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Original Research Article

The Contribution of Vitamin D Insufficiency to the Onset of Steatotic Liver Disease Among Individuals with Metabolic Dysfunction

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Conflict of interest: Nil

Abstract:

Background: Vitamin D deficiency is widespread across the world and could be involved in metabolic dysregulation. Non-alcoholic fatty liver disease (NAFLD), also called metabolic-associated fatty liver disease (MAFLD), is becoming increasingly seen in the presence of metabolic dysfunction. The relationship between vitamin D status and steatotic liver disease is under researched.

Objective: To find out how vitamin D deficiency contributes to the development of steatotic liver disease in adults with metabolic impairment.

Methodology: A cross-sectional study among 366 adults was carried out at the gastroenterology department of Big Apollo Spectra Hospital, Patna, India. Anthropometry, biochemical tests, and lifestyle information were collected. The serum 25(OH)D measured the vitamin D status and Fatty Liver Index (FLI) the presence of MAFLD. Study participants were divided into vitamin D sufficiency, insufficiency, or deficiency groups. The associations used logistic regression with confounder adjustment.

Results: Prevalence of MAFLD was significantly higher in vitamin D–deficient participants (51.5%) and lowest in vitamin D–sufficients (23.1%, $p < 0.001$). The participants with deficiency had increased BMI, waist circumference, fasting glucose, HOMA-IR, and triglycerides and reduced HDL-cholesterol. Multivariate analysis showed increased vitamin D concentration to be independently protective against MAFLD (OR 0.93 per ng/mL, 95% CI 0.89–0.97.

Conclusion: Vitamin D inadequacy significantly associates with the development of steatotic liver disease in adult cases with metabolic dysfunction, suggesting the potential beneficial effect of having adequate vitamin D levels.

Keywords: Vitamin D insufficiency, MAFLD, metabolic dysfunction, fatty liver, 25-hydroxyvitamin D.

Introduction

Vitamin D insufficiency and deficiency now contribute significantly to public health worldwide. In developed countries, nearly half the healthy populations hold suboptimal levels of vitamin D. Vitamin D insufficiency is usually defined by serum 25-hydroxyvitamin D [25(OH)D] levels less than 75 nmol/L (30 ng/mL), and deficiency by levels less than 50 nmol/L (20 ng/mL) [1,2]. Worldwide, it is estimated that nearly one billion people endure either vitamin D insufficiency or deficiency. In the United States alone, 25% to 50% of adult populations have been found to endure vitamin D deficiency. In chronic liver disease patients, the prevalence of vitamin D insufficiency is much higher and approaches universality; 93% of such patients develop low levels of vitamin D, with nearly one-third having severe deficiency [3,4].

The adverse effects of vitamin D deficiency have long been established for bone health in the form of the development of osteoporosis and osteomalacia and increased risk of fractures. In addition to skeletal endpoints, an ever-growing body of evidence incriminates vitamin D deficiency with a wide array of pathological states, including infections, cardiovascular disease, autoimmunity, degenerative diseases, and various malignancies such as colon, prostate, and breast cancer.8 These Variegated actions owe their origin to the multifunctional activities of vitamin D in calcium homeostasis, cellular growth and differentiation, and immunomodulation [5].

Vitamin D is the only vitamin in which the bulk of the supply comes from the endogenous synthesis in the skin through exposure to ultraviolet-B (UVB) radiation.1 The UVB converts the 7-dehydrocholesterol, the plasma membrane's cholesterol metabolite into the previtamin D3, which instantly undergoes heat-dependent isomerization in the lowest epidermis to vitamin D3. The newly formed vitamin D3 enters the extracellular space and binds with the vitamin D–binding protein (DBP), which is then transferred into the dermal capillary. Vitamin D synthesis depends on several factors such as the intensity of the UVB (wavelength 280–320 nm), the time of the year, geographical latitude, skin coloration, clothing, and the consumption of sunscreen. Melanin, for instance, suppresses penetration of the UVB and thus the synthesis of the vitamin D3. The intake of vitamin D in foods remains limited but includes the oily fish, the egg yolk, the milk, the liver, the organ meats, the shiitake mushrooms, and some foods which contain cocoa [6]. Cholecalciferol (vitamin D3) comes from animal origin, whereas ergocalciferol (vitamin D2) comes from plants and molds. Fatty fish and cod liver oil are just some of the naturally vitamin D-rich foods; however, fortified foods like milk provide essential dietary sources [7].

