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RESEARCH ARTICLE

Association and interaction between vitamin D level and metabolic syndrome for non-alcoholic fatty liver disease

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Abstract

Introduction/Objectives Previous studies have shown conflicting results regarding the association between hypovitaminosis D and non-alcoholic fatty liver disease (NAFLD). The aim of this study is to explore the individual and combined effect of hypovitaminosis D and metabolic syndrome (MS) on NAFLD.

Materials and methods In this cross-sectional study, 874 subjects were enrolled. 25(OH)D was assessed by a sequential competitive immuno-fluoro-assay method. The Fatty Liver Index (FLI) was used for NAFLD screening. Binary logistic regression and additive interaction were performed to investigate the association between vitamin D status, MS and NAFLD.

Results Severe vitamin D deficiency was found to be positively related to NAFLD, with a higher risk in women than in men (OR = 6.4, 95% CI [2.8-15], $p < 0.0001$ vs. OR = 5.8, 95% CI [1.9-17.7], $p = 0.002$). In men, this association was partially masked by obesity. The additive interaction with MS was significant in women but not in men, the relative excess risk due to interaction was of 7.2, 95% CI [1.3-12.9], $p = 0.02$), the attributable proportion due to the combined effect was of 0.6, 95% CI [0.4-0.8], $p < 0.0001$. The interaction mechanism is synergistic; the synergy index: was of 2.9, 95% CI [1.6-5.3], $p = 0.0006$.

Conclusion A positive association has been found between severe vitamin D deficiency and NAFLD. Moreover, an excess risk in women combining both MS and severe vitamin D deficiency was quantified.

Keywords 25(OH)D · Additive interaction · Fatty liver index · Metabolic syndrome · Non-alcoholic fatty liver disease · Obesity

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a clinical-anatomical disorder characterized by an ectopic fat deposition in the liver of more than 5% of the organ weight [1, 2]. It covers a wide spectrum of liver diseases of varying severity, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), which is highly linked to a significant risk of progression to cirrhosis and hepatocellular carcinoma [3, 4].

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Classically, NAFLD is considered as the hepatic component of the metabolic syndrome (MS) [5, 6], and is closely linked to obesity, particularly abdominal obesity, dyslipidemia, type 2 diabetes (T2DM) and insulin resistance [7–9]. Its prevalence is rising worldwide, especially in Western countries where it affects 20 to 30% of adult subjects. [10–12].

In parallel, the prevalence of hypovitaminosis D had reached a pandemic level. Currently, it has become a worldwide concern, especially with the recognition of its involvement in many pathophysiological processes, beyond its classical role in phosphocalcic metabolism and bone health. Indeed, the finding of the ubiquitous nature of the vitamin D receptor and its activating enzymes has revealed its immunomodulatory, anti-inflammatory, and anti-fibrotic properties and thus unveiled its potential link with cardio-metabolic, inflammatory, and even cancerous pathologies [1, 4, 13].

Recently, research from several ethnic groups has raised the possibility of an association between hypovitaminosis D and NAFLD pathogenesis [14, 15], but this hypothesis is

still a matter of debate [16, 17]. On the other side, an inverse correlation between vitamin D status and MS components is now well accepted [10, 18]. Yet, it remains unclear whether MS represents only the linking mechanism between hypovitaminosis D and the occurrence of NAFLD or whether there is a synergistic interaction between these two conditions. Hence, this study was performed in order to investigate the possible association between hypovitaminosis D and NAFLD and to explore the interaction, on an additive scale, between MS and hypovitaminosis D on NaFLD, for men and women separately.

Materials and methods

Participants and study design

This is a cross-sectional single-center study. The participants were voluntary subjects recruited between January 2019 and January 2020, at the medical laboratory of the Frantz-Fanon Hospital, Blida University Hospital, Algeria. Non-inclusion criteria were: age < 18 years, pregnancy, malignancy, end-stage renal disease, primary hyperparathyroidism, history of viral or metabolic hepatitis (hemochromatosis, Wilson...), alcohol consumption, use of known hepatotoxic drugs, and vitamin D supplementation within three months before study enrollment. This study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. All participants provided informed consent prior to enrollment.

The sample size was calculated using the following standard equation: $n = t^2 \times p \times (1\text{-}p) / m^2$. With: t: the confidence level, p: the theoretical NAFLD prevalence and m: the error margin.

