Original Article

Regression of Non-Alcoholic Fatty Liver by Vitamin D Supplement: A Double-Blind Randomized Controlled Clinical Trial

Hamid Lorvand Amiri MSc[1], Shahram Agah MD[2], Seyedeh Neda Mousavi PhD[3], Agha Fatemeh Hosseini PhD[4], Farzad Shidfar PhD•[1,2]

Abstract

Objective: Evidence indicates that NAFLD patients are vitamin D deficient. Vitamin D has new roles in lipid and glucose metabolism. The aim of this study was to examine the effects of calcitriol supplementation on the NAFLD progression based on liver lipid accumulation, serum lipid profile and insulin resistance.

Methods: We randomly assigned 73 patients with NAFLD-confirmed by ultra-sonography to 12 weeks of treatment with hypocaloric diet (reduction of 500 kcal per day) plus 25 µg of calcitriol supplement or a hypocaloric diet plus placebo. Before and after treatment, we assessed anthropometric parameters, grade of fatty liver, serum lipoproteins, liver enzymes and insulin level.

Result: Baseline variables were not significantly different between groups. A statistically significant reduction in anthropometric measures was observed over the 12 weeks in both groups. However, no significant differences were seen between groups after intervention. Compared with the placebo, reductions in triglyceride and an increase in HDL cholesterol were seen over the 12 weeks of intervention in the calcitriol group ($P = 0.002$ and $P = 0.004$). AST level was decreased in the calcitriol group (-4.2 ± 4.3 µmol/L, $P < 0.001$), but increased in the placebo group (12.6 ± 6.1 µmol/L, $P = 0.02$) after 12 weeks. Reductions in mean difference of ALT, insulin and HOMA were significantly higher in the calcitriol than placebo group ($P = 0.01$, $P = 0.007$ and 0.01).

Conclusions: Calcitriol supplementation combined with weight loss diet showed no significant effects on anthropometric measures in NAFLD patients. However, it may have positive effects on lipid profile, liver enzyme tests and insulin sensitivity during a weight-loss program.

Keywords: Anthropometry, calcitriol, insulin resistance, non-alcoholic fatty liver disease

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Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD), a common metabolic disorder, causes abnormal functions of the liver due to accumulation of fat cells.[1,2] NAFLD is a spectrum which varies from fatty liver disease alone to non-alcoholic steatohepatitis (NASH).[3] Simple steatohepatitis is the first step of disease, but it may lead to ulcers, inflammation, fibrosis and cirrhosis with disease progression,[4] which has a direct link with visceral obesity, glucose intolerance and dyslipidemia.[5,6] Visceral obesity leads to insulin resistance and inflammation which are the main causes of NAFLD. Prevalence of NAFLD is estimated to be 20% – 30% in the Western countries[7,8] and 15% in Asia.[9] Weight loss remains the standard of care because no pharmacologic therapy has conclusively proved effective for treatment of this condition. Multiple pharmacologic interventions have been attempted with variable success; these include pentoxifilline[10] orlistat,[11] vitamin E,[12,13] ursodeoxycholic acid,[14] and lipid-lowering agents.[15] Trials of glucose-lowering agents such as metformin[12,16] and thiazolidinediones[16–19] have yielded promising results, but to our knowledge, no randomized, placebo-controlled studies have provided conclusive support for their use.

Vitamin D is a fat-soluble vitamin that exists in a number of food products such as oils and dairy products. In recent decades, a decreasing trend in serum 25 hydroxy vitamin D (25(OH) D) levels has been associated with decreasing rate of milk consumption, avoidance of sunlight exposure, and increasing rate of body mass index (BMI).[20] Vitamin D deficiency (VDD) is one of the most prevalent micronutrient deficiencies worldwide, and the rate of this deficiency is estimated to be 30% – 50%.[21] Vitamin D is traditionally known as the regulator of calcium and phosphorus metabolism, but in recent years a significant relationship has been observed between serum levels of Vitamin D and risk of chronic diseases such as diabetes and cardiovascular diseases.[22] Vitamin D receptors are present in more than 38 tissues and its receptors affect genes controlling oxidative stress and inflammation.[23] Several studies have shown a significant relationship between vitamin D deficiency and NAFLD,[24–26] obesity and insulin resistance.[2,27,28] However, in several studies, there was no association between Vitamin D concentration and severity of fatty liver.[29,30] Serum levels of 25(OH)D are associated with chronic metabolic diseases including insulin resistance (IR), type 2 diabetes, cardiovascular disease, metabolic syndrome and fatty liver.[31] Vitamin D receptor (VDR) regulates lipid and glucose metabolism in the liver.[32,33] Unfortunately, dietary vitamin D is not enough and the solar ultraviolet radiation to the skin is a better source of vitamin D

