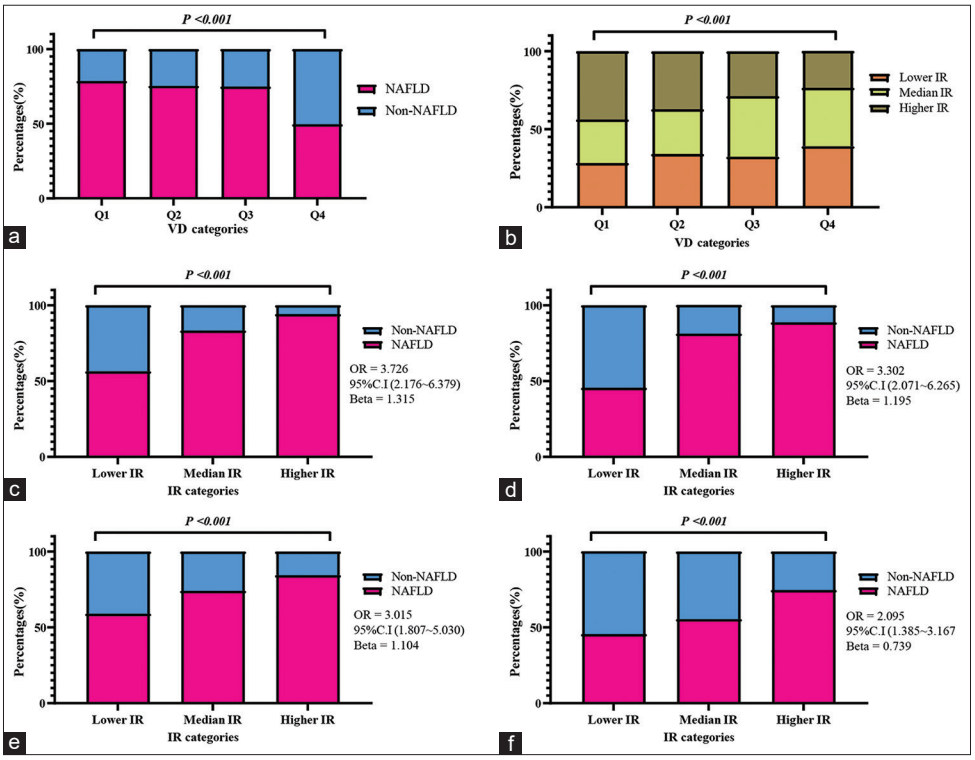

Figure 2: The prevalence of NAFLD in different VD categories and IR categories. (a) The prevalence of NAFLD in different VD categories; (b) The distribution of IR categories in different VD categories; (c-f) The prevalence of NAFLD in different IR categories at different VD categories. IR = insulin resistance, NAFLD = non-alcoholic fatty liver disease, VD = vitamin D

cells pretreated with a mixture of oleic acid/palmitic acid (the lipid group), 381 hepatic genes changed their expression profile in HepaRG cells treated with a mixture of oleic acid/palmitic acid and synthetic VD (10 nM) (the VD group). Using a cutoff threshold of | log2FC > 2| and P.adj < 0.05, 211 and 170 genes were upregulated and downregulated, respectively [Figure 3a]**.** Heatmaps were constructed to determine the expression of different genes in samples [Figure 3b].

Functional Enrichment of DEGs

The DAVID database (https://david.ncifcrf.gov/) was used to assess the underlying functions and pathways of DEGs, and the results are shown in Figure 4. (1) Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations showed significant enrichment of these DEGs in various metabolic-related terms, such as alcoholic liver disease, IR, and interleukin-17 (IL-17) signaling pathways. This result

indicated that IR plays an essential role in the relationship between VD and NAFLD. Seven DEGs were enriched in the IR pathway, including solute carrier family 2 (facilitated glucose transporter), member 2 (SLC2A2), protein phosphatase 1 regulatory subunit 3E (PPP1R3E), cAMP responsive element binding protein 3-like 3 (CREB3L3), interleukin-6 (IL-6), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NFKBIA), and phosphoenolpyruvate carboxykinase 2 (PCK2). The expression of DEGs in VD and lipid groups is displayed in Figure 4 (2) SLC2A2 and PPP1R3E were highly expressed, and CREB3L3, IL-6, PPARGC1A, NFKBIA, and PCK2 were lowly expressed in the VD group.