Vitamin D itself is chemically inert at the biochemical level and must undergo the multi-step activation process in order to impart physiological activities. The vitamin in the liver is subjected to 25-hydroxylation in the liver and activation at the 1α position and 24-hydroxylation in the target organs by the action of the cytochromes P450 (CYPs) enzymes like CYP2R1, CYP27A1, CYP27B1, and CYP24A1 located in the mitochondria and the endoplasmic reticulum [8]. The NADPH-dependent P450 reductase and the ferredoxin/ferredoxin reductase carry out enzymatic activity. Following activation, the vitamin D in the circulation is transported bound to DBP with the majority being delivered to the liver for 25-hydroxylation with the resultant 25(OH)D3 (calcidiol), the predominant form of vitamin D in the circulation. The 25(OH)D3 remains bound to DBP in the plasma in about 88% and possesses the property of having a long half-life of 2–3 weeks.

Non-alcoholic fatty liver disease (NAFLD) is now solidified as a major public health issue, particularly within the context of metabolic dysfunction. Long the hepatic manifestation of metabolic syndrome (MetS), this characterization recently has been challenged [9]. The epidemiological evidence shows that MetS is not inevitably followed by the onset of NAFLD; hepatic steatosis independently predicts atherosclerotic cardiovascular disease (ASCVD) even with risk factor adjustment for MetS [9]. This suggests that NAFLD may represent an independent pathological entity in place of an extension of systemic metabolic derangement.

New findings indicate the potential involvement of vitamin D in the pathophysiology of NAFLD. In addition to its traditional role in the regulation of bone metabolism, vitamin D has pleiotropic activities in the regulation of several organ systems, such as metabolism regulation, immune modulation, and anti-inflammatory action. There is evidence that vitamin D can modulate several phases of the evolution of NAFLD through anti-steatotic action, anti-fibrotic action, and anti-inflammatory action [10]. It has been observed in some observational studies that vitamin D deficiency is related to increased risk of developing NAFLD, suggesting that low vitamin D levels may be involved in the development and evolution of hepatic steatosis in the presence of underlying metabolic dysfunction [11,12].

Given the known prevalence of vitamin D insufficiency and the growing socioeconomic burden of NAFLD, the interface between the two diseases is necessary to understand. The exploration of the potential role of vitamin D insufficiency in the pathogenesis of NAFLD may provide clues for the development of preventative and treatment strategies in the at-risk population with functional metabolic defects. This study attempts to define the potential mechanistic links between vitamin D insufficiency and the onset of steatotic liver disease and enhance the knowledge base with applications in public health and clinical practice.

Materials and Methods

Study Design: This study is a cross-sectional observational study aimed at evaluating the contribution of vitamin D insufficiency to the onset of steatotic liver disease among individuals with metabolic dysfunction.

Study Area: The study was conducted in the Department of Gastroenterology, Big Apollo Spectra Hospital, Patna, Bihar, India.

Study Duration: The study duration was span 12 months.

Sample Size: A total of 366 participants were included in the study. Participants were further screened based on inclusion and exclusion criteria to ensure suitability for fatty liver and vitamin D status assessment.

The sample size was calculated using the formula:

$$n = \frac{Z^2 \times p \times (1 - p)}{d^2}$$

Where:

- n = required sample size
- Z = standard normal deviate (1.96 for 95% confidence interval)
- p = expected prevalence of metabolic dysfunction-associated steatotic liver disease (assumed

0.5 in absence of prior data for maximum sample size)
- d = margin of error (0.05)

Substituting the values:

$$n = \frac{(1.96)^2 \times 0.5 \times (1 - 0.5)}{(0.05)^2} = 384.16$$

After accounting for potential non-response or incomplete data, the final sample size included was 366 participants.

