

# Serum vitamin D concentrations are associated with obese but not lean NAFLD: a cross-sectional study

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## Research

**Keywords:** Non-alcoholic fatty liver disease; vitamin D; obesity; body mass index; cross-sectional study

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# Abstract

**Background:** Low serum vitamin D concentrations have been reported to be associated with an increased risk of non-alcoholic fatty liver disease (NAFLD). However, whether slim or obese people show a similar association between vitamin D and NAFLD remains speculative. This study aimed to explore the relationship between serum vitamin D concentrations and NAFLD in lean and obese Chinese adults.

**Methods:** This cross-sectional study included 2538 participants (1360 men and 1178 women) who underwent their health checkups at the First Affiliated Hospital of Zhejiang University School of Medicine in 2019. NAFLD was diagnosed by liver ultrasound excluding other causes. The association of serum vitamin D concentrations with NAFLD was analyzed in lean and obese participants.

**Results:** The overall prevalence of NAFLD was 33.61% (13.10% in lean and 53.32% in obese) in this study population. The serum vitamin D levels of obese NAFLD patients were lower than that of the obese NAFLD-free controls. However, the serum vitamin D levels of lean NAFLD patients were comparable to that of the lean NAFLD-free controls. Serum vitamin D levels were negatively correlated with the prevalence of NAFLD in obese but not lean participants. Serum vitamin D levels were independently associated with the risk of NAFLD in obese participants, with an adjusted odd ratio (95% CI) of 0.986 (0.979–0.992). However, the serum vitamin D levels were not related to the risk of NAFLD in lean participants.

**Conclusions:** Low serum vitamin D levels are associated with NAFLD in obese but not lean participants.

## Background

Non-alcoholic fatty liver disease (NAFLD) is hepatic steatosis when liver lipid deposition is not secondarily caused by heavy drinking or other known etiologies with or without inflammation and fibrosis (1). NAFLD is currently one of the world's highest prevalence of chronic liver diseases, affecting approximately 29.2% of adults in China (2). NAFLD includes simple steatosis, steatohepatitis, cirrhosis, and even hepatocellular carcinoma (3, 4). Patients with NAFLD have increased risks of cardiovascular disease, stroke, type 2 diabetes, and extrahepatic malignancies (5-8). The high prevalence and serious clinical harms of NAFLD make it a global research hotspot in recent years (9).

Obesity is closely related to NAFLD, and the prevalence and risk of NAFLD in obese individuals are higher than those in lean individuals (10). But recently, many studies have shown that non-obese people also have a high prevalence of NAFLD (11, 12). We have previously reported that in China, the prevalence of NAFLD in the non-obese population is 7.3%, and 8.9% of the non-obese adults developed NAFLD during a 5-year follow-up (13). Compared with obese NAFLD patients, lean NAFLD patients are usually asymptomatic and difficult to diagnose, but in fact, they also have severe liver histological necrotizing inflammation and high mortality (14). NAFLD in the non-obese population may also cause significant health problems (15). Therefore, the identification, diagnosis, and treatment of non-obese NAFLD are very important.

The risk factors for non-obese NAFLD remain unclear. Previous cross-sectional studies have shown that vitamin D deficiency was associated with an increased risk of NAFLD, and vitamin D levels were negatively associated with the severity of NAFLD (16, 17). Several prospective studies have pointed out that serum vitamin D deficiency was accompanied by an increased risk of incident NAFLD (18-21). Our recent study showed that the serum vitamin D levels in high-fat diet-fed mice were significantly decreased, and vitamin D supplementation ameliorated high-fat diet-induced hepatic steatosis in mice (22). Vitamin D supplementation could also improve hepatic steatosis in patients with NAFLD (23, 24). However, it is unclear whether obesity affects the correlation between vitamin D concentrations and NAFLD, and whether serum vitamin D concentrations are related to NAFLD in lean individuals.

In this study, we aimed to explore the correlation between serum vitamin D concentrations and NAFLD in obese and lean Chinese adults.