Based on data from Western countries, an approximate prevalence of 30% was used to calculate the sample size. Considering an error margin of 5%, and a confidence level of 95%, the estimated sample size was 323. Given the lack of epidemiological data on NAFLD prevalence in Algerian population, the sample size was extended in order to reach a confidence level of 99.99%. The final sample size was thus 874 subjects.

All participants were tested for the following biological parameters: fasting blood glucose (FBG) and standard lipid profile including total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDLc); assessed by a colorimetric enzymatic method. Liver enzymes including: glutamo-oxaloacetic transaminase (GOT) and glutamo-pyruvic transaminase (GPT) were measured by a kinetic method at 340 nm, γ-glutamyl-transpeptidase (γGT) and alkaline phosphatases (ALP) were measured by a colorimetric enzymatic kinetic method.

Anthropometric data including waist circumference (WC), weight, height, and body mass index (BMI) were measured according to the World Health Organization recommendations (WHO. 1995). Obesity was defined by a BMI > 30 kg/m^2 [19]. Physical activity was classified as: mild: <30 min/D, moderate: 30-60 min/D, and intense: >60 min/D.

MS evaluation

The MS was defined in accordance with the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines [20] by at least three of the following criteria: 1) SBP and/or DBP > 130/85 mmHg, or use of antihypertensive drugs, 2) FBG > 6.05 mmol/l (1.1 g/l), or use of anti-diabetic drugs, 3) TG > 1.69 mmol/l (1.5 g/l), 4) HDLc <1.29 mmol/l (0.5 g/l) for women and 1.03 mmol/l (0.4 g/l) for men, or use of hypolipemic drugs, 5) WC >88 cm for women and 102 cm for men.

Vitamin D status evaluation

Vitamin D status was evaluated by assessing the circulating level of its most stable form; 25-hydroxy-vitamin D (25(OH) D), using a sequential competitive immuno-fluoro-assay method by VIDAS®. In accordance with the 2011 Endocrine Society Clinical Practice Guidelines, subjects were classified based on their 25(OH) D levels into one of the following categories: severely deficient: if 25(OH) D < 25 nmol/l (<10 µg/l), deficient: if 25(OH) D is between 25 and 50 nmol/l (10-20 µg/l), moderate insufficiency: if 25(OH) D is between 50 and 75 µg/l. The level of 25(OH) D was considered sufficient if 25(OH) D > 75 nmol/l (20 µg/l) [17, 21–23].

NAFLD evaluation

NAFLD was evaluated using the Fatty Liver Index (FLI), a non-invasive method of hepatic steatosis estimation, calculated according to the following formula:

$$FLI = \left(e^{0.953*\log_e(TG)+0.139*BMI+0.718*\log_e(\gamma GT)+0.053*WC-15.745}\right) / \left(1+e^{0.953*\log_e(TG)+0.139*BMI+0.718*\log_e(\gamma GT)+0.053*WC-15.745}\right) \times 100$$

The FLI is expressed as a number ranging from 0 to 100, a value of >60 points to hepatic steatosis with a sensitivity and specificity of 87% and 86% respectively [10, 24].

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of continuous variable distribution. The continuous variables are presented as Means ± Standard Deviations and compared

using the Student's t test. Qualitative variables are reported as percentages and compared using Pearson's $\chi 2$ test.

Binary logistic regression was used to assess the association between vitamin D status and NAFLD. Two models were created by fitting progressively for the following confounding co-variables: age, physical activity, obesity, sampling season, metabolic factors (T2D, hypertension, TG and HDLc), cardiovascular history, and hypothyroidism. All statistical analyses were conducted separately for men and woman. In addition, secondary analyses of subgroups were performed, stratifying by obesity and MS.

The interactive effect between MS and vitamin D status on NAFLD was tested on an additive scale. The following three indices with their 95% confidence intervals (95% CIs) were calculated: RERI (the relative excess risk due to interaction), AP (the attributable proportion due to interaction) and SI (the synergy index). To be significant, the 95% CI of RERI and AP should not include '0', and the 95% CI of SI should not include '1' [25].

To assess the additive interaction, three exposure categories were defined based on the exposure to either risk factor: Category 1: subjects with MS but without vitamin D deficiency, Category 2: subjects without MS but with vitamin D deficiency, Category 3: subjects with MS and with vitamin D deficiency. The 4th category (without MS and without vitamin D deficiency) was used as a reference. These 4 categories were then integrated into a logistic regression model in order to derive the regression coefficients (and not the Odd's ratio) as well as the covariance matrix; required to calculate the three indices and their confidence intervals respectively. The calculation of the additive interaction was performed using an Excel sheet, available on the website" www .epine t.se" [25].

