

# Does vitamin D replacement therapy cause a regression in fatty liver disease? A case control study of comparison of vitamin D and other common therapy modalities

Vusal Abdurahmanov<sup>1</sup>, Arzu Güntürk<sup>1</sup>, Fatma Ferda Kartufan<sup>2</sup>, Rahman Nurmammedov<sup>1</sup>, Yaşar Küçükardalı<sup>1</sup>

<sup>1</sup> Yeditepe University, Internal Medicine  
Department, Istanbul, Turkey  
<sup>2</sup> Yeditepe University, Anesthesia and  
Reanimation Department, Istanbul, Turkey

## ORCID ID of the author(s)

VA: 0000-0002-8229-9784  
AG: 0000-0002-1941-8699  
FFK: 0000-0002-5592-2366  
RN: 0000-0002-7345-6741  
YK: 0000-0002-8719-8886

## Corresponding Author

Arzu Güntürk  
Department of Internal Medicine, Yeditepe  
University Faculty of Medicine  
Hastane yolu st; no:4 4-1, 34752, Istanbul, Turkey  
E-mail: arzu.gunturk@yeditepe.edu.tr

## Ethics Committee Approval

This study is approved by Clinical Ethical  
Committee of the Yeditepe University (Approval  
form number: 1267, Approval date: Oct.  
27.2016).

All procedures in this study involving human  
participants were performed in accordance with  
the 1964 Helsinki Declaration and its later  
amendments.

## Conflict of Interest

No conflict of interest was declared by the  
authors.

## Financial Disclosure

The authors declared that this study has received  
no financial support.

## Published

2021 September 25

Copyright © 2021 The Author(s)

Published by JOSAM

This is an open access article distributed under the terms of the Creative  
Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC  
BY-NC-ND 4.0) where it is permissible to download, share, remix,  
transform, and buildup the work provided it is properly cited. The work  
cannot be used commercially without permission from the journal.



## Abstract

**Background/Aim:** Non-alcoholic fatty liver is quite common among modern populations, and simpler methods are researched for its early diagnosis and therapy. Studies are stating that vitamin D deficiency could play a role in the etiopathogenesis of fatty liver. This study aimed to compare the efficacy of metformin and vitamin D therapy in improving fatty liver disease.

**Methods:** A total of 86 patients with non-alcoholic fatty liver disease were included in this case control study and classified into four groups according to the treatment received. In the study group, 23 patients were using metformin only, and 21 patients were using both metformin and vitamin D. Twenty-one patients were using vitamin D only, and 21 patients were on a diet and an exercise regimen (control group). Weight, BMI, waist circumference, fatty liver index (FLI), HOMA-IR, AST, ALT, GGT, triglyceride parameters were evaluated before and after four weeks of therapy.

**Results:** There was a significant regression in the fatty liver disease of the patients who used both metformin and vitamin D (FLI-%5, 90 (11.1)  $P=0.025$ ). Among patients who used only metformin and only vitamin D, the decrease in FLI was not significant ( $P>0.05$ ); however, FLI was observed to significantly decrease in the control group ( $-7.30$ ,  $P=0.018$ ). The serum CRP levels were also observed to significantly decrease in the control, Met and Met-D vit groups ( $P=0.025$ ,  $P=0.002$ ,  $P=0.006$ , respectively).

**Conclusions:** The combination of vitamin D and metformin therapy could positively contribute to the improvement of NAFLD in patients with vitamin D deficiency.

**Keywords:** Fatty liver, Metformin, Vitamin D

## Introduction

Non-alcoholic fatty liver disease (NAFLD) describes the visceral adiposity of the liver without secondary causes (e.g., heavy alcohol use). NAFLD is the known leading cause of cryptogenic cirrhosis [1]. NAFL and nonalcoholic steatohepatitis (NASH) are the two subgroups of NAFLD. NAFL is the adiposity of the liver without significant inflammation signs, while NASH indicates inflammatory steatohepatitis [2].

The pathogenesis of NAFLD is still controversial. The dominant hypothesis is the “double-hit model” suggested by Day and James [3]. The first hit is made by free fatty acids in the liver, accumulation of the triglycerides (TG), insulin resistance (via lipolysis and hyperinsulinemia) and obesity (leptin resistance). Because of these mechanisms, proinflammatory cytokines are secreted and oxidative stress occurs, resulting in a chronic inflammatory state. Both mechanisms make the second hit, which is the progression of the liver damage to steatohepatitis and fibrosis. Insulin resistance is important in NAFLD pathogenesis. The fat content of the liver affects insulin sensitivity more than visceral adiposity [4]. This supports the hypothesis that fatty liver has a direct role in the insulin resistance pathogenesis.