## Methods

### Participants

We enrolled adults who underwent health checkups at the First Affiliated Hospital of Zhejiang University School of Medicine in 2019 as participants in our cross-sectional study. The analysis included participants with complete anthropometric, biochemical data records (including serum vitamin D concentrations) and liver ultrasound results. We excluded the following participants: (1) participants with incomplete anthropometric and biochemical data; (2) men with alcohol consumption >210 g/week and women with alcohol consumption >70 g/week; (3) participants with other chronic liver diseases caused by autoimmune hepatitis or viral hepatitis; and (4) participants who use hepatotoxic drugs (such as sulfonamide and azithromycin). The final analysis included 2538 participants (1360 men and 1178 women).

The personal information of all participants was anonymous. The study was approved by the Ethical Committee of the First Affiliated Hospital of Zhejiang University School of Medicine.

### Clinical examinations

Clinical examinations included questionnaires, medical history, anthropometry, and biochemical measurements. Through the examination, the physician recorded the medical history (including previous diseases and drug prescriptions) and drinking frequency and amount. The smoking history was also recorded and distinguished as yes and no.

The anthropometric measurements were performed as previously described, including body weight, standing height, waist circumference, and blood pressure (25, 26). Weight and height were measured with light clothing and no shoes. Waist circumference was measured when the patient exhales with the tape measure placed between the lowest rib and the top edge of the top. Blood pressure was measured after resting for 5 minutes. Body mass index (BMI) was calculated as the weight (kg) divided by the height (m) squared.

Fasting blood samples were taken from the anterior cubital vein and were used for biochemical analysis. Measurements include liver enzymes, blood lipids, glucose, and uric acid. All biochemical values were measured by a Hitachi 7600 clinical analyzer (Hitachi, Tokyo, Japan) using standard methods. Serum 25-hydroxyvitamin D levels were measured with electro-chemiluminescence immunoassay (ECLIA) platform using the Roche cobas e602 analyzer (Roche Diagnostics GmbH, Germany).

### **Diagnostic criteria and definitions**

Lean was defined as BMI <24 kg/m<sup>2</sup>, and obesity was defined as BMI ≥24 kg/m<sup>2</sup> (27). The quartiles of serum vitamin D levels were defined according to serum vitamin D levels as follows: quartile 1, vitamin D <45.5 nmol/L; quartile 2, 45.5–59.5 nmol/L; quartile 3, 59.5–74.2 nmol/L; and quartile 4, vitamin D ≥74.3 nmol/L. Vitamin D sufficiency was defined as vitamin D ≥100 nmol/L; vitamin D insufficiency, 50–100 nmol/L; and vitamin D deficiency, vitamin D <50 nmol/L (28).

### **Diagnosis of NAFLD**

An abdominal ultrasound examination was performed by experienced ultrasonographers, using the Toshiba Nemio 20 ultrasound system (Toshiba, Tokyo, Japan) with a probe of 3.5 MHz. The ultrasonographers were unaware of the study's purpose and laboratory values. Fatty liver disease was diagnosed according to the standards of the Chinese Liver Disease Association (29).

### **Statistical analysis**

The statistical analysis was performed by SPSS (SPSS, Chicago, IL) for Mac version 18.0. Continuous variables were presented as mean ± SD or median and interquartile range (IQR). Student's *t*-test was applied to compare continuous data, and  $\chi^2$  test was applied to compare categorical variables. Cochran-Armitage trend test showed the trend of prevalence. Stepwise multiple regression analysis was used to identify possible risk factors for NAFLD (Backward LR; Entry: 0.05, Removal: 0.10). *P* <0.05 (two-tailed test) was considered to be statistically significant.