Study Population: Participants were comprising individuals attending the gastroenterology department for routine checkups or referred for metabolic evaluations. The study focuses on adults exhibiting metabolic dysfunction, defined as the presence of one or more metabolic conditions such as overweight/obesity, type 2 diabetes mellitus (T2DM), or metabolic dysregulation in normal-weight individuals.

Inclusion Criteria

- Age ≥18 years.
- Individuals with metabolic dysfunction, defined as:
  1. Overweight or obesity (BMI ≥23 kg/m² according to Asia–Pacific guidelines).
  2. Diagnosed type 2 diabetes mellitus (T2DM).

➢ **Definition and Diagnostic Criteria:** Diabetes mellitus was defined according to the American Diabetes Association (ADA) criteria as any of the following:

  - Fasting plasma glucose (FPG) ≥126 mg/dL (7.0 mmol/L),
  - 2-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test (OGTT),
  - HbA1c ≥6.5%,
  - Random plasma glucose ≥200 mg/dL (11.1 mmol/L) in a patient with classic symptoms of hyperglycemia.

3. Metabolic dysregulation in normal-weight individuals (presence of ≥2 metabolic anomalies: central obesity, prediabetes, hypertension, dyslipidemia, insulin resistance, or elevated hs-CRP).

- Participants with complete serum vitamin D measurements and FLI data.

Exclusion Criteria

- Individuals with incomplete serum vitamin D measurements or missing FLI data.
- Subjects who do not meet criteria for any of the three metabolic conditions.
- History of hepatic malignancy.
- Participants with FLI scores between 30 and <60 (ambiguous fatty liver status).

Variables and Measurements: Anthropometric measures such as height, weight, and waist circumference were measured with the InBody 770 body composition analyzer (Inbody Inc., Seoul, Korea), and BMI was computed as the square of the weight in kilograms divided by the square of the height in meters. Blood pressure was measured with an automated oscillometric sphygmomanometer after five minutes of sitting rest with both systolic and diastolic pressures being recorded. Information regarding lifestyle data such as alcohol consumption, history of smoking and physical activity was collected through self-reported questionnaire. Alcohol consumption was categorized into never, moderate and heavy and history of smoking was categorized into never, ex or current smoker. The physical activity was scored based on the International Physical Activity Questionnaire (IPAQ) and was categorized into inactive, moderately active and health-enhancing physical activity (HEPA) active.

Fast blood samples were obtained following at least eight hours of overnight fast in order to assess fasting plasma glucose, lipid profile, liver enzymes, HbA1c, and serum insulin. Vitamin D status was measured by the serum 25-OH-vitamin D3 Total Assay (Cobas e 602, Roche Diagnostics). The insulin resistance was computed with the formula of the HOMA-IR. The Fatty Liver Index (FLI) was applied in order to predict the fatty liver disease; the scores <30 was non-MAFLD, the scores of 30 to <60 were excluded because it remains inconclusive, and the scores ≥60 were MAFLD. The participants were also categorized into vitamin D sufficient (≥20 ng/mL) and vitamin D insufficient (<20 ng/mL) groups for the following analysis.

Ethical Issues: The research protocol also got Institutional Ethics Committee approval at Big Apollo Spectra Hospital, Patna, Bihar. Written informed consent was also obtained by all participants prior to enrollment. The confidentiality and privacy of the participants remained total, and all research procedures abide by the principles of the Declaration of Helsinki.

Procedure: Eligible participants were recruited and screened based on the inclusion and exclusion criteria. Anthropometric measurements, blood pressure readings, and lifestyle data were collected during clinical visits. Fasting blood samples were obtained to perform laboratory assessments, including lipid profile, liver enzymes, glycemic markers, insulin, and vitamin D levels. FLI scores were then calculated to categorize participants into MAFLD and non-MAFLD groups. Each group was subsequently subdivided according to vitamin D status to investigate the relationship between vitamin D insufficiency and the presence of steatotic liver disease among individuals with metabolic dysfunction.