Metformin was first used in the 1950s and is the first step therapy for type 2 diabetes mellitus patients today [5]. It reduces gluconeogenesis in the liver, stimulates glucose uptake at the muscles and increases fatty acid oxidation at the adipose tissue, causing decreased blood glucose [6]. As a result, peripheral insulin sensitivity increases. Metformin prevents adipose tissue growth not only by the direct inhibition of adipogenesis, but also with the modulation of the synthesis or secretion of adipokines [7]. Adiponectin, induced by metformin, stimulates AMPK (AMP-activated protein kinase) directly and prevents hepatic lipid accumulation by increasing free fatty acid oxidation and decreasing its synthesis. In a hepatic steatosis rat model, metformin was shown to downgrade hepatomegaly, hepatic fat accumulation, and cause a regression in elevated liver functions test by reducing hepatic tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) expression [8].

Vitamin D receptors (VDR) are localized in the mononuclear cells in the peripheral blood and in various tissues containing activated T cells in the human body. There are epidemiologic proofs which show that vitamin D deficiency is an independent risk factor for NAFLD [9]. In healthy individuals with normal liver enzymes, low vitamin D levels were strongly and independently associated with NAFLD [10]. Another study shows that the severity of vitamin D deficiency is related with the histopathological severity of NAFLD and the patients with low vitamin D levels are at more advanced stages of liver steatosis and fibrosis [11]. According to the meta-analyses, the probability of an NAFLD patient having vitamin D deficiency is increased 1.26-fold [12].

This study aimed to research the effect of metformin and vitamin D monotherapies and combination therapies.

## Materials and methods

This retrospective study was performed on the patients over 18 years of age who visited the Yeditepe University Internal

Medicine outpatient clinic between 2015-2018. The ethics approval for the study was obtained from Yeditepe University Clinical Trials Ethical Committee (Approval form number: 1267 / Chairperson: Prof. Dr. T. CELIK) on 27 October 2016.

The study was conducted per the principles of the Declaration of Helsinki, and in compliance with all international and national laws and regulations. Patients gave their written informed consent before any procedures were performed.

The inclusion criteria were as follows:

- Being over 18 years old
- Having mild and moderately elevated levels of liver function tests and/or hepatosteatosis in ultrasonography
- >%50 FLI
- <30 ng/dl vitamin D levels
- Complete anthropometric and laboratory measurements

The exclusion criteria included having any of the following:

- Acute viral hepatitis
- Alcoholic hepatitis
- Autoimmune hepatitis
- Toxic ischemic hepatitis
- A liver mass
- Pancreatitis and/or cholangitis attack
- Steroid, antiepileptic, antiviral, antifungal drug use

The following parameters were evaluated at baseline and at the end of 6 months: Age (year), Height (cm), Weight (kg), waist circumference (cm), vitamin D level (ng/ml), HOMA-IR, CRP (mg/dl), AST (U/l), ALT (U/l), GGT (U/l), and TG (mg/dl). Fatty liver index (FLI) is calculated using waist circumference, triglyceride and GGT and shows the percentage of fat deposition of the liver. It is correlated with hepatosteatosis in ultrasonography. Values below 30% indicate no hepatosteatosis those >60% are significantly indicative of steatosis [13].

### Calculation of FLI

FLI

$$= \frac{e^{0,953 \cdot \log(TG) + 0,139 \cdot BMI + 0,718 \cdot \log(GGT) + 0,053 \cdot \text{waist circumference} - 15,74}}{1 + e^{0,953 \cdot \log(TG) + 0,139 \cdot BMI + 0,718 \cdot \log(GGT) + 0,053 \cdot \text{waist circumference} - 15,74}} \cdot 100 [13]$$

### Creation of study groups

- MetDvit Group: 21 patients using Metformin+ D vitamin
- Met Group: 23 patients using only Metformin
- Dvit Group: 21 patients using only D vitamin
- Control group: 21 patients using neither Metformin, nor D vitamin

Metformin was used at 1000-3000 mg/day for 6 months.

Vitamin D was used at 50000 Units/week for 4-6 weeks.

### Statistical analysis

Minimum-maximum, mean and standard deviation values were included in descriptive statistics. The distribution of the variables was assessed by a coefficient of variation, Skewness – Kurtosis tests, histogram, detrended plot, and the normality test of Kolmogorov-Smirnov. The data were considered parametric if three or more tests mentioned above were positive.