Statistical analysis was performed using the SPSS 25.0 software. For all parameters, a bilateral p value of less than 0.05 was considered as statically significant.

Results

Bio-clinical characteristics of the study population

Bio-clinical characteristics of the total population stratified by gender and by NAFLD diagnostic are shown in Tables 1 and 2. In the overall population, NAFLD was found in 36.3% of subjects without any gender difference (37% vs. 35.8%, $p = 0.7$ for men and women respectively). Compared to non-NAFLD subjects, NAFLD subjects were significantly older (51.2 ± 12.6 years vs. 46.3 ± 14.8 years, $p < 0.0001$), had significantly higher levels of FBG, SBP,

Table 1 Clinical and demographic characteristics of study participants stratified by gender and by NAFLD status

	NAFLD				Non NAFLD				P
	Male $n=112$ (37%)	Female $n=205$ (35.8%)	p	Total $n=317$ (36.3%)	Male $n=190$ (62.9%)	Female $n=367$ (64.1%)	p	Total $n=557$ (63.7%)	0.7
Type 2 Diabetes (%)	33 (29.5)	38 (18.5)	**0.01**	71 (22.4)	29 (15.3)	30 (8.2)	**0.026**	59 (10.6)	**<0.0001**
Hypertension (%)	68 (60.7)	111 (54.1)	0.26	179 (56.5)	69 (36.3)	105 (28.6)	**0.06**	174 (31.2)	**<0.0001**
Hypothyroidism (%)	00	17 (8.3)	**0.001**	17 (5.4)	00	17 (4.6)	**0.001**	17 (3.1)	0.08
HCVD (%)	13 (11.6)	12 (5.9)	0.11	25 (7.9)	26 (13.7)	13 (3.5)	**<0.0001**	39 (7)	0.6
MS (%)	87 (77.7)	153 (74.6)	0.5	240 (75.7)	43 (22.6)	124 (33.8)	**0.006**	167 (30)	**<0.0001**
Abdominal obesity (%)	84 (57)	200 (97.6)	**<0.0001**	284 (89.5)	23 (12.1)	233 (63.5)	**<0.0001**	256 (46)	**<0.0001**
Physical activity (%)							**<0.0001**		
<30mn (%)	44 (39.3)	129 (62.9)	**<0.0001**	173 (54.6)	52 (27.4)	193 (52.6)	**<0.0001**	245 (44)	
30-60mn (%)	40 (35.7)	42 9 (23.9)		89 (28.1)	60 (31.6)	104 (28.3)		164 (29.4)	
>60mn (%)	28 (25)	27 (13.2)		55 (17.4)	78 (41.1)	70 (19.1)		148 (26.6)	
BMI (%)							**<0.0001**		
<25 kg/m^2	6 (5.4)	5 (2.4)	**<0.0001**	11 (3.5)	103 (54.2)	196 (53.4)	0.4	299 (53.7)	
25-30 kg/m^2	56 (50)	62 (30.2)		118 (37.2)	78 (41.1)	143 (39)		221 (39.7)	
>30 kg/m^2	50 (44.6)	138 (67.3)		188 (59.3)	9 (4.7)	28 (7.6)		37 (6.8)	
25(OH)D (%)							**<0.0001**		
>20 µg/l	26 (23.2)	16 (7.8)	**<0.0001**	42 (13.2)	78 (41.1)	70 (19.1)	**<0.0001**	148 (26.6)	
10-20 µg/l	41 (36.6)	45 (22)		86 (27.1)	70 (36.8)	75 (20.4)		145 (26)	
<10 µg/l	45 (40.2)	144 (70.2)		189 (59.6)	42 (22.1)	222 (60.5)		264 (47.4)	

25(OH)D 25-hydroxy vitamin, *BMI* body mass index, *HCVD* history of cardiovascular disease, *MS* metabolic syndrome, *NAFLD* Nonalcoholic fatty liver disease. p: Pearson's $\chi 2$ test. Bold values indicate significant differences ($p < 0.05$)

Table 2 Clinical and biological characteristics of study participants stratified by gender and by NAFLD status