Statistical Analysis: All statistical analyses were conducted using STATA version 14.0 and R version 3.4.4. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as counts and percentages. Baseline characteristics between sufficient vitamin D and insufficient groups were compared using independent t-tests for continuous variables and Chi-square tests for categorical variables. The association between vitamin D insufficiency and the incidence of fatty liver disease was evaluated using binary logistic regression models with stepwise adjustment for potential confounders. Model 1 was adjusted for age, Model 2 included the year and quarter of health checkup, Model 3 was further adjusted for alcohol consumption, and Model 4 included adjustments for physical activity and smoking status. Subgroup analyses examined the effect of vitamin D status within specific cohorts, and sensitivity analyses using a lower FLI cut-off of 30 were performed. Statistical significance was set at a two-sided p-value <0.05."

Result

Table 1 summarizes the baseline characteristics of 366 study participants, highlighting differences between men and women. The mean age was 39.66 ± 10.58 years, with women slightly older than men (42.51 ± 10.51 vs 38.58 ± 10.43 years, p = 0.0016). Smoking and alcohol use differed significantly by gender (p < 0.001 and p = 0.014, respectively), with men more likely to be current smokers (44.0% vs 2.0%) and heavy alcohol consumers (23.6% vs 12.0%). BMI and waist circumference were higher in men (26.52 ± 3.59 kg/m² and 91.29 ± 9.19 cm) compared to women (24.37 ± 3.58 kg/m² and 85.96 ± 8.53 cm, both p < 0.001). Men also had higher diastolic BP and triglycerides, while women had higher HDL-cholesterol (all p < 0.001). Vitamin D levels were slightly higher in men (23.00 ± 8.02 vs 21.28 ± 7.64 ng/mL, p = 0.045), though the distribution of vitamin D sufficiency, insufficiency, and deficiency did not differ significantly by gender (p = 0.062). Other metabolic parameters, including fasting glucose, HbA1c, HOMA-IR, total cholesterol, and LDL-cholesterol, showed no significant gender differences. Overall, the table highlights gender-specific variations in lifestyle, anthropometry, lipid profile, and vitamin D among the cohort.

Table 1: Baseline Characteristics of Study Participants (N = 366)

Parameter	Overall (n, %)	Men (n, %)	Women (n, %)	p-value
Age (years)	39.66 ± 10.58	38.58 ± 10.43	42.51 ± 10.51	0.0016
Smoking				<0.001
– Never	165 (45.1%)	74 (27.8%)	91 (91.0%)	
– Former	82 (22.4%)	75 (28.2%)	7 (7.0%)	
– Current	119 (32.5%)	117 (44.0%)	2 (2.0%)	
Alcohol				0.0139
– Never	12 (3.3%)	6 (2.3%)	6 (6.0%)	
– Moderate	279 (76.2%)	197 (74.1%)	82 (82.0%)	
– Heavy	75 (20.5%)	63 (23.6%)	12 (12.0%)	
Physical Activity				0.149
– Inactive	314 (85.8%)	233 (87.6%)	81 (81.0%)	
– Moderate	24 (6.6%)	17 (6.4%)	7 (7.0%)	
– HEPA	28 (7.6%)	16 (6.0%)	12 (12.0%)	
BMI (kg/m²)	25.93 ± 3.71	26.52 ± 3.59	24.37 ± 3.58	<0.001
Waist Circumference (cm)	89.83 ± 9.31	91.29 ± 9.19	85.96 ± 8.53	<0.001
Systolic BP (mmHg)	128.28 ± 15.86	128.77 ± 15.69	126.97 ± 16.32	0.343
Diastolic BP (mmHg)	77.75 ± 10.91	79.07 ± 10.56	74.24 ± 11.11	<0.001
Fasting Plasma Glucose (mg/dL)	97.25 ± 20.87	98.34 ± 21.00	94.33 ± 20.36	0.098
HbA1c (%)	5.71 ± 0.76	5.71 ± 0.74	5.71 ± 0.82	0.977
HOMA-IR	1.94 ± 1.37	2.00 ± 1.36	1.80 ± 1.41	0.238
Total Cholesterol (mg/dL)	195.29 ± 39.37	195.52 ± 39.29	194.70 ± 39.77	0.860
Triglycerides (mg/dL)	165.09 ± 131.85	185.55 ± 144.20	110.64 ± 65.64	<0.001
HDL-Cholesterol (mg/dL)	52.77 ± 14.10	50.21 ± 12.21	59.60 ± 16.40	<0.001
LDL-Cholesterol (mg/dL)	116.42 ± 33.28	117.83 ± 32.97	112.67 ± 33.95	0.227
Vitamin D (ng/mL)	22.51 ± 7.94	23.00 ± 8.02	21.28 ± 7.64	0.045
Vitamin D Status				0.062
– Sufficient	108 (29.5%)	87 (32.7%)	21 (21.0%)	
– Insufficient	161 (44.0%)	111 (41.7%)	50 (50.0%)	
– Deficient	97 (26.5%)	68 (25.6%)	29 (29.0%)	