The investigators used the Post Hoc test to identify if the sample size was sufficient. Then, the data were grouped into Control group ( $G_{\text{control}}$ ), Dvit group ( $G_{\text{Dvit}}$ ), Metformin group

(G<sub>Met</sub>) and Metformin and Dvit groups (G<sub>Met-Dvit</sub>) before and after the treatment. The groups were not compared with each other according to all treatment outcomes, because the study was retrospective. The groups were not randomized, and power analysis was not performed. Even the control group's outcomes were noted from the files of the patients examined in the Internal Medicine Department. Therefore, the investigators performed a One Way Anova test to all pre-treatment values. The non-differing data were selected, and their outcomes after the treatment were compared between the groups with a Mann Whitney U test.

The values obtained before and after the treatment were compared within the groups with a paired sample t-test or Wilcoxon test. The post-treatment data were evaluated for correlation with Pearson or Spearman Correlation Tests.  $P < 0.012$  was considered significant in the Post Hoc Bonferroni test, and  $P < 0.05$  was considered significant in others.

### Results

Forty-four (51%) patients were male, and forty-two (49%) were female. The mean age and height of the participants were 41.2 (11.4) years, and 170.8 (9.3) cm, respectively.

The demographic data and the pre- and post-treatment values are shown in Table 1.

Table 1: Descriptive pre-treatment data

Pre-treatment data	Female Mean (SD) (n)	Male Mean (SD) (n)	Overall Mean (SD) (n)
Age (years)	41.5 (11.5)(42)	41.6 (11.6) (44)	41.2 (11.4) (86)
Height (cm)	163.7(5.1) (42)	177.7(5.7) (44)	170.8 (9.3) (86)
Weight (kg)	78.4(13.7) (42)	94.5(14.5) (44)	86.5 (16.2) (86)
BMI (kg/m <sup>2</sup> )	29.2 (4.9) (42)	30.0 (4.7) (44)	29.5(4.8) (86)
Waist C. (cm)	98.4(13.1) (42)	105.4(9.8) (44)	102.0 (12.0) (86)
D vit (ng/ml)	16.5(8.4) (42)	18.0 (7.7) (44)	17.8(7.5) (86)
FLI (%)	53.2(24.1) (42)	73.4(18.1) (44)	63.3(24.1) (86)
HOMA IR	4.0(2.3) (39)	4.5(2.5) (37)	4.2(2.3) (76)
CRP (mg/dl)	3.5(4.8) (34)	3.9(3.2) (40)	3.7(3.5) (74)
ALT (U/l)	22.1(9.3) (42)	54.2(41.6) (43)	38.1(36.1) (85)
AST (U/l)	21.9(6.9) (42)	33.2(20.6) (42)	27.6(16.5) (84)
GGT (U/l)	22.7(13.8) (42)	52.3(52.5) (44)	37.4(41.1) (86)
TG (mg/dl)	140.2(52.8) (42)	198.7(118.0)(44)	171.5(98.8) (86)

### Within-group comparison

**G<sub>control</sub>:** The decrease in TG and FLI and the increase in vitamin D following treatment were significant ( $P=0.034$ ,  $P=0.013$ , and  $P=0.01$ ). There were no significant differences between morphological (weight, BMI, waist circumference), or laboratory values (IR, CRP, AST, ALT, GGT) between the pre- and post-treatment periods.

**G<sub>Dvit</sub>:** Vitamin D had significantly increased after the treatment ( $P=0.001$ ). There were no significant differences between the morphological (weight, BMI, waist circumference), or laboratory values (IR, CRP, AST, ALT, GGT, TG) and FLI between the pre- and post-treatment periods.