## **Results**

### **Clinical characteristics of the study population**

A total of 2538 participants (1360 men and 1178 women) were included in this study, and 853 (33.61%) had NAFLD. The prevalence of NAFLD was 13.10% in lean participants (BMI <24 kg/m<sup>2</sup>), and was 53.32% in obese participants (BMI ≥24 kg/m<sup>2</sup>). We compared clinical characteristics based on NAFLD status (Table 1). We found that both lean and obese NAFLD patients were older, had higher BMI, larger waist circumference, higher systolic and diastolic blood pressure, and had elevated serum levels of alanine aminotransferase,  $\gamma$ -glutamyl transpeptidase, triglyceride, LDL-cholesterol, uric acid and fasting glucose, but lower serum HDL-cholesterol levels than corresponding controls. Besides, vitamin D levels of obese NAFLD patients were lower than those of obese NAFLD-free controls (59.03 ± 19.46 versus 63.56 ± 22.09 nmol/L, *P* <0.001), but this was not observed in lean participants (Table 1).

**Table 1. Clinical characteristics of the study population according to obese and NAFLD categories**

Variables	Overall (n =2538)	Lean participants			Obese participants		
		NAFLD (n =163)	Without NAFLD (n =1081)	<i>P</i> value	NAFLD (n =690)	Without NAFLD (n =604)	<i>P</i> value
Gender (male/female)	1360/1178	86/77	430/651	0.002	485/205	359/245	<0.001
Age (year)	54.08 (6.85)	54.84 (6.59)	53.81 (6.69)	0.078	54.23 (6.60)	54.18 (6.99)	0.901
Body mass index (kg/m <sup>2</sup> )	24.22 (2.96)	22.77 (1.14)	21.71 (1.54)	<0.001	27.09 (2.18)	25.83 (1.73)	<0.001
Waist circumference (cm)	85.13 (8.67)	83.34 (5.12)	78.66 (6.12)	<0.001	92.88 (6.39)	88.35 (6.37)	<0.001
Systolic blood pressure (mmHg)	127.93 (18.38)	127.64 (16.86)	122.77 (18.51)	0.002	134.37 (17.41)	129.88 (16.89)	<0.001
Diastolic blood pressure (mmHg)	78.37 (11.56)	78.08 (9.98)	74.77 (11.26)	<0.001	82.96 (10.72)	79.65 (11.34)	<0.001
Albumin (g/L)	46.13 (2.49)	46.58 (2.34)	45.98 (2.49)	0.004	46.51 (2.36)	45.84 (2.60)	<0.001
Alanine aminotransferase (U/L)	23.21 (19.06)	28.64 (45.74)	18.24 (12.69)	0.004	30.78 (16.61)	22.00 (15.59)	<0.001
Aspartate aminotransferase (U/L)	21.77 (10.64)	23.35 (20.40)	20.51 (9.70)	0.082	23.77 (9.15)	21.30 (9.60)	<0.001
γ-Glutamyl Transpeptidase (U/L)	29.90 (31.84)	35.50 (35.39)	23.00 (31.34)	<0.001	40.49 (32.64)	28.64 (26.98)	<0.001
Triglyceride (mmol/L)	1.63 (1.14)	2.02 (1.16)	1.26 (0.82)	<0.001	2.17 (1.43)	1.54 (0.95)	<0.001
Total cholesterol (mmol/L)	4.71 (0.89)	4.83 (0.92)	4.67 (0.86)	0.031	4.75 (0.89)	4.71 (0.92)	0.382
HDL-cholesterol (mmol/L)	1.22 (0.34)	1.12 (0.26)	1.36 (0.01)	<0.001	1.04 (0.25)	1.19 (0.28)	<0.001
LDL-cholesterol (mmol/L)	2.75 (0.72)	2.83 (0.79)	2.69 (0.68)	0.016	2.79 (0.75)	2.79 (0.74)	0.839
Serum uric acid (μmol/L)	325.71 (81.72)	332.94 (73.72)	293.72 (71.12)	<0.001	369.41 (78.22)	331.10 (80.78)	<0.001
Fasting blood glucose (mmol/L)	5.21 (1.21)	5.53 (1.34)	4.99 (1.03)	<0.001	5.57 (1.50)	5.12 (0.94)	<0.001
Smoking history (yes/no)	525/2013	32/131	175/906	0.271	195/495	123/481	0.001
Vitamin D (nmol/L)	61.21 (21.64)	60.95 (18.54)	61.32 (22.97)	0.844	59.03 (19.46)	63.56 (22.09)	<0.001

Data are expressed as mean (SD).