Discussion

The present study investigated the relationship between serum levels of VD and NAFLD, as well as the potential mediating role of IR in this association. Our findings demonstrated that lower levels of serum VD were associated with a higher prevalence of NAFLD. Furthermore, negative correlations were observed between serum VD levels and CAP, LSM, HOMA-IR, and Fins, suggesting that VD deficiency may contribute to the pathogenesis of IR and the development of hepatic steatosis.

The relationship between VD levels and NAFLD has yielded conflicting results in previous studies. A case-control study showed no significant difference in VD levels between 409 patients with NAFLD and 803 healthy controls.[22] Another study involving 4,614 NAFLD cases and 4,568 controls also did not find an association between genetically predicted VD levels and NAFLD risk.[23] Contrarily, a previous meta-analysis of 17 cross-sectional and case-control studies found that patients with NAFLD exhibited reduced VD levels and

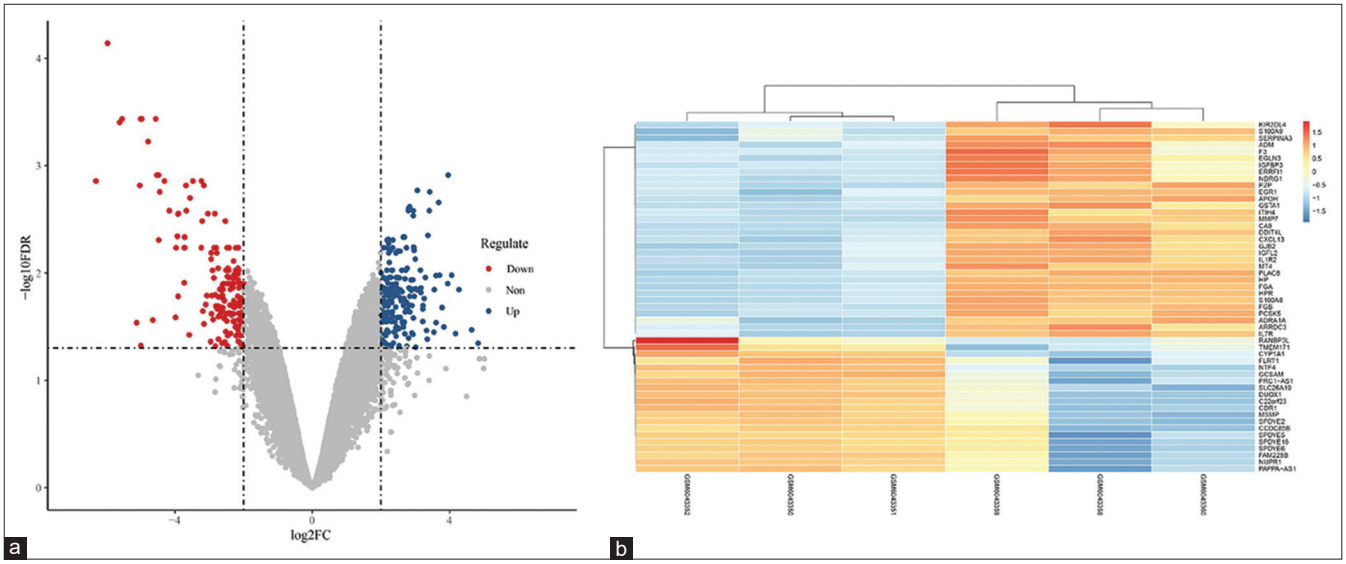

Figure 3: The volcanic map and heat map to show the DEGs between the lipid group and VD group. (a) The volcanic map. (b) The heatmap. CAP = controlled attenuation parameter, VD = vitamin D