Table 2: Inter-group comparison

	G <sub>control</sub> (n:21)			G <sub>Dvit</sub> (n:21)			G <sub>Met</sub> (n:23)			G <sub>Met-Dvit</sub> (n:21)		
	Before Mean(SD)	After Mean(SD)	P-value	Before Mean(SD)	After Mean(SD)	P-value	Before Mean(SD)	After Mean(SD)	P-value	Before Mean(SD)	After Mean(SD)	P-value
Weight	81.1(10.5)	80.4(9.8)	0.4 <sup>p</sup>	76.7(11.6)	76.8(11.2)	0.79 <sup>p</sup>	91.2(16.1)	88.6(16.4)	0.012 <sup>*p</sup>	95.6(18.7)	93(17.9)	0.007 <sup>**p</sup>
BMI	27.95(2.8)	28.2(2.6)	0.37 <sup>p</sup>	26.7(3.5)	26.8(3.5)	0.76 <sup>p</sup>	30.7(4.5)	29.8(4.5)	0.009 <sup>**w</sup>	32.3(6)	31.1(5.3)	0.010 <sup>*w</sup>
Wcirc	100(9.9)	98.2(9.3)	0.06 <sup>p</sup>	94.4(7.5)	94.5(7.7)	0.93 <sup>p</sup>	106.1(11)	103.6(10.2)	0.034 <sup>*p</sup>	107.2(14.3)	104.3(13.2)	0.010 <sup>*w</sup>
Dvit	20.5(7.5)	24.8(9.4)	0.01 <sup>**w</sup>	13.9(5.7)	29.1(13.8)	<0.001 <sup>***p</sup>	18.8(6.3)	21.9(8.6)	0.08 <sup>w</sup>	17.6(9)	27.8(9.5)	<0.001 <sup>***p</sup>
FLI	57.4(23.4)	50.1(24.8)	0.018 <sup>*p</sup>	50.6(21.9)	46(22.1)	0.18 <sup>p</sup>	73.4(22.9)	68.9(22.9)	0.09 <sup>p</sup>	72.2(22.5)	66.3(26.9)	0.025 <sup>*p</sup>
HOMA-IR	3.4(1.1)	3.2(1.2)	0.38 <sup>w</sup>	2.8(0.9)	2.7(0.9)	0.90 <sup>w</sup>	5.6(3.1)	3.5(1.6)	0.020 <sup>*w</sup>	5.2(2.8)	3.9(1.9)	0.02 <sup>*w</sup>
CRP	4.0(4.2)	3.7(3.8)	0.66 <sup>w</sup>	2.7(3.1)	2.3(2.7)	0.60 <sup>w</sup>	4.8(3.5)	4.6(3.4)	0.90 <sup>w</sup>	2.8(2.8)	1.9(1.6)	0.21 <sup>w</sup>
ALT	46.4(31.8)	46.7(45)	0.84 <sup>w</sup>	32.3(23.5)	33.9(31)	0.86 <sup>w</sup>	40.5(21.2)	36.9(17.5)	0.58 <sup>w</sup>	34.1(23.4)	31.2(22.2)	0.41 <sup>w</sup>
AST	32.4(28.9)	29.3(16.9)	0.18 <sup>w</sup>	23.2(7.8)	24(10.6)	0.89 <sup>w</sup>	28.8(9.3)	29.1(11.8)	0.81 <sup>w</sup>	25.9(11)	24.5(9.8)	0.36 <sup>w</sup>
GGT	34.4(23.2)	33.1(24.2)	0.75 <sup>p</sup>	33.9(23.8)	30.4(23.8)	0.25 <sup>p</sup>	50.7(70)	48.6(67.9)	0.24 <sup>w</sup>	28(24)	29.3(21.9)	0.71 <sup>w</sup>
TG	150.8(114.1)	120(64.9)	0.034 <sup>*w</sup>	166.3(72.7)	139.9(72.5)	0.14 <sup>w</sup>	180.3(53.9)	168(62)	0.36 <sup>p</sup>	172.7(131.5)	181.8(139)	0.40 <sup>w</sup>

\* P<0.05, \*\* P<0.01, \*\*\* P<0.001, p: Paired sample t test, w: Wilcoxon test

**G<sub>Met</sub>:** The decrease in morphological data (weight, BMI, waist circumference,  $P=0.013$ ,  $P=0.019$ , and  $P=0.008$ , respectively), along with that in HOMA-IR values ( $P=0.02$ ) was significant, while the increase of vitamin D and the decrease of CRP, AST, ALT, GGT, and TG, and FLI were not.

**G<sub>Met-Dvit</sub>:** The decrease in morphological data (weight, BMI, waist circumference,  $P=0.07$ ,  $P=0.016$ , and  $P=0.011$ , respectively), the increase in vitamin D, and the decrease in HOMA-IR ( $P<0.001$ , and  $P=0.015$  respectively), and the decrease in FLI were significant ( $P=0.025$ ). The decrease in CRP, AST, ALT, GGT, and TG were insignificant (Table 2).

### Inter-group comparison

The groups were similar in terms of pre-treatment CRP, AST, ALT, GGT, TG values ( $P>0.05$ ). Therefore, they were included in the intra-group comparison tests (Table 3).