Authors' affiliations: [1]Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran, [2]Colorectal Research Center , Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran, [3]Department of Biochemistry and Nutrition, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran, [4]Department of Biostatistics, School of Public Health, Iran University of Medical Sciences, Tehran, Iran.
•Corresponding author and reprints: Farzad Shidfar PhD, Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran. Tel/fax: +98-21-88622533, E-mail: shidfar.f@iums.ac.ir.
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for the body.[34] Nakano, et al.[35] investigated the effect of sunlight therapy and vitamin D supplementation on NAFLD progression in a sample of rats. Sunlight therapy increased the active form of vitamin D in serum of rats and additionally attenuated insulin resistance and hepatic steatosis. The results of a recent study by Roth, et al.[36] also showed that lack of vitamin D intake in rats led to NAFLD progression through increased gene expression involved in inflammatory and oxidative stress pathways. According to previous studies and the major role of vitamin D in insulin resistance, we speculate that vitamin D may have a role in NAFLD progression via its effect on IR and lipid profile. So, the aim of this study was to examine the effects of calcitriol supplementation on NAFLD progression based on liver lipid accumulation, serum lipid profile and insulin resistance.

Materials and Methods

Study design and participants

A randomized double blind placebo-controlled trial was conducted to compare calcitriol supplementation on NAFLD progression risk based on liver lipid accumulation, serum lipid profile and insulin resistance in patients with fatty liver in Rasool Akram Hospital, Tehran, Iran.

Patients who met the following inclusion criteria entered this study: 1) diagnosis of NAFLD according to ultra-sonography; 2) BMI < 35 kg/m^2; 3) age 18 – 65 years; 4) serum 25 (OH) D level < 15 ng/mL, and 5) Iranian ethnicity. The exclusion criteria included: 1) BMI < 25 kg/m^2; 2) lactating or pregnancy; 3) diagnosis of chronic diseases including inflammatory diseases, heart, liver and renal failure, cancer, acute myocardial infarction, diabetes, stroke, or serious injuries; 4) consumption of hepatotoxic drugs such as phenytoin, amiodarone, levothyroxine, tamoxifen; 5) receiving multivitamin or antioxidant supplements at least 3 months; 6) smoking or alcohol consumption; 7) any malabsorption diseases such as celiac disease or steatorrhea; 8) liver hereditary diseases such as iron or copper storage disease, and 9) athletes.

The study was approved by the Bioethics Committee of Iran University of Medical Sciences, Tehran, Iran (IRCT 201408312709N29). The written consent forms were signed by all the participants before participation. Eighty patients were randomly assigned to receive a 1000 IU supplement of vitamin D (25 µg/d as calcitriol; Jalinus Arya Co. Iran) or exactly the same placebo (25 µg/d as lactose; Jalinus Arya Co. Iran), after lunch with a glass of water for 12 weeks. Shape, color and packaging of placebo were similar to the calcitriol in the supplemented group. The products were administered by a blinded researcher assistant to blinded patients. First, calcitriol and placebo were divided into similar packages for one week intake. Then, residuals of each package were assessed to determine the compliance of the patients.

The participants were instructed to maintain a low calorie diet, 500 kcal reduced from previous eating habits, and not to change their routine physical activity during the study period. The participants were randomly assigned to one of the two groups via computer-generated numbers.

Outcome measures

Blood samples were collected from the antecubital vein after 12 h overnight fasting. After centrifugation for 20 min (3000 *g*), the serum samples were frozen simultaneously and stored at -80˚C until analyzed. In order to eliminate the probable effects of sex hormones on blood lipids, blood sampling was not performed between days 1 and 5 of the menstrual cycle in women. At the baseline and after 12 weeks of treatment, liver fat accumulation, serum lipid profile, fasting plasma glucose and insulin levels were measured for both groups. Fasting plasma glucose, lipid profile and liver enzymes were measured by an enzymatic method (Pars Azmoon Co. kit, Tehran, Iran) using Liasys autoanalyzer while insulin was measured by IRMA method (Immunotech Co. kit); homeostasis model assessment for insulin resistance (HOMA-IR) (fasting glucose (mg/dL) × fasting insulin (µmol/mL) /405) was used as the major outcome measurement. 25-OH vitamin D was measured by ELISA kit (SE120139, Sigma Aldrich).