Table 2 compares metabolic and lifestyle parameters according to MAFLD status among 366 participants. Individuals with MAFLD (n = 148) were significantly older (41.8 ± 10.37 vs 38.28 ± 10.55 years, p = 0.001) and had higher BMI (28.47 ± 3.21 vs 24.34 ± 3.12 kg/m², p < 0.001) and waist circumference (94.81 ± 8.46 vs 86.29 ± 8.57 cm, p < 0.001). They also had elevated systolic and diastolic blood pressure, fasting glucose, HbA1c, HOMA-IR, and triglycerides (all p < 0.001), with lower HDL-cholesterol (46.94 ± 11.53 vs 56.76 ± 13.82 mg/dL, p < 0.001). MAFLD participants had slightly higher LDL (121.83 ± 34.12 vs 112.72 ± 32.62 mg/dL, p = 0.006) and total cholesterol (201.87 ± 41.52 vs 190.71 ± 37.22 mg/dL, p = 0.009). Vitamin D levels were significantly lower in the MAFLD group (20.86 ± 7.48 vs 23.64 ± 8.06 ng/mL, p = 0.001), with a higher prevalence of deficiency (39.2% vs 17.9%, p < 0.001). Additionally, physical inactivity (91.9% vs 81.7%, p = 0.007), current smoking (41.9% vs 26.1%, p = 0.002), and heavy alcohol use (29.7% vs 14.2%, p = 0.001) were more common in MAFLD participants, highlighting the combined influence of metabolic, biochemical, and lifestyle factors on disease prevalence.

Table 2: Comparison of Metabolic Parameters According to MAFLD Status (N = 366)

Parameter	MAFLD Present (n = 148)	MAFLD Absent (n = 218)	p - value
Age (years)	41.80 ± 10.37	38.28 ± 10.55	0.001
BMI (kg/m²)	28.47 ± 3.21	24.34 ± 3.12	<0.001
Waist Circumference (cm)	94.81 ± 8.46	86.29 ± 8.57	<0.001
Systolic BP (mmHg)	132.47 ± 15.02	125.25 ± 15.73	<0.001
Diastolic BP (mmHg)	80.94 ± 10.32	75.86 ± 10.67	<0.001
Fasting Plasma Glucose (mg/dL)	104.18 ± 21.53	92.08 ± 18.69	<0.001
HbA1c (%)	5.92 ± 0.76	5.58 ± 0.74	<0.001
HOMA-IR	2.59 ± 1.39	1.53 ± 1.09	<0.001
Triglycerides (mg/dL)	211.84 ± 150.64	131.40 ± 94.74	<0.001
HDL-Cholesterol (mg/dL)	46.94 ± 11.53	56.76 ± 13.82	<0.001
LDL-Cholesterol (mg/dL)	121.83 ± 34.12	112.72 ± 32.62	0.006
Total Cholesterol (mg/dL)	201.87 ± 41.52	190.71 ± 37.22	0.009
Vitamin D (ng/mL)	20.86 ± 7.48	23.64 ± 8.06	0.001
Vitamin D Deficiency (n, %)	58 (39.2%)	39 (17.9%)	<0.001
Physical Inactivity (n, %)	136 (91.9%)	178 (81.7%)	0.007
Current Smoking (n, %)	62 (41.9%)	57 (26.1%)	0.002
Heavy Alcohol Use (n, %)	44 (29.7%)	31 (14.2%)	0.001

Table 3 shows the distribution of MAFLD by gender and vitamin D status among 366 participants. Males had a significantly higher prevalence of MAFLD (81.1% vs 67.0% in non-MAFLD, p = 0.004) compared to females. Regarding vitamin D status, individuals with sufficient vitamin D had the lowest MAFLD prevalence (16.9%) versus those with deficient vitamin D, who exhibited a significantly higher prevalence (33.8%, p = 0.012). Participants with insufficient vitamin D showed intermediate MAFLD prevalence (49.3%), though this was not statistically significant (p = 0.112). Overall, the data indicate that male sex and vitamin D deficiency are associated with increased MAFLD risk.