Table 3: Inter-group comparison of post-treatment CRP, ALT, AST, GGT, and TG

Comparable Data	Group-Group (n-n) Mean(SD)	Z	P-value
CRP-at	G <sub>control</sub> -G <sub>Dvit</sub> (n21-n21) (6.2(8.8) - 2.3(2.5))	-2.245	0.025 <sup>*w</sup>
	G <sub>control</sub> -G <sub>Met</sub> (n21-n23) (6.2(8.8) - 5.1(3.9))	-0.554	0.579
	G <sub>control</sub> -G <sub>Met-Dvit</sub> (n21-n21) (6.2(8.8) - 2.3(2.1))	-1.935	0.053
	G <sub>Dvit</sub> -G <sub>Met</sub> (n21-n23) (2.3(2.5) - 5.1(3.9))	-3.039	0.002 <sup>**w</sup>
	G <sub>Dvit</sub> -G <sub>Met-Dvit</sub> (n21-n21) (2.3(2.5) - 2.3(2.1))	-0.080	0.936
	G <sub>Met</sub> -G <sub>Met-Dvit</sub> (n23-n21) (5.1(3.9) - 2.3(2.1))	-2.708	0.006 <sup>**w</sup>
ALT-at	G <sub>control</sub> -G <sub>Dvit</sub> (n21-n21) (49.8(48.4) - 33.9(31))	-0.793	0.118
	G <sub>control</sub> -G <sub>Met</sub> (n21-n23) (49.8(48.4) - 36.9(17.5))	-0.231	0.817
	G <sub>control</sub> -G <sub>Met-Dvit</sub> (n21-n21) (49.8(48.4) - 31.2(22.2))	-0.849	0.396
	G <sub>Dvit</sub> -G <sub>Met</sub> (n21-n23) (33.9(31) - 36.9(17.5))	-1.823	0.068
	G <sub>Dvit</sub> -G <sub>Met-Dvit</sub> (n21-n21) (33.9(31) - 31.2(22.2))	-0.170	0.865
	G <sub>Met</sub> -G <sub>Met-Dvit</sub> (n23-n21) (36.9(17.5) - 31.2(22.2))	-1.526	0.127
AST-at	G <sub>control</sub> -G <sub>Dvit</sub> (n21-n21) (30.6(16.5) - 24(10.6))	-1.563	0.428
	G <sub>control</sub> -G <sub>Met</sub> (n21-n23) (30.6(16.5) - 28.6(11.7))	-0.183	0.855
	G <sub>control</sub> -G <sub>Met-Dvit</sub> (n21-n21) (30.6(16.5) - 34.5(44.7))	-0.862	0.389
	G <sub>Dvit</sub> -G <sub>Met</sub> (n21-n23) (24(10.6) - 28.6(11.7))	-2.155	0.031 <sup>*w</sup>
	G <sub>Dvit</sub> -G <sub>Met-Dvit</sub> (n21-n21) (24(10.6) - 34.5(44.7))	-0.562	0.574
	G <sub>Met</sub> -G <sub>Met-Dvit</sub> (n23-n21) (28.6(11.7) - 34.5(44.7))	-1.174	0.240
GGT-at	G <sub>control</sub> -G <sub>Dvit</sub> (n21-n21) (33.9(22.4) - 30.4(23.8))	-0.667	0.505
	G <sub>control</sub> -G <sub>Met</sub> (n21-n23) (33.9(22.4) - 48.6(67.9))	-0.400	0.689
	G <sub>control</sub> -G <sub>Met-Dvit</sub> (n21-n21) (33.9(22.4) - 28(24))	-1.007	0.314
	G <sub>Dvit</sub> -G <sub>Met</sub> (n21-n23) (30.4(23.8) - 48.6(67.9))	-1.070	0.285
	G <sub>Dvit</sub> -G <sub>Met-Dvit</sub> (n21-n21) (30.4(23.8) - 28(24))	-0.290	0.772
	G <sub>Met</sub> -G <sub>Met-Dvit</sub> (n23-n21) (48.6(67.9) - 28(24))	-1.835	0.067
TG-at	G <sub>control</sub> -G <sub>Dvit</sub> (n21-n21) (124.7(68.9) - 139.9(72.5))	-1.032	0.302
	G <sub>control</sub> -G <sub>Met</sub> (n21-n23) (124.7(68.9) - 168(625))	-2.503	0.012 <sup>*w</sup>
	G <sub>control</sub> -G <sub>Met-Dvit</sub> (n21-n21) (124.7(68.9) - 72.7(131.5))	-1.887	0.059
	G <sub>Dvit</sub> -G <sub>Met</sub> (n21-n23) (139.9(72.5) - 168(62))	-1.821	0.069
	G <sub>Dvit</sub> -G <sub>Met-Dvit</sub> (n21-n21) (139.9(72.5) - 172.7(131.5))	-1.120	0.263
	G <sub>Met</sub> -G <sub>Met-Dvit</sub> (n23-n21) (168(62) - 172.7(131.5))	-0.729	0.466

\* P<0.05, \*\* P<0.01, Mann Whitney U test

The post-treatment decrease in the CRP level of  $G_{Dvit}$  was significant ( $P=0.025$ ) compared with that in the  $G_{control}$ , just as the post-treatment increase of TG in  $G_{Met}$  ( $P=0.012$ ) compared to the  $G_{control}$ . The decrease in the CRP and TG levels of  $G_{Met-Dvit}$  groups were insignificant ( $P=0.053$ , and  $P=0.059$  respectively) compared with those in the  $G_{control}$ . The post-treatment decrease in the CRP and AST levels of  $G_{Dvit}$  were significant ( $P=0.002$ , and  $P=0.031$ , respectively) compared to those in  $G_{Met}$ . The post-treatment data of  $G_{Dvit}$  and  $G_{Met-Dvit}$  were similar. The post-treatment decrease in the CRP level of  $G_{Met-Dvit}$  was significant ( $P=0.006$ ) compared to that of  $G_{Met}$  (Table 4). The laboratory values not mentioned above yielded insignificant results in the intra-group analysis.