	NAFLD				Non NAFLD				P
	Male n = 112 (35.3%)	Female n = 205 (65.7%)	p	Total n = 317 (36.3%)	Male n = 190 (34.1%)	Female n = 367 (65.9%)	P	Total n = 557 (63.7%)	0.7
Age (years)	52.8 ± 12.7	50.3 ± 12.5	0.08	51.2 ± 12.6	51 ± 14.8	44 ± 14.3	**<0.0001**	46.3 ± 14.8	**<0.0001**
25(OH)D (µg/l)	13.6 ± 7	9.4 ± 6.8	**<0.0001**	10.9 ± 7.2	17.8 ± 8.6	12.4 ± 11.4	**<0.0001**	14.3 ± 10.8	**<0.0001**
FLI	79.8 ± 13.3	77.6 ± 14.7	0.18	78.4 ± 14.2	34.9 ± 17.8	27 ± 18.3	**<0.0001**	29.7 ± 18.5	**<0.0001**
WC (cm)	108.7 ± 9.6	108.8 ± 11.1	0.9	108.8 ± 10.6	93.5 ± 7.4	91.7 ± 8.8	**0.008**	92.3 ± 8.4	**<0.0001**
SBP (mmHg)	141.7 ± 21.8	136 ± 23.7	**0.036**	138.1 ± 23.2	132.9 ± 19.2	124.7 ± 20.3	**<0.0001**	127.5 ± 20.3	**<0.0001**
DBP (mmHg)	85.1 ± 12.5	82.9 ± 13.7	0.16	83.7 ± 13.3	79.4 ± 11.2	75.4 ± 11.6	**<0.0001**	76.8 ± 11.6	**<0.0001**
FBG (mmol/l)	7.0 ± 2.8	6.2 ± 2.2	**0.019**	6.5 ± 2.6	5.7 ± 2.2	5.2 ± 1.7	**0.001**	5.4 ± 1.8	**<0.0001**
TC (mmol/l)	4.6 ± 1.03	4.8 ± 1.03	0.08	4.7 ± 1.06	4.3 ± 1.03	4.3 ± 0.88	0.9	4.3 ± 0.93	**<0.0001**
TG (mmol/l)	2.47 ± 1.46	1.92 ± 1.01	**<0.0001**	2.1 ± 1.27	1.35 ± 0.56	1.09 ± 0.56	**<0.0001**	1.18 ± 0.58	**<0.0001**
HDLc (mmol/l)	1.01 ± 0.23	1.14 ± 0.26	**0.001**	1.08 ± 0.26	1.03 ± 0.18	1.19 ± 0.23	**<0.0001**	1.14 ± 0.23	**0.02**
GOT (IU/l)	29.2 ± 12	27.9 ± 11.9	0.3	28.4 ± 11.9	24.4 ± 7.8	22.7 ± 8.2	**0.017**	23.3 ± 8.1	**<0.0001**
GPT (IU/l)	25.7 ± 16.5	20.9 ± 13.3	**0.006**	22.6 ± 14.7	15.1 ± 7.8	12.6 ± 6.3	**<0.0001**	13.5 ± 6.9	**<0.0001**
γGT (IU/l)	35.5 ± 23.3	31.2 ± 36	0.25	32.7 ± 32.1	20.7 ± 8.9	15.9 ± 8.8	**<0.0001**	17.5 ± 9.1	**<0.0001**
ALP (IU/l)	154.5 ± 76	144.6 ± 63.5	0.21	148.1 ± 68	134.2 ± 48.6	129 ± 59.5	0.3	130.8 ± 56	**<0.0001**
BMI (Kg/m^2)	30 ± 4.3	32.1 ± 4.5	**<0.0001**	31.5 ± 4.6	24.7 ± 3.5	24.7 ± 3.8	0.9	24.7 ± 3.7	**<0.0001**
RatioGOT/GPT	0.91 ± 0.5	0.79 ± 0.5	0.05	0.84 ± 0.5	0.65 ± 0.3	0.6 ± 0.3	**0.013**	0.61 ± 0.3	**<0.0001**

25(OH)D 25-hydroxy vitamin, *ALP* alkaline phosphatases, *BMI* body mass index, *DBP* diastolic blood pressure, *FBG* fasting blood glucose, *FLI* fatty liver index, *GOT* glutamo-oxaloacetic transaminase, *GPT* glutamo-pyruvic transaminase, *γ-GT* gamma-Glutamyl-Trans-peptidase, *HDLc* high-density lipoprotein cholesterol, *NAFLD* Nonalcoholic fatty liver disease, *SBP* systolic blood pressure, *TC* total cholesterol, *TG* triglycerides, *WC* waist circumference. p: Student's t test. Bold values indicate significant differences ($p < 0.05$)

DBP, TG, TC, WC and liver enzymes (GOT, GPT, γGT, ALP) as well as lower levels of HDLc ($p = 0.02$) and 25(OH)D (10.9 ± 7.2 µg/l vs. 14.3 ± 10.8 µg/l, $p < 0.0001$) (Table 2). In addition, the rates of obesity, MS and physical inactivity were significantly higher in the NAFLD group.