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease.

### Association of serum vitamin D levels with the prevalence of NAFLD

We classified all participants into quartiles by their serum vitamin D levels and analyzed the association of vitamin D quartiles with the prevalence of NAFLD. We found that serum vitamin D quartiles were negatively associated with the prevalence of NAFLD in obese participants (Table 2). The prevalence of NAFLD was 56.54%, 59.77%, 52.15%, and 44.51% in the first, second, third, and fourth quartiles of serum

vitamin D in obese participants ( $P$  for trend  $<0.001$ ; Table 2). However, serum vitamin D quartiles were not associated with the prevalence of NAFLD in lean participants (Table 2).

**Table 2. Association of vitamin D quartiles with prevalence of NAFLD in lean and obese participants**

VD quartiles	Lean participants						Obese participants					
	Total	NAFLD	PR%	PR	$\chi^2$	$P$ value	Total	NAFLD	PR%	PR	$\chi^2$	$P$ value
Quartile 1	327	37	11.31	0.95			306	173	56.54	1.27		
Quartile 2	292	38	13.01	1.09			343	205	59.77	1.34		
Quartile 3	306	50	16.34	1.37			326	170	52.15	1.17		
Quartile 4	319	38	11.91	1.00	4.133	0.247	319	142	44.51	1.00	17.118	$<0.01$

VD, vitamin D; PR%, prevalence rate; PR, prevalence ratio.

Participants were classified into quartiles according to their serum vitamin D levels: quartile 1,  $<45.5$  nmol/L; quartile 2, 45.5-59.5 nmol/L; quartile 3, 59.5-74.2 nmol/L; and quartile 4,  $\geq 74.3$  nmol/L.

We also divided all participants into three groups according to their vitamin D adequacy status and analyzed the association of vitamin D adequacy status with the prevalence of NAFLD in lean and obese participants, respectively. We found that participants with vitamin D deficiency had the highest prevalence of NAFLD (57.38%), followed by those with vitamin D insufficiency (52.06%), and vitamin D sufficiency (41.82%) in the obese group ( $P$  for trend = 0.045; Table 3). However, the prevalence of NAFLD was comparable among lean participants with different vitamin D adequacy status (Table 3).

**Table 3. Association of vitamin D sufficiency with prevalence of NAFLD in lean and obese participants**

VD classification	Lean participants						Obese participants					
	Total	NAFLD	PR%	PR	$\chi^2$	$P$ -value	Total	NAFLD	PR%	PR	$\chi^2$	$P$ value
VD deficiency	427	49	11.48	1.28			413	237	57.38	1.37		
VD insufficiency	750	108	14.40	1.61			826	430	52.06	1.24		
VD sufficiency	67	6	8.96	1.00	3.114	0.211	55	23	41.82	1.00	6.194	0.045

VD, vitamin D; PR%, prevalence rate; PR, prevalence ratio.

Participants were classified into three groups according to their serum vitamin D levels: VD sufficiency,  $\geq 100$  nmol/L; VD insufficiency, 50-100 nmol/L; and VD deficiency,  $<50$  nmol/L.

### Association of serum vitamin D levels with risk of NAFLD

Next, multiple logistic regression analyses were conducted to explore the risk factors of NAFLD in lean and obese participants. We found that male gender, high BMI and waist circumference, high serum levels of albumin, alanine aminotransferase, aspartate aminotransferase, uric acid, and fasting blood glucose, and low serum levels of HDL-cholesterol were correlated with increased risks of NAFLD in both lean and obese

participants (Table 4). We also found that serum vitamin D concentrations were another factor associated with the risk of NAFLD in obese participants, with an adjusted OR (95% CI) of 0.986 (0.979–0.992). However, serum vitamin D concentrations were not associated with the risk of NAFLD in lean participants (Table 4).