Table 4: Inter-group comparison of post-treatment CRP

CRP-at Groups (Mean (SD))	$G_{Dvit}$	$G_{Met}$	$G_{Met-Dvit}$ Mean (SD) 2.3(2.1)
	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value
$G_{control}$ (6.2(8.8))	0.025*	0.579	0.053
$G_{Dvit}$ (2.3(2.5))		0.002**	0.936
$G_{Met}$ (5.1(3.9))			0.006**

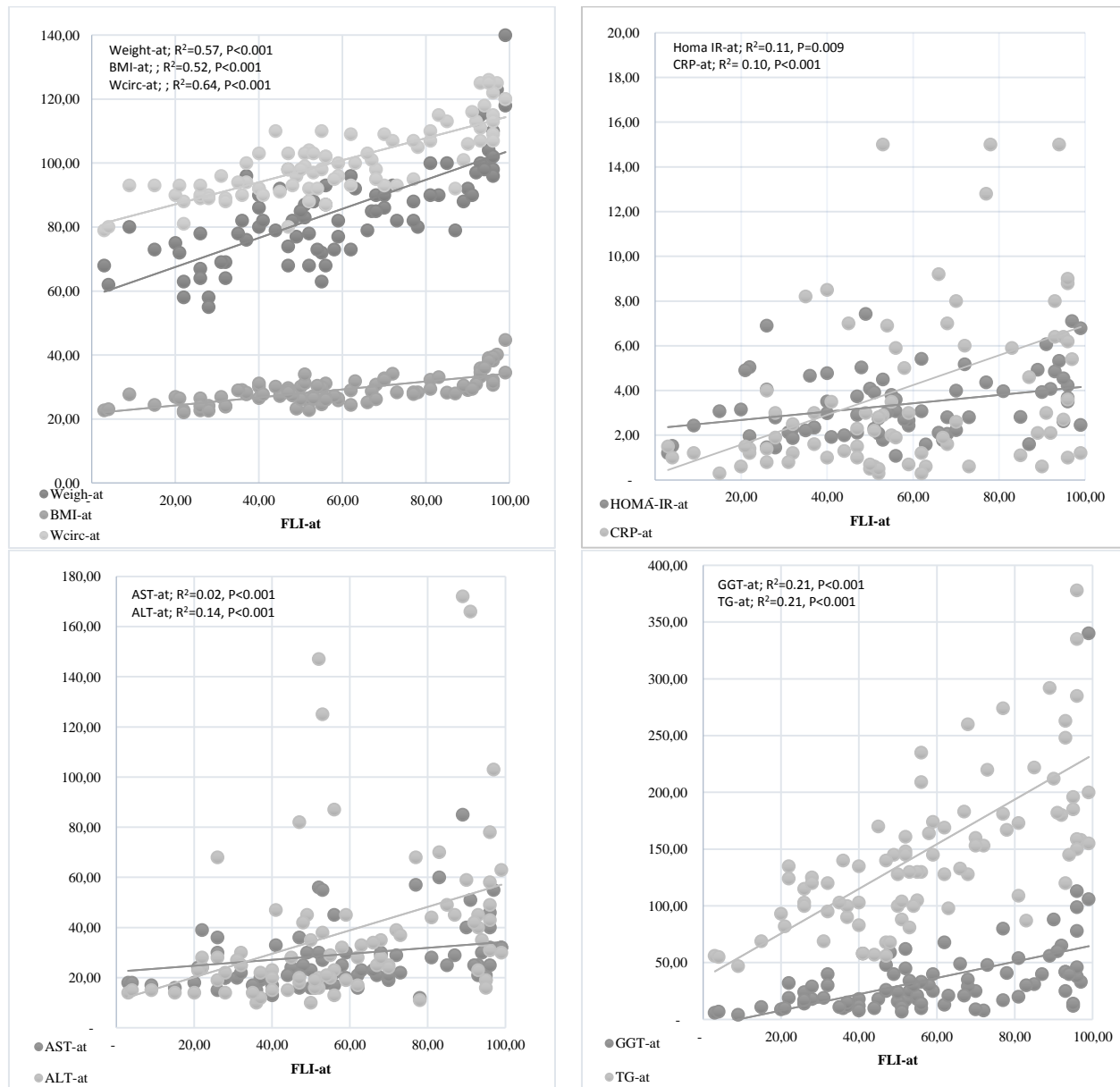
\*  $P<0.05$ , \*\* $P<0.01$ , Mann Whitney U test