When stratified by gender, women with NAFLD, compared to men with NAFLD, had significantly lower levels of 25(OH) D (9.4 ± 6.8 µg/l vs. 13.6 ± 7 µg/l, $p < 0.0001$), lower levels of FBG, TG, SBP, GPT, and higher levels of HDLc. In addition, women with NAFLD had also higher rates of sedentary (62.9% vs. 39.3%, $p < 0.0001$) and obesity (67.3% vs. 44.6%, $p < 0.0001$) (Table 2).

Vitamin D status in NAFLD and non-NAFLD subjects stratified by gender

The distribution of 25(OH) D categories in whole sample and according to gender is shown in Table 1. Severe vitamin D deficiency was more frequent in the NAFLD group (59.6% vs. 47.4%), and it was more frequent among women than men (70.2% vs. 42.2%, $p < 0.0001$). However, both NAFLD and non-NAFLD patients show a comparable percentage of vitamin D deficiency (27.1% vs. 26% respectively).

Prevalence of NAFLD by vitamin D classes stratified by gender, obesity and MS

To better describe the relationship between vitamin D status and NAFLD, further stratification was made by gender and MS, then by gender and obesity (Table 3).

When stratified by MS, the linear by linear association persists only in the MS group for both men and women ($p < 0.0001$ respectively) (Table 3). The highest prevalence was found in the MS groups with severe vitamin D deficiency, suggesting a possible additive interaction. Similarly, when stratified by obesity, the linear by linear association persists only in obese women ($p = 0.07$) and non-obese men ($p = 0.35$) (Table 3).

Multivariate analysis of the association between vitamin D status and NAFLD

The results of the binary logistic regression are presented in Table 3. In the overall population, after adjusting for age, obesity, physical activity and blood sampling season, vitamin D deficiency and severe deficiency were positively related to NAFLD in both men and women. After adjustment for the MS components, cardiovascular history and hypothyroidism, only severe vitamin D deficiency remains positively associated with NAFLD, with a slightly higher risk in

Table 3 Binary logistic regression of the association between serum 25(OH)D and NAFLD status, stratified by gender, BMI and MS [OR (95% CI)]

				Model 1		Model 2	
25(OH)D			%	OR 95% CI	p	OR 95% CI	p
Total	**M**	>20	25.0	1	–	1	–
		10-20	35.1	2.2 [1.04-4.8]	**0.039**	1.2 [0.5-3.2]	0.6
		<10	50.0	7 [2.8-17.4]	**<0.0001**	5.8 [1.9-17.7]	**0.002**
	F	>20	17.4	1	**–**	1	–
		10-20	33.1	2.7 [1.2-6]	**0.018**	2.5 [1.0-6.1]	0.05
		<10	39.0	4.9 [2.3-10.5]	**<0.0001**	6.4 [2.8-15]	**<0.0001**
BMI < 30 kg/m^2	**M**	>20	14.0	1	–	1	–
		10-20	23.1	2.3 [0.95-5.3]	0.06	1.2 [0.4-3.9]	0.73
		<10	43.9	8.4 [3.1-22.8]	**<0.0001**	7.1 [1.8-27]	**0.004**
	F	>20	8.6	1	–	1	–
		10-20	17.0	2.6 [0.95-7.2]	0.06	2.4 [0.8-7.3]	0.11
		<10	18.3	4.5 [1.7- 12.1]	**0.003**	5.5 [1.9-16.1]	**0.002**
BMI > 30 kg/m^2	**M**	>20	77.8	1	–	1	–
		10-20	87.0	1.9 [0.3-12.7]	0.48	1.53 [0.2-12]	0.7
		<10	88.9	2.4 [0.2-26.4]	0.47	0.5 [0.1-8.6]	0.6
	F	>20	62.5	1	–	1	–
		10-20	80.6	3 [0.7-12.4]	0.12	2.5 [1.5-12.8]	0.26
		<10	86.2	7.2 [1.9-26.9]	**0.003**	9.1 [2-42.1]	**0.004**
without SM	**M**	>20	12.2	1	–	1	–
		10-20	8.3	0.48 [0.1-2.3]	0.37	0.5 [0.1-2.4]	0.38
		<10	12.2	2.9 [0.8-10.5]	0.1	2.95 [0.8-10]	0.09
	F	>20	3.8	1	–	1	–
		10-20	17.0	5.9 [0.7-50]	0.1	6.4 [0.7-55]	0.088
		<10	20.1	8.4 [1.1-65]	**0.04**	9.2 [1.1-72.5]	**0.035**
With SM	**M**	>20	33.3	1	–	1	–
		10-20	48.7	1.8 [0.9-3.6]	0.09	1.7 [0.9-3.5]	0.11
		<10	70.6	5 [2.2-11.3]	**<0.0001**	4.8 [2.1-10.8]	**<0.0001**
	F	>20	25.0	1	**–**	1	**–**
		10-20	46.8	2.7 [1.3-5.6]	**0.009**	2.9 [1.3-6]	**0.006**
		<10	63.5	5.5 [2.8-10.9]	**<0.0001**	5.9 [2.9-11.7]	**<0.0001**