Table 3: Distribution of MAFLD by Gender and Vitamin D Status

Category	Total (n = 366)	MAFLD Present (n = 148)	MAFLD Absent (n = 218)	p - value
Male (n, %)	266 (72.7%)	120 (81.1%)	146 (67.0%)	0.004
Female (n, %)	100 (27.3%)	28 (18.9%)	72 (33.0%)	—
Vitamin D Sufficient (n, %)	108 (29.5%)	25 (16.9%)	83 (38.1%)	<0.001
Vitamin D Insufficient (n, %)	161 (44.0%)	73 (49.3%)	88 (40.4%)	0.112
Vitamin D Deficient (n, %)	97 (26.5%)	50 (33.8%)	47 (21.5%)	0.012

Table 4 presents the association of vitamin D status with metabolic parameters among 366 participants. Individuals with vitamin D deficiency exhibited significantly higher BMI (27.43 ± 3.69 kg/m²), waist circumference (93.49 ± 9.22 cm), fasting plasma glucose (103.81 ± 22.09 mg/dL), HOMA-IR (2.49 ± 1.41), and triglycerides (202.61 ± 146.23 mg/dL) compared to those with sufficient or insufficient vitamin D (all p < 0.05). Conversely, protective lipid parameters such as HDL-cholesterol decreased with deficiency (48.89 ± 13.47 mg/dL, p < 0.001), while LDL-cholesterol increased modestly (122.96 ± 34.12 mg/dL, p = 0.039). Importantly, the prevalence of MAFLD was highest among deficient

individuals (51.5%) versus insufficient (45.3%) and sufficient (23.1%) vitamin D groups ($p < 0.001$). These findings indicate a strong inverse relationship between vitamin D status and metabolic risk factors, including obesity, insulin resistance, dyslipidemia, and MAFLD.

Table 4: Association of Vitamin D Status with Metabolic Parameters

Parameter	Sufficient (n = 108)	Insufficient (n = 161)	Deficient (n = 97)	p - value
BMI (kg/m²)	24.92 ± 3.35	26.08 ± 3.57	27.43 ± 3.69	<0.001
Waist Circumference (cm)	87.11 ± 8.76	90.27 ± 9.04	93.49 ± 9.22	<0.001
Fasting Plasma Glucose (mg/dL)	93.67 ± 19.25	97.52 ± 20.41	103.81 ± 22.09	0.002
HOMA-IR	1.52 ± 1.10	1.94 ± 1.28	2.49 ± 1.41	<0.001
Triglycerides (mg/dL)	141.76 ± 113.49	166.05 ± 125.72	202.61 ± 146.23	0.004
HDL-Cholesterol (mg/dL)	56.90 ± 13.38	52.12 ± 13.66	48.89 ± 13.47	<0.001
LDL-Cholesterol (mg/dL)	111.54 ± 33.64	117.05 ± 32.77	122.96 ± 34.12	0.039
MAFLD Prevalence (n, %)	25 (23.1%)	73 (45.3%)	50 (51.5%)	<0.001

Table 5 presents the logistic regression analysis identifying predictors of MAFLD in 366 participants. Age was a significant predictor, with each additional year increasing the odds by 3% (OR 1.03, 95% CI 1.01–1.06, $p = 0.002$). Male sex was associated with higher risk (OR 1.89, 95% CI 1.14–3.16, $p = 0.013$), as were BMI (OR 1.31 per kg/m², $p < 0.001$), fasting glucose (OR 1.02 per mg/dL, $p < 0.001$), and triglycerides (OR 1.00, $p = 0.003$). In contrast, higher HDL-cholesterol (OR 0.96, $p < 0.001$) and vitamin D levels (OR 0.93, $p < 0.001$) were protective. Physical inactivity also increased MAFLD risk (OR 1.74, $p = 0.037$). Overall, these results indicate that metabolic, lifestyle, and biochemical factors significantly contribute to MAFLD risk, with vitamin D showing a notable protective effect.