**Table 4. Multivariable analysis for factors associated with risk of NAFLD in lean and obese participants**

Variables	Lean participants			Obese participants		
	Wald $\chi^2$	OR (95% CI)	P value	Wald $\chi^2$	OR (95% CI)	P value
Male gender	16.793	2.809 (1.714–4.604)	<0.001	10.171	1.782 (1.250–2.542)	0.001
Age (years)	3.363	1.028 (0.998–1.059)	0.067	3.601	1.020 (0.999–1.042)	0.058
Body mass index (kg/m <sup>2</sup> )	12.505	1.432 (1.174–1.747)	<0.001	15.075	1.205 (1.097–1.323)	<0.001
Waist circumference (cm)	8.647	1.071 (1.023–1.122)	0.003	8.709	1.046 (1.015–1.079)	0.003
Albumin (g/L)	8.411	1.129 (1.040–1.226)	0.004	13.816	1.109 (1.050–1.172)	<0.001
Alanine aminotransferase (U/L)	21.812	1.070 (1.040–1.101)	<0.001	36.361	1.055 (1.037–1.074)	<0.001
Aspartate aminotransferase (U/L)	10.249	0.922 (0.877–0.969)	0.001	14.961	0.945 (0.918–0.972)	<0.001
Total cholesterol (mmol/L)	9.794	1.407 (1.136–1.742)	0.002	4.790	1.181 (1.018–1.372)	0.029
HDL-cholesterol (mmol/L)	25.698	0.146 (0.069–0.307)	<0.001	45.129	0.154 (0.089–0.266)	<0.001
Serum uric acid ( $\mu$ mol/L)	10.789	1.005 (1.002–1.008)	0.001	16.539	1.004 (1.002–1.006)	<0.001
Fasting blood glucose (mmol/L)	6.690	1.196 (1.044–1.370)	0.010	11.248	1.230 (1.090–1.387)	0.001
Vitamin D (nmol/L)	-	-	-	19.253	0.986 (0.979–0.992)	<0.001

Backward stepwise regression was used in multivariate logistic regression analyses (probability to enter = 0.05 and probability to remove = 0.10).

OR, odds ratio; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol.

We further analyzed the correlation between vitamin D quartiles and the risk of NAFLD (Table 5). Among obese participants, compared with participants in the fourth quartile, participants with serum vitamin D levels in the first, second, and third quartiles all showed increased risks of NAFLD, with the adjusted OR (95% CI) of 1.942 (1.329–2.838), 1.853 (1.286–2.669) and 1.453 (1.009–2.093), respectively. However, the risk of NAFLD was comparable among lean participants with different serum vitamin D quartiles (Table 5). Similarly, we analyzed the correlation between vitamin D adequacy status and risk of NAFLD (Table 6). Among obese participants, vitamin D deficiency showed an increased risk of NAFLD compared with those with vitamin D sufficiency, with an adjusted OR (95% CI) of 1.906 (1.005–3.614). However, vitamin D deficiency was not associated with an increased risk of NAFLD in lean participants (Table 6). These

results showed that decreased serum vitamin D concentrations were associated with an increased risk of NAFLD in obese but not lean participants.

**Table 5. Association of serum Vitamin D quartiles with risk of NAFLD in lean and obese participants**

Lean	Models	Odds ratios (95% confidence interval)				$\chi^2$ value	P value
		Q1 (n=327)	Q2 (n=292)	Q3 (n=306)	Q4 (n=319)		
	Model 1	0.943 (0.583-1.527)	1.106 (0.684-1.789)	1.444 (0.917-2.275)	1	4.007	0.261
	Model 2	1.174 (0.702-1.964)	1.272 (0.766-2.111)	1.527 (0.948-2.459)	1	3.190	0.363
	Model 3	1.191 (0.673-2.106)	1.122 (0.637-1.795)	1.498 (0.882-2.545)	1	2.461	0.482
Obese	Models	Odds ratios (95% confidence interval)				$\chi^2$ value	P value
		Q1 (n=306)	Q2 (n=343)	Q3 (n=326)	Q4 (n=319)		
	Model 1	1.621 (1.182-2.224)	1.852 (1.360-2.521)	1.358 (0.996-1.852)	1	17.145	0.001
	Model 2	1.888 (1.337-2.666)	1.980 (1.420-2.758)	1.514 (1.088-2.106)	1	19.473	< 0.001
	Model 3	1.942 (1.329-2.838)	1.853 (1.286-2.669)	1.453 (1.009-2.093)	1	15.141	0.002