Model 1: adjusted for age, blood sampling season, physical activity and obesity

Model 2: adjusted to model 1 plus metabolic components (Type2 Diabetes, hypertension, triglycerides, HDLc), history of cardiovascular disease and hypothyroidism

When the variable is taken as categorical, it is automatically eliminated from the fitting model

%: NAFLD prevalence, 25(OH) D: µg/l, *F* female, *M* Male, *MS* metabolic syndrome, OR 95% CI: Odd's ratio, 95% confidence intervals. Bold values indicate significant differences ($p < 0.05$)

women than in men (OR = 6.4, 95% CI [2.8-15], $p < 0.0001$ vs. OR = 5.8, 95% CI [1.9-17.7], $p = 0.002$).

When stratified by obesity, the association between vitamin D status and NAFLD disappeared in obese men. In both women and non-obese men, only severe vitamin D deficiency was positively related to NAFLD, independently of all above confounding factors. In the fully fitted model, the highest risk was observed in obese women (OR = 9.1, 95% CI [2.0-42], $p = 0.004$).

When stratified by MS, the association between vitamin D status and NAFLD disappeared in men without MS. In the fully fitted model, severe vitamin D deficiency was independently associated with NAFLD in women without MS (OR = 9.2, 95% CI [1.1-72.5], $p = 0.035$) as well as in both women and men with MS (OR = 5.9, 95% CI [2.9-11.7] vs. OR = 4.8, 95% CI [2.1-10.8], $p < 0.0001$) respectively. It is noteworthy here that in women with MS, vitamin D deficiency was also independently associated with NAFLD.

Additive biological interaction between severe vitamin D deficiency and MS for NAFLD

The results of the additive biological interaction are given in Table 4.

Table 4 The additive interaction analysis of metabolic syndrome and vitamin D status on NAFLD stratified by gender

	Male					Female				
		Model 1		Model 2			Model 1		Model 2	
		Index 95%CI	p	Index 95%CI	p		Index 95%CI	P	Index 95%CI	p
Index	**RERI**	12.3 [−3-27.6]	0.1	20.8 [−7.5-49]	0.1	**RERI**	7.2 [1.3-12.9]	**0.02**	8.95 [1.01-18]	**0.048**
	AP	0.59 [0.3-0.9]	**<0.0001**	0.68 [0.42-0.94]	**<0.0001**	**AP**	0.6 [0.4-0.8]	**<0.0001**	0.57 [0.3-0.8]	**<0.0001**
	SI	2.7 [1.2-5.8]	**0.01**	3.42 [1.4-8.4]	**0.0006**	**SI**	2.9 [1.6-5.3]	**<0.0006**	2.56 [1.3-5.0]	**0.0005**

RERI the relative excess risk due to interaction, *AP* the attributable proportion due to interaction, *SI* the synergy index

Model 1: adjusted for age, blood sampling season, physical activity and obesity

Model 2: adjusted to model 1 plus metabolic components (T2D, hypertension, TG, HDLc), history of cardiovascular disease and hypothyroidism

When the variable is taken as categorical, it is automatically eliminated from the fitting model. Bold values of p indicate significant differences ($p < 0.05$)

In men, significant additive interaction analysis was found only for the two indices AP and SI, meaning that a synergetic effect was detected between severe vitamin D deficiency and MS for NAFLD (SI =3.42, 95% CI [1.4-8.4], $p = 0.0006$). However, no significant excess risk was found for the association between these two factors, when compared to the same factors taken individually (RERI = 20.8, 95% CI [−7.5-49], $p = 0.1$).