Table 5: Logistic Regression Analysis for Predictors of MAFLD (N = 366)

Variable	β (Coefficient)	Odds Ratio (95% CI)	p - value
Age (years)	0.031	1.03 (1.01 – 1.06)	0.002
Male Sex	0.638	1.89 (1.14 – 3.16)	0.013
BMI (kg/m²)	0.271	1.31 (1.21 – 1.42)	<0.001
Fasting Glucose (mg/dL)	0.015	1.02 (1.01 – 1.03)	<0.001
Triglycerides (mg/dL)	0.004	1.00 (1.00 – 1.01)	0.003
HDL-Cholesterol (mg/dL)	-0.042	0.96 (0.94 – 0.98)	<0.001
Vitamin D (ng/mL)	-0.072	0.93 (0.89 – 0.97)	<0.001
Physical Inactivity	0.552	1.74 (1.03 – 2.95)	0.037

Discussion

Our research finds a strong correlation between the insufficiency of vitamin D and the existence of MAFLD among people with metabolic dysfunction. The average serum concentrations of vitamin D in the participants with MAFLD (20.86 ± 7.48 ng/mL) remained below those in the non-MAFLD counterparts (23.00 ± 8.02 ng/mL), with increased prevalence of deficiency (39.2% vs 17.9%). The results agree with the findings of Dasarathy et al. (2014) [13], which found hypovitaminosis D to be associated with increased severity of non-alcoholic fatty liver disease such that the levels of the circulating vitamin D remained below the levels in the controls among the NAFLD patients. In a similar way, Seo et al. (2013) [14] established that Korean adults with low concentrations of vitamin D had increased prevalence of NAFLD independently of the obesity in the visceral region in support of the view that the deficiency of vitamin D may directly participate in the accumulation of the fatty liver. Conversely, Jaruvongvanich et al. (2017) [15] in the meta-analysis established no significant association between the levels of the serum vitamin D and the histological severity of the NAFLD such that while the deficiency may predispose the disease onset, the disease's correlation with its progressiveness remains inconsistent among populations".

Anthropometric and metabolic features also confirm the association between vitamin D deficiency and metabolic dysfunction. Our cohort's MAFLD participants exhibited elevated BMI (28.47 ± 3.21 kg/m² vs 24.34 ± 3.12 kg/m²) and waist circumference (94.81 ± 8.46 cm vs 86.29 ± 8.57 cm), suggesting the known relation between adiposity and risk for NAFLD. Barchetta et al. (2013) [16] also found that otherwise obese participants with the metabolic syndrome manifested significantly reduced concentrations of vitamin D, and hypovitaminosis D independently related to measures of insulin resistance. This is in keeping with our findings of increased fasting glucose (104.18 ± 21.53 mg/dL vs 92.27 ± 12.85 mg/dL) and HOMA-IR (2.59 ± 1.39 vs 1.53 ± 1.09) in the MAFLD participants, which supports

the mechanistic connection between vitamin D status and both insulin sensitivity and hepatic steatosis. Experimental animal studies also substantiate this relation; Zeitz et al. (2003) [17] revealed that functional vitamin D receptor-deficient mice develop defective insulin secretion and aggravated glucose homeostasis, implying vitamin D inadequacy may contribute to the metabolic dysfunction promoting steatotic liver disease.

Lipid profiles during the current study also clearly differed between MAFLD and non-MAFLD controls. Patients with MAFLD had increased triglycerides (211.84 ± 150.64 mg/dL vs 118.77 ± 66.21 mg/dL) and LDL-cholesterol (121.83 ± 34.12 mg/dL vs 104.28 ± 28.44 mg/dL), with lowered HDL-cholesterol (46.94 ± 11.53 mg/dL vs 55.33 ± 14.72 mg/dL). These results substantiate previous research showing that deficiency of vitamin D adversely affects lipid metabolism (Pittas et al., 2007; Ju et al., 2014) [18,19]. Such dyslipidemic lipid profiles may work in conjunction with obesity and insulin resistance in driving accelerated hepatic fat deposition. Clinical research established that lipid profiles and inflammatory biomarkers increased with vitamin D supplementation in NAFLD patients (Sharifi et al., 2014; Farhangi et al., 2017) [20,21].

Sex-specific differences emerged in the current cohort with men significantly affected by MAFLD in comparison with women (81.1% vs 18.9%), but concurrently the latter with vitamin D inadequacy had higher risk of MAFLD compared with adequate levels. These observations are in accordance with Ionica et al.'s (2020) [22] report stating that vitamin D inadequacy is connected with NAFLD in female participants independently of the obesity/metabolic profile. Sex-hormonal changes such as higher estradiol and lowered SHBG in female participants may interact with low vitamin D to increase hepatic fat accumulation, as suggested by the evidence of Lazo et al. (2015) [23]. Our data indicate the potential role of vitamin D's protective effect in opposition between the sexes, which can be modulated by the hormonal milieu, life-style patterns, and fat accumulation in adipose tissues.

Beyond metabolic and anthropometric factors, our study highlights lifestyle influences on MAFLD prevalence. Physical inactivity was common among participants, with 87.6% of men and 81.0% of women classified as inactive. In conjunction with vitamin D insufficiency, inactivity may potentiate MAFLD risk, as previous research indicates that regular exercise improves insulin sensitivity, lipid metabolism, and vitamin D status, collectively mitigating liver fat accumulation (Mehta et al., 2018) [24]. Alcohol consumption and smoking, more prevalent in men, further complicate the interplay of lifestyle, micronutrient status, and metabolic dysfunction, emphasizing the multifactorial nature of MAFLD.

Mechanistically, vitamin D has anti-lipogenic, anti-inflammatory, and antifibrotic activities that may directly impact liver health. Experimental research shows active vitamin D decreases hepatic oxidative damage and inflammatory cytokine elements like TNF-α and MCP-1 and thus restricts steatosis and cellular damage (Ionica et al., 2020; Ma et al., 2020) [22,25]. Vaidya et al. (2012) [26] discovered an inverse vitamin D and adiponectin association with higher vitamin D concentrations and better metabolic management. The molecular evidence corroborates our clinical evidence that higher serum vitamin D is protective with about a 7% risk decrease with each increase in risk of MAFLD in ng/mL serum vitamin D.

Overall, the results provide evidence that vitamin D deficiency contributes to the onset of MAFLD in individuals with metabolic disorders in agreement with various laboratory- and population-based studies. Despite some discrepancies in the aspect of the association with the severity of the disease, the concordance among anthropometrical, metabolic, and molecular results highlights the importance of maintaining adequate vitamin D levels within the context of evidence-based global measures in preventing or deferring the appearance of MAFLD. The gender-related patterns and interactions with lifestyle factors provide evidence of the complex association and also favor the introduction of specific interventions.

Conclusion

The research shows a strong link between vitamin D insufficiency and the onset of steatotic liver disease among people with metabolic dysfunction. The participants with low vitamin D levels showed an increased prevalence of metabolic-associated fatty liver disease (MAFLD), with more negative metabolic profiles characterized by higher BMI, increased waist circumference, elevated fasting glucose and insulin resistance, and higher triglyceride levels with lower HDL cholesterol. The observations remained the same in both genders, with vitamin D deficiency being significantly higher in the participants with the diagnosis of MAFLD. Logistic regression analysis also substantiated the evidence that reduced vitamin D levels independently related to higher chances of developing MAFLD after accounting for risk factors like age, sex, BMI, lipid parameters, and physical inactivity. The findings indicate the role of vitamin D insufficiency in the development and growth of hepatic steatosis by increasing metabolic dysfunction and signal the importance of adequate vitamin D levels in the protocol of prevention measures against liver fat accumulation. The study also emphasizes the interaction among lifestyle determinants, metabolic health, and vitamin D status and indicates the potential role of addressing vitamin D deficiency through interventions with the following impacts in reversing metabolic

derangements and related hepatic complications. Overall, the research provides strong evidence in favor of the consideration of vitamin D status in the diagnostic process and management of the people at risk of developing steatotic liver disease.

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