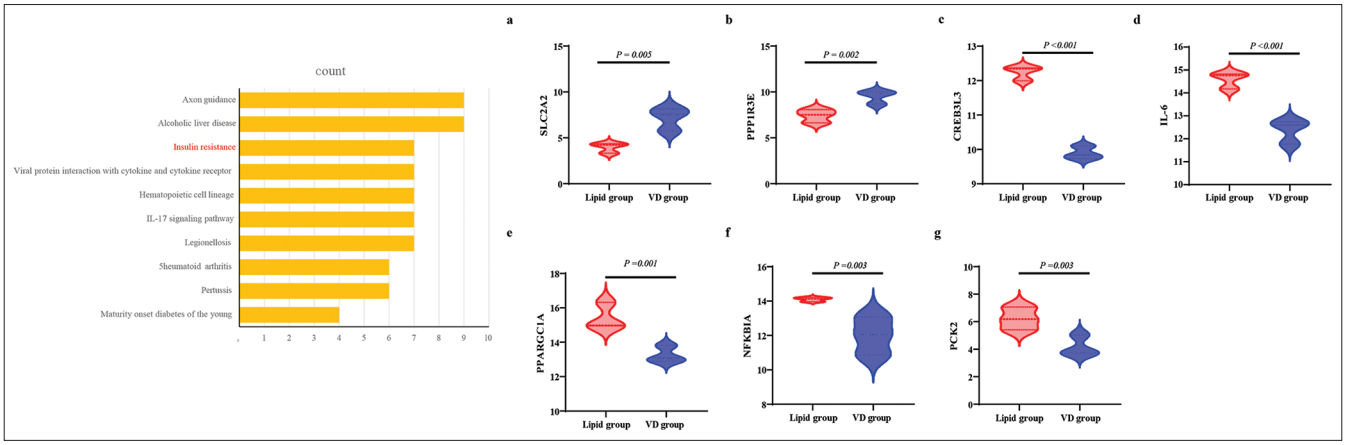

Figure 4: The pathways of DEGs after enriched by KEGG and the expression of seven genes enriched in the IR pathway. The horizontal histogram showed the pathway of DEGs enriched by KEGG. a~f showed the RNA expression of seven genes. DEGs = differentially expressed genes, IR = insulin resistance, KEGG = Kyoto Encyclopedia of Genes and Genomes

a higher probability of being VD deficient compared to those without NAFLD.[24,25] This inverse association has been consistently observed in recently published studies — independent of metabolic characteristics — and VD levels have been found to be inversely associated with the degree of liver steatosis and fibrosis.[26-28] Our results are consistent with studies that reported an inverse relationship between serum VD level and NAFLD prevalence and severity as well.[29-32] The discrepancy in findings could potentially be explained by ethnicity-specific features and different population characteristics.[31]

Moreover, our bioinformatics analysis revealed seven DEGs in the HepaRG cells with low vs. high VD levels. Of these genes, two (SLC2A2 and PPP1R3E) were upregulated, and five (CREB3L3, IL-6, PPARGC1A, NFKBIA, and PCK2) were downregulated in cells treated with VD. These findings suggest that VD may play a role in regulating glucose and lipid metabolism in liver cells. SLC2A2 (also known as glucose transporter 2 (GLUT2)) is a glucose transporter that plays a key role in hepatic glucose uptake and metabolism.[33] PPP1R3E is a regulatory subunit of glycogen synthase phosphatase, which regulates glycogen synthesis in the liver.[34] The upregulation of these genes in the VD group suggests that VD may promote glucose uptake and metabolism in insulin-sensitive tissues, which could potentially improve insulin sensitivity, reduce IR, and prevent the development of NAFLD.

The downregulation of CREB3L3, IL-6, PPARGC1A, NFKBIA, and PCK2 in the VD group suggests that VD may also modulate the expression of genes involved in inflammation, oxidative stress, and gluconeogenesis, which are key contributors to the development of IR and NAFLD. CREB3L3 is a transcription factor involved in lipid metabolism and glucose homeostasis.[35] IL-6 is a pro-inflammatory cytokine that has been implicated in the development of NAFLD. PPARGC1A is a transcriptional coactivator that regulates lipid metabolism and mitochondrial biogenesis.[36] NFKBIA is an inhibitor of nuclear factor kappa B (NF-κB), a transcription factor that plays a central role in inflammation and immune responses.[37] Finally, PCK2 is a key enzyme in gluconeogenesis, which is dysregulated in patients with NAFLD and IR.[38] Previous animal studies indicated that appropriate intervention on these abnormally expressed genes would greatly improve the steatosis of hepatocytes.[39-41] This suggests that altered expression of these genes participates in the insulin signaling pathway, which highlights the pharmacological inhibition of this pathway as a potentially useful approach not only for controlling glycemia but also for mitigating the development and/or progression of NAFLD.[39]

Our study has several strengths, including the use of a large, well-characterized NHANES dataset of NAFLD patients with detailed clinical and laboratory data. We also performed rigorous statistical analyses to examine the associations between VD, IR, and NAFLD and used mediation analysis to examine the potential mediating effect of IR on these associations. Furthermore, we performed bioinformatics analysis of gene expression profiles using the DAVID database to identify potential biological pathways linking VD deficiency to NAFLD.

However, this study also has some limitations. First, the serum VD level was measured only once at baseline. Thus, we could not account for the potential confounding factors (such as dietary intake or sun exposure) or temporal changes in the serum VD level. Second, we did not measure the functional activity of the proteins encoded by the detected DEGs. Therefore, it is not clear whether the observed changes in gene expression translate into changes in protein function. Further studies are needed to investigate the functional consequences of the observed changes in gene expression, especially in patients with NAFLD.