**Correlations between post-treatment outcomes**

A positive correlation was found between FLI, morphological outcomes (weight, BMI, waist circumference) and FLI ( $P<0.001$ ,  $P<0.001$ , and  $P<0.001$  respectively), and various post-treatment laboratory values (HOMA-IR, CRP, AST, ALT, GGT, TG) ( $P=0.009$ ,  $P<0.001$ ,  $P<0.001$ ,  $P<0.001$ ,  $P<0.001$ , and  $P<0.001$  respectively). Post-treatment vitamin D was not correlated with FLI, while it was positively correlated with waist circumference ( $P=0.019$ ) (Figure 1).

HOMA-IR was positively correlated with the morphological outcomes (weight, BMI, waist circumference) and the ALT level after treatment ( $P=0.005$ ,  $P=0.013$ ,  $P=0.007$ , and  $P=0.027$ , respectively). Post-treatment CRP was also positively correlated with the morphological outcomes (weight, BMI, waist circumference) ( $P=0.016$ ,  $P=0.004$ , and  $P=0.062$ , respectively) and the ALT, AST, GGT, TG level after the treatment ( $P=0.012$ ,  $P=0.029$ ,  $P=0.026$ , and  $P=0.010$ , respectively).

Figure 1: Correlation of FLI with treatment outcomes



## Discussion

This is the first study investigating the therapeutic efficacy of vitamin D alone and in combination with metformin in non-alcoholic liver fattening. We used the "Fatty Liver Index" for liver fattening and treatment outcomes were evaluated with FLI, laboratory and anthropometric parameters. The most interesting result of this study is that the FLI decrease was significant in the control group, which did not use metformin or vitamin D, and in the  $G_{\text{Met-Dvit}}$ . According to the rest of our results, it can be stated that vitamin D has a reductive pleotropic effect on the fatty liver when combined with Metformin.

CRP is an inflammation parameter, and it shows the pleotropic effect better than FLI, because the FLI is affected by TG, BMI and waist circumference. The decrease in CRP levels is another striking result of this study. In the Dvit and Met-Dvit Groups, CRP levels were significantly decreased after therapy.

The first study on the relationship between fatty liver and vitamin D was performed on 262 patients with metabolic syndrome at Sapienz University in 2011. Vitamin D deficiency was more common in fatty liver patients, and FLI and 25 (OH) vitamin D levels were negatively correlated [10]. In this study, there was no correlation between vitamin D and FLI, the probable reason being the inclusion of patients with low vitamin D levels only.

In another study conducted on 82 NAFLD cases, the changes in the fat parameters with exercise and diet were investigated, and despite the reduction in calories and vitamin D intake an increment was observed in serum 25-hydroxy vitamin D levels of the NAFLD patients. This resulted in an improvement in serum vitamin D levels and metabolic parameters without vitamin D supplementation in NAFLD patients [14].

Another study investigated the effects of different doses of Metformin on liver biochemistry (aminotransferases), histology and metabolic syndrome [15]. In 2001, Marchesini et al. [16] performed a non-randomized study using metformin (administered for 4 months, 1.5g/day) in 20 patients with non-diabetic NASH and found a significant improvement in insulin resistance, aminotransferase levels, liver morphology and volume in the treated group compared to the diet group. Histologic recovery could not be evaluated because biopsy was not performed. In another study on 17 non-diabetic patients who received metformin, the effect of metformin (twice a day 850 mg) vs. dieting on the fatty liver was investigated by biopsy and no histologic difference was detected. However, ALT, AST, BMI, and insulin resistance markers significantly improved when compared with the control group [17]. In this study, there was no change in liver enzymes or cholestasis tests but BMI, waist circumference and HOMA-IR results decreased in the Met group, while Vitamin D did not increase.

Metformin was compared with placebo among NAFLD patients in a meta-analysis including 417 cases evaluating 4-12 months of follow-up results. In the treated group, there were significant improvements in ALT (-8.12 U/I), AST (-4.52 U/I), HOMA-IR (-0.61), and BMI (-0.82 kg/m<sup>2</sup>), and insignificant improvements in histological response (steatosis, inflammation, hepatocellular ballooning, and fibrosis). Current information shows that metformin improves liver function, HOMA-IR, and

BMI to some extent, but does not improve histological response in NAFLD patients [18].