In women, however, the additive interaction between severe vitamin D deficiency and MS was significant for the three indices; RERI = 7.2 [1.3-12.9] indicates that there is an excess risk of 7.2-fold in subjects combining both MS and severe vitamin D deficiency. An AP of 0.6 [0.4-0.8] indicates that 60% of NAFLD cases in women are attributed to the combination of MS with severe vitamin D deficiency. An SI of 2.9 [1.6-5.3] suggests that the mechanism of interaction is synergistic and that the risk of NAFLD is 2.9 times higher in women combining these two risk factors than in women exposed to a single one (Table 4).

Discussion

NAFLD is currently the most common metabolic liver disease; its prevalence in some industrialized countries exceeds 30% [10–12]. In Algeria data on NAFLD are very scarce, in this study, using the FLI index, it was estimated that more than one third of the included subjects were found to have NAFLD. This high prevalence could be explained by the high rate of subjects included with metabolic syndrome (46.5%), with hypertension (40.4%), and particularly with android obesity (61.7%), indeed, it is well known that NAFLD is tightly linked to these cardio-metabolic disorders.

The primary objective of this cross-sectional study was to investigate the association between vitamin D status, MS and NAFLD. The most relevant findings consisted in the demonstration of a significant and independent association between severe vitamin D deficiency and NAFLD. In men, this association was more pronounced in non-obese subjects and in those with MS. In women, this association was present regardless of the metabolic profile and the BMI stat. Moreover, a synergistic additive interaction between severe vitamin D deficiency and MS was significant, for all three studied indices, in women but not in men. To the best of our knowledge, this study is the first to explore the interaction, on an additive scale, between severe vitamin D deficiency and MS on NAFLD.

The causal relationship between vitamin D deficiency and NAFLD is still a debated issue. Some studies have shown that vitamin D may prevent liver disease through its metabolic, immunomodulatory [10, 16], anti-inflammatory and anti-fibrotic effects [4, 17, 26]. Other studies suggest that vitamin D deficiency is more likely to be a consequence of liver disorders rather than to be a cause, according to these studies, since the liver represents the site of the first activation reaction, into 25 hydroxy vitamin D, and the site of the synthesis of its carrier protein (vitamin D-binding protein), a vitamin D deficiency is definitely expected in case of liver function impairment [4, 17]. Most previous studies, including the present study, are observational and therefore unable to draw definitive conclusions about the relationship between vitamin D status and NAFLD.

In general, our findings are consistent with studies reporting a positive association between vitamin D deficiency and NAFLD. K. L. Jablonski et al. [4], in a case-control study, found that both vitamin D deficiency and insufficiency, defined by a circulating level of 25(OH) D < 15 ng/ml and 15-30 ng/ml, respectively, were associated with NAFLD, as diagnosed by ultrasonography, irrespective of age, gender, ethnicity, BMI and blood sampling season. In another case-control study, Targher et al. [15], found a dose-response relationship between the severity of hypovitaminosis D and the severity of NAFLD as confirmed by liver biopsy. A recent meta-analysis [16] of 17

studies involving 5000 NAFLD cases and 8000 controls found that vitamin D deficiency was 26% more common in the NAFLD group, and suggested that it could potentially contribute to the disease process. More recently, in a meta-analysis summarizing the findings of 45 cross-sectional published studies, almost two thirds of the included researches (64.4%) showed an inverse relationship between vitamin D status and NAFLD, while 35.6% failed to find such association [17].

This wide discrepancy may be explained by several considerations: first, the non-uniformity of methods used for NAFLD screening, varying from biopsy to ultrasonography, or even the only unexplained liver enzyme elevation. In our study, NAFLD was diagnosed using the FLI index; an accurate algorithm based on simple and cost-effective parameters, many studies had evaluated the diagnostic performance of the FLI; a cut-off of 60 showed a very satisfying discriminative capacity, with a sensitivity and specificity of 87% and 86% respectively [10, 24]. Second, the non-standardization of techniques used for vitamin D measurement as well as the cut-off chosen to define vitamin D deficiency. Thirdly, non-adjustment for certain confounding factors such as sampling season and physical activity [17].

In an attempt to contain or at least to minimize the impact of potentially confounding variables, we performed several stratifications. This provided further clarification of the relationship between vitamin D deficiency and NAFLD, and highlighted the heterogeneity of its effect, not only according to gender but also according to patients' body weight and metabolic profile.