In conclusion, our study showed that VD deficiency was associated with higher prevalence and severity of NAFLD and provides insights into the potential role of VD in regulating IR pathways and gene expression. This study adds to the growing body of literature on the role of VD in metabolic disorders and may have important clinical implications for the prevention and treatment of NAFLD. While the findings are promising, further studies are needed to validate and expand on our results.

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Ethics approval and consent to participate

The study was reviewed and approved by the ethics committee of the Xuzhou Central Hospital. The approval number of the ethics committee is XZXY-LJ-20201110-060.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this manuscript.

Author's contribution

CY Zou and XK Liu drafted this manuscript and collected the sample. Y Sun, YQ Sang, YM Ma, and GS Peng analyzed the data. MS He provided great help in the area of ultrasonic medicine for this study. J Liang and HF Geng are responsible for the integrity of the work as a whole.

All authors read and approved the final version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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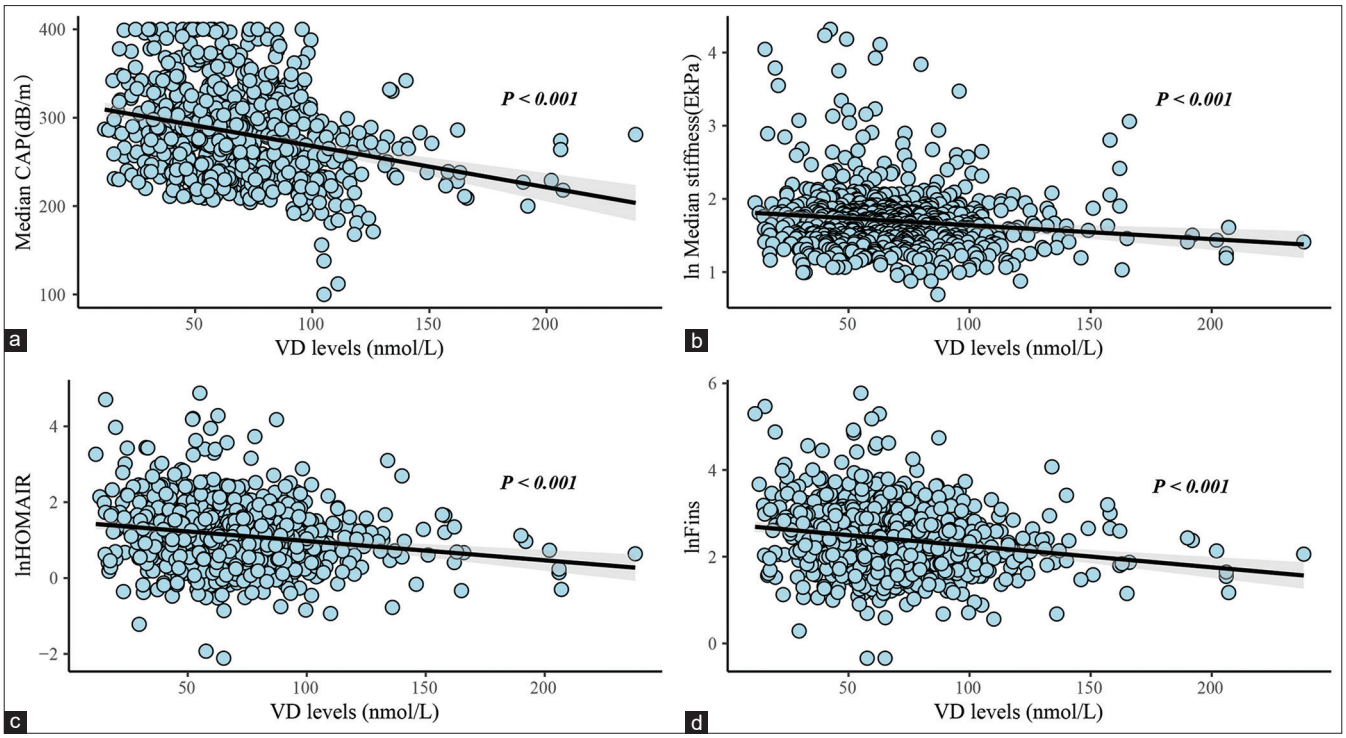

Supplement Figure 1: The association of VD with (a) median CAP, (b) median stiffness, (c) HOMA-IR, and (d) Fins. CAP = controlled attenuation parameter, Fin = fasting insulin, HOMA-IR = homeostatic model assessment of insulin resistance, VD = vitamin D