In this study, HOMA-IR was significantly reduced by 2.10 in the Met group, by 1.30 in the MetDvit group, and insignificantly reduced by -0.121 in the Dvit group. In the control group, however, HOMA-IR did not change. Reduction in AST, ALT, GGT levels were significant in none of the groups. This might be due to the fact that initially, the baseline values were not high.

Although vitamin D was not administered to the control group, Fatty Liver Index and TG were decreased. These changes could be explained by doing more exercise, more sunbathing and adaptation to diet in the control group patients, who focused mostly on a lifestyle change.

All findings considered, it is safe to state that a combination of Metformin and vitamin D is more effective on liver fattening than single therapies. Also, various new peptide hormones, for example, preptin, can be associated with vitamin D and insulin resistance and cause NASH [19].

## Limitations

The low number of cases prevented strong interpretations of the results. Also, due to the retrospective and single center nature of the study, no randomization could be performed. Further larger, prospective and randomized controlled trials evaluating histological outcomes are needed to shed light on the effect of metformin and vitamin D on fatty liver disease.

## Conclusion

Our study showed that a combination of vitamin D and metformin could positively contribute to the regression of fatty liver. Clinical trials with metformin give hope in managing liver diseases by improving the metabolic features of fatty liver disease.

## References

- Caldwell SH, Oelsner DH, Iezzoni JC, Hespeneide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology*. 1999;29:664-9.
- Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc*. 1980;55:434-8.
- Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology*. 1998;114:842-5.
- Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, et al. Identification and characterization of metabolically benign obesity in humans. *Arch Intern Med*. 2008;168(15):1609-16.
- He L, Sabet A, Djedjos S, Miller R, Sun X, Hussain MA, et al. Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. *Cell*. 2009;137(4):635-46.
- Turlin B, Mendler MH, Moirand R, Guyader D, Guillygomarc'h A, Deugnier Y. Histologic features of the liver in insulin resistance-associated iron overload. A study of 139 patients. *Am J Clin Pathol*. 2001;116:263.
- Huyppens P, Quartier E, Pipeleers D, Van De Castele M. Metformin reduces adiponectin protein expression and release in 3T3-L1 adipocytes involving activation of AMP activated protein kinase. *Eur J Pharmacol*. 2005;518:90-5.
- Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM. Metformin reverses fatty liver disease in obese, leptin deficient mice. *Nat Med*. 2000;6:998-1003.
- Dasarthy J, Periyalwar P, Allampati S, Bhinder V, Hawkins C, Brandt P, et al. Hypovitaminosis D is associated with increased whole-body fat mass and greater severity of non-alcoholic fatty liver disease. *Liver Int*. 2014;34:118-27. doi: 10.1111/liv.12312.
- Barchetta I, Angelico F, Ben MD, Baroni MG, Pozzili P, Morini S, et al. Strong association between non-alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. *BMC Med*. 2011;9:85.
- Targher G, Bertolini L, Scala L, et al. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis*. 2007;17(7):517-24. doi: 10.1016/j.numecd.2006.04.002.
- Eliades M, Spyrou E, Agrawal N, Lazo M, Brancati FL, Potter JJ, et al. Meta-analysis: vitamin D and non-alcoholic fatty liver disease. *Alimentary Pharmacology and Therapeutics*. 2013;38:246-54.
- Bedogni G, Bellentani S, Miglioli L, Massuti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol*. 2006;6:33.
- Lee SM, Jun DW, Cho YK, Jang KS. Vitamin D deficiency in non-alcoholic fatty liver disease: The chicken or the egg? *Clin Nutr*. 2015 Epub ahead of print.
- Cowin GJ, Jonsson JR, Bauer JD, Ash S, Ali A, Osland EJ, et al. Magnetic resonance imaging and spectroscopy for monitoring liver steatosis. *J Magn Reson Imaging*. 2008;28(4):937-45.
- Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in non-alcoholic steatohepatitis. *Lancet*. 2001 Sep 15;358(9285):893-4. doi: 10.1016/S0140-6736(01)06042-1.

17. Abrams GA, Kunde SS, Lazenby AJ, Clements RH. Portal fibrosis and hepatic steatosis in morbidly obese subjects: A spectrum of non-alcoholic fatty liver disease. *Hepatology*. 2004;40:475– 83.
18. Li Y, Liu L, Wang B, Wang J, Chen D. Metformin in non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Biomedical reports*. 2013;1(1):57–64.
19. Arğun D, Basim P, Uysal BB, Argun F. Association of circulating preptin with non-alcoholic fatty liver disease: A case-control study. *J Surg Med*. 2021;5(6):628-32.

This paper has been checked for language accuracy by JOSAM editors.

The National Library of Medicine (NLM) citation style guide has been used in this paper.