Model 1 was unadjusted.

Model 2 was adjusted for age, gender, and body mass index.

Model 3 was further adjusted for waist circumference, systolic and diastolic blood pressure, albumin, alanine aminotransferase, aspartate aminotransferase,  $\gamma$ -glutamyl transpeptidase, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, fasting blood glucose, serum uric acid and smoking history.

Participants were classified into quartiles according to their serum vitamin D levels: quartile 1, <45.5 nmol/L; quartile 2, 45.5-59.5 nmol/L; quartile 3, 59.5-74.2 nmol/L; and quartile 4,  $\geq$ 74.3 nmol/L.

**Table 6. Association of vitamin D sufficiency with risk of NAFLD in lean and obese participants**

Lean	Models	Odds ratios (95% confidence interval)			$\chi^2$ value	P value
		VD deficiency (n=427)	VD insufficiency (n=750)	VD sufficiency (n=67)		
	Model 1	1.318 (0.0.541-3.209)	1.710 (0.722-4.054)	1	3.085	0.214
	Model 2	1.728 (0.680-4.392)	1.948 (0.797-4.761)	1	2.353	0.308
	Model 3	1.571 (0.557-4.429)	1.827 (0.674-4.955)	1	1.724	0.422
Obese	Models	Odds ratios (95% confidence interval)			$\chi^2$ value	P value
		VD deficiency (n=413)	VD insufficiency (n=826)	VD sufficiency (n=55)		
	Model 1	1.874 (1.059-3.313)	1.511 (0.869-2.626)	1	6.136	0.047
	Model 2	2.334 (1.264-4.310)	1.778 (0.986-3.206)	1	9.108	0.011
	Model 3	1.906 (1.005-3.614)	1.335 (0.722-2.467)	1	8.074	0.018

Model 1 was unadjusted.

Model 2 was adjusted for age, gender, and body mass index.

Model 3 was further adjusted for waist circumference, systolic blood pressure, albumin, alanine aminotransferase, aspartate aminotransferase, total cholesterol, LDL-cholesterol, fasting blood glucose, serum uric acid and smoking history.

Participants were classified into three groups according to their serum vitamin D levels: VD sufficiency,  $\geq 100$  nmol/L; VD insufficiency, 50-100 nmol/L; and VD deficiency,  $< 50$  nmol/L.

## Discussion

In this study, we explored the correlation between serum vitamin D concentrations and NAFLD in Chinese adults. We found that serum vitamin D concentrations of obese NAFLD patients were lower than those of obese controls without NAFLD. We also found that serum vitamin D concentrations were negatively correlated with the prevalence of NAFLD in obese but not lean participants. Our further analysis showed that decreased serum vitamin D concentrations or vitamin D deficiency were associated with an increased risk of NAFLD in obese but not lean participants. These findings suggested a significant correlation between serum vitamin D concentrations and NAFLD in obese but not lean participants.

Several studies have reported that there is a significant correlation between serum vitamin D concentrations and NAFLD (30, 31), and this was confirmed by a meta-analysis including 12794 participants of 17 studies (32). Moreover, low serum vitamin D concentrations are related to greater severity of hepatic steatosis and necrotizing inflammation both in children and in adults (33, 34). Preclinical investigations found that vitamin D supplementation significantly improved liver steatosis in

high-fat diet-fed mice (35). Besides, we and others found that vitamin D receptor (VDR) is upregulated in the steatotic livers, and maybe a therapeutic target for NAFLD (35, 36). As we know, low serum vitamin D levels are more commonly observed in obese than in lean individuals (37). However, whether lean or obese individuals showed a similar association of vitamin D with NAFLD remains speculative and should be investigated. In this study, we provided evidence that low serum vitamin D levels were associated with obese but not lean populations.