Height was measured with a wall-mounted stadiometer to the nearest 0.5 cm. Weight was measured on a calibrated Seca scale to the nearest 100 gram, in fasting with minimal clothing and no shoes. Body Mass Index (BMI) was calculated according to the formula: BMI = weight / height^2 (kg/m^2). Body fat was measured by Bioelectrical Impedance Analyzer (BIA) setting at the beginning and end of the study. BIA was performed with an impedance plethysmograph, which emitted 800 µA and 50 kHz alternating sinusoidal current (model BIA-109 RJL/Akern Systems, Detroit, Michigan, USA) and was connected to surface electrodes (standard, tetrapolar placement on the right hand and foot). The demographic data were collected during the initial anthropometric assessment.[37]

Dietary intake and physical activity assessment

Energy, macronutrient and micronutrient intake were estimated using 24 h dietary recall in three days (two regular days and one holiday) and validated FFQ.[38] At the beginning and end of the study, a blinded nutritionist completed the questionnaires through a direct interview. The dietary intake data were analyzed using Nutritionist IV software (version 4.1; First Databank Division, The Hearst Corporation) to assess macronutrient and micronutrient contents of the food. Physical activity level was assessed by international physical activity questionnaire (IPAQ)[39] in three days (two regular days and one holiday) at the beginning and end of the study.

Grades of fatty liver classification

Liver ultrasound device Siemens brand Sonoline G50 series and 3.5 to 5 MHz probe made in Germany were used for liver sonography. Liver steatosis was classified through sonographic echogenicity of liver as: 1) normal: echogenicity as the same as renal cortex; 2) grade I: mild steatosis; increased hepatic echogenicity with visible periportal and diaphragmatic echogenicity; 3) grade II: moderate steatosis; increased hepatic echogenicity with imperceptible periportal echogenicity, without obscuration of diaphragm; 4) grade III: sever steatosis; increased hepatic echogenicity with imperceptible periportal echogenicity and obscuration of diaphragm.[40]

Primary and secondary outcomes

The primary outcome was an improvement in grade of fatty liver disease. Secondary outcomes included changes in serum aminotransferase levels, anthropometric measures, insulin resistance and lipid profile.

Statistical Analysis

In designing the study, we considered a power of 80% with a

two-sided test with α = 0.05 (type I error) and standard deviation (SD) difference of 1.64 for ALT. On the basis of SDs, reported in similar studies,[41] the number of subjects needed to be treated in order to detect this difference was 36/group. Given an anticipated dropout rate of 10%, we set the enrollment target at 40 subjects.

All data were expressed by means ± SD. The level of significance was set at $P < 0.05$. Statistical analyses were performed with IBM SPSS Statistics software (version 18; IBM Corp). Normal distribution of the variables was checked by Kolmogorov-Smirnov test. Baseline parameters were analyzed by Chi-square test for homogeneity assessment. ANCOVA test was used to adjust the effects of baseline parameters on outcome measures in the calcitriol compared with the placebo group. Independent student's *t*-test was used to test whether the differences between the mean values of the items studied in both groups were significant. The comparison of mean values of variables before and after the intervention in each group was examined by paired *t*-tests. Chi-square test was used to compare the qualitative variables before and at the end of the study.

Results

Baseline characteristics

From 85 patients with serum 25 (OH) D levels lower than 15 ng/mL, 5 patients did not meet the inclusion criteria. The remaining patients gave written consent, and totally 36 patients were analyzed in the placebo and 37 patients in the calcitriol group. There was no significant difference in energy and nutrient intake at the beginning of the study between two groups and also no significant change was seen at the end of the study (Table 1). The level of physical activity was not different between the two groups at baseline and also at the end of the study. At baseline, there was no significant difference between the two groups (Tables 1, 2 and 3).

Anthropometric measurements

As shown in Table 4, there was significant weight reduction in both groups after 12 weeks of the study (placebo = -1.84 ± 1.75 kg, calcitriol = -1.8 ± 2.01 kg, $P < 0.001$ and $P < 0.001$). As shown in Table 4, there was not a significant difference between the groups after 12 weeks of the intervention ($P = 0.06$).

BMI reduction was significant in the calcitriol group (-1.09 ± 0.77 kg/m^2, $P < 0.001$). However, there was no significant difference between the groups in BMI reduction after 12 weeks of intervention.

In both groups, body fat mass, waist and hip circumferences had decreased significantly after 12 weeks of intervention ($P < 0.001$). Body fat decline was -2.25% ± 2.2% in the calcitriol and -2.35% ± 2.9% in the placebo group. The waist and hip circumferences decline were -3.1 ± 3.5 cm and -2.4 ± 2.04 cm in the calcitriol, as well as -2.5 ± 2.8 cm and -1.4 ± 2.4 cm in the placebo group (Table 4). There was no significant difference between the groups after 12 weeks of intervention in BMI, weight, fat mass, waist and hip circumferences reduction, adjusted for age.

Biochemical measurements

Reductions in triglyceride and ALT levels and an increase in HDL cholesterol were seen over the 12 weeks of intervention in the calcitriol group ($P = 0.002$, $P < 0.001$ and $P = 0.004$, respectively). Furthermore, according to the ANCOVA test, there were significant improvements in triglyceride and HDL cholesterol in the calcitriol group compared with the placebo group over 12 weeks (Table 4). Reduction of ALT level was significantly higher