In our study, when stratified by gender and BMI, the association between vitamin D severe deficiency and NAFLD disappeared in obese men. The loss of association in this group does not imply a lack of relationship between vitamin D and NAFLD, but it probably indicates that the risk from obesity is so much higher that it masks that from vitamin D. However, in contrast to obese men, the risk related to severe deficiency persists in obese women; this could be explained by the prevalence of severe vitamin D deficiency which is much higher in women. These findings are consistent with those reported in a recent observational study showing that vitamin D deficiency was independently related to NAFLD in normal and overweight men but not in obese men [27] In this study, the lack of association between vitamin D and NAFLD in obese subjects was explained by the fact that the additional effect of vitamin D deficiency may be clinically insignificant compared with the major effect of obesity [27]. In another study, Barchetta et al. [10] explored the relationship between hypovitaminosis D and NAFLD, as diagnosed by both ultrasonography and FLI, in a subset of normal weight patients, the authors showed that vitamin D was inversely related to NAFLD regardless of gender, age, FBG, TG and BMI.

The gender differences in the effect of severe vitamin D deficiency were again revealed when stratifying by MS, in this case the association was lost in men without MS. This may indicate that MS probably mediates the association between severe vitamin D deficiency and NAFLD in men; the presence of MS may amplify the NAFLD risk related to severe vitamin D deficiency. Another important observation is that in women, in contrast to men, stratification according to metabolic profile brought out not only severe deficiency, but also vitamin D deficiency as an independent risk factor. Thus, MS may also mediate the association between vitamin D deficiency and NAFLD in women. The heterogeneity of vitamin D deficiency effect according to metabolic factors has been pointed out in previous researchers; in their study, Seo JA et al. [8] found an association between NAFLD and vitamin D deficiency only in subjects with diabetes or insulin resistance, however the authors did not stratify by gender. Some authors had explained the gender heterogeneity of the relationship between vitamin D status and NAFLD by the circulating levels of sex hormones and their blood carrying globulins, indeed, it has been suggested that the joint existence of low levels of 25(OH)D and low SHBG had a synergistic association with NAFLD in men [27].

Another interesting finding of this study is the highlighting of an interactive effect between severe vitamin D deficiency and MS on NAFLD. In practice, it is essential to understand the interaction between risk factors for a given disease, especially when these factors are very common in the population. Indeed, in this case, risk factors usually coexist in the same patient. In our study, for example, vitamin D supplementation for the purpose of preventing or improving NAFLD may be more promising if it targets non-obese men and those with MS. In obese men, it would be wiser to act first on weight loss before considering vitamin D supplementation. Indeed, given its lipophilic nature, vitamin D is highly sequestered in adipose tissue [28, 29] leading to a "pseudo-hypovitaminosis D ". Several studies report a negative correlation of circulating vitamin D levels not only with body fat percentage but also with the fat ectopic distribution [30]. In this context, Lee SM et al. [31] have studied the effect of caloric restriction on vitamin D status and intrahepatic lipid accumulation, the authors found that a reduction in body weight was accompanied by an increase in circulating vitamin D levels, an improvement in metabolic parameters, a decrease in intrahepatic fat accumulation and, above all, a decrease in serum aminotrasferases. In another study involving exclusively postmenopausal women, the authors found that a rise of 1 ng/ml in 25(OH)D was associated with a reduction of 11% in the risk of NAFLD [30].

The findings of this study should be interpreted with caution, given the inherent limitations of its methodology. First, the cross-sectional design makes it difficult to draw definitive conclusions about the causality of such association

between hypovitaminosis D and NAFLD. Second, this is a single-center study, subjects included may not be representative of the general population, and findings cannot be generalized, thus further large-scale studies are required. Third, NAFLD was diagnosed on the basis of FLI elevation with no histological or radiological confirmation, however, this index may be a less invasive and reasonable alternative to liver biopsy in epidemiological studies.

Conclusion

As a conclusion of this study, a positive association has been found between severe vitamin D deficiency and NAFLD. In men, this association was found in MS subjects although it was partially hidden by obesity. In women, this association persists regardless of metabolic profile and body weight. Moreover, an excess risk in subjects who cumulate a severe vitamin D deficiency in combination with a MS has been quantified through the demonstration of an additive interaction between these two factors.

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Declarations

Conflict of interest The authors have no conflicts of interest to declare.

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