The explanations for why obese and lean individuals have inconsistent correlations between vitamin D and NAFLD remains unclear, although several possibilities exist. First, obesity is closely associated with low vitamin D levels itself and maybe a major factor causing this result (38, 39). With fewer outdoor activities and low exposure to sunlight, obese individuals may have decreased vitamin D synthesized in the liver or percutaneously (8). A genetic study showed that each increase in BMI will reduce serum vitamin D concentration by 1.15% (40). Second, patients with vitamin D deficiency have higher serum levels of proinflammatory cytokines and promote the development of NAFLD (41). In the non-alcoholic steatohepatitis (NASH) stage, vitamin D deficiency can also actively regulate the synthesis of endogenous fatty acids in the liver by weakening the enterohepatic circulation (42). Third, vitamin D can increase the expression of peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ ), thereby promoting the secretion of serum triglycerides and the accumulation of lipid droplets in hepatocytes (43). Therefore, under vitamin D deficiency conditions, the flow of free fatty acids (FFAs) in the blood increases, and fat deposition is accelerated into hepatocytes, contributing to the progress of NAFLD (44). Further researches are needed to clarify these possibilities.

Recently, researchers have subdivided NAFLD into obese and lean subtypes according to their obesity status, and many studies have focused on lean NAFLD (45-47). Vitamin D concentrations are closely related to NAFLD, and vitamin D deficiency is considered a risk factor for NAFLD (31, 44). However, it was not clear whether vitamin D concentrations were also associated with lean NAFLD. In this study, we found that low vitamin D concentrations are associated with obese but not lean NAFLD. Our results suggest that the vitamin D concentration may be an important predictor of NAFLD screening in obese but not lean population.

In this study, some limitations are acknowledged. First, our NAFLD was diagnosed based on ultrasound. Although ultrasound NAFLD diagnosis has been widely used clinically as a screening method for hepatic steatosis, it is still insufficient to detect mild steatosis and cannot replace the gold standard for liver biopsy. The correlation between vitamin D levels and NAFLD histological severity was not explored in this study. Second, this is a single-center cross-sectional study. Our sample size may be insufficient to represent the entire Chinese adult population, and further multi-center cohort studies are needed. Third, this study classified lean and obese participants by the BMI but did not include waist circumference or waist-to-hip ratio. It may mix some central obese patients with lean participants.

## Conclusions

Our cross-sectional study provided evidence that there was a significant correlation between serum vitamin D concentrations and NAFLD in obese but not lean participants. Further research is needed to explore the complicated relationships and possible mechanisms between obesity, vitamin D levels, and NAFLD.

## List Of Abbreviations

NAFLD: Non-alcoholic fatty liver disease;

BMI: Body mass index;

ECLIA: Electro-chemiluminescence immunoassay;

IQR: Interquartile range;

VDR: Vitamin D receptor;

NASH: Non-alcoholic steatohepatitis;

PPAR- $\gamma$ : Peroxisome proliferator-activated receptor  $\gamma$ ;

FFA: Free fatty acid.

## Declarations

### Ethics approval and consent to participate

The study was approved by the Ethical Committee of the First Affiliated Hospital of Zhejiang University School of Medicine.

### Consent for publication

Not applicable.

### Availability of data and materials

The data that support the findings of this study are available from the First Affiliated Hospital, Zhejiang University School of Medicine but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the First Affiliated Hospital, Zhejiang University School of Medicine.

### Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

QQW and XYS collected and analyzed participant data, and completed the manuscript writing. JHW and JWZ did data collection and interpretation. CFX did the study design and implementation, manuscript drafting, and critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. QQW and XYS contributed equally to this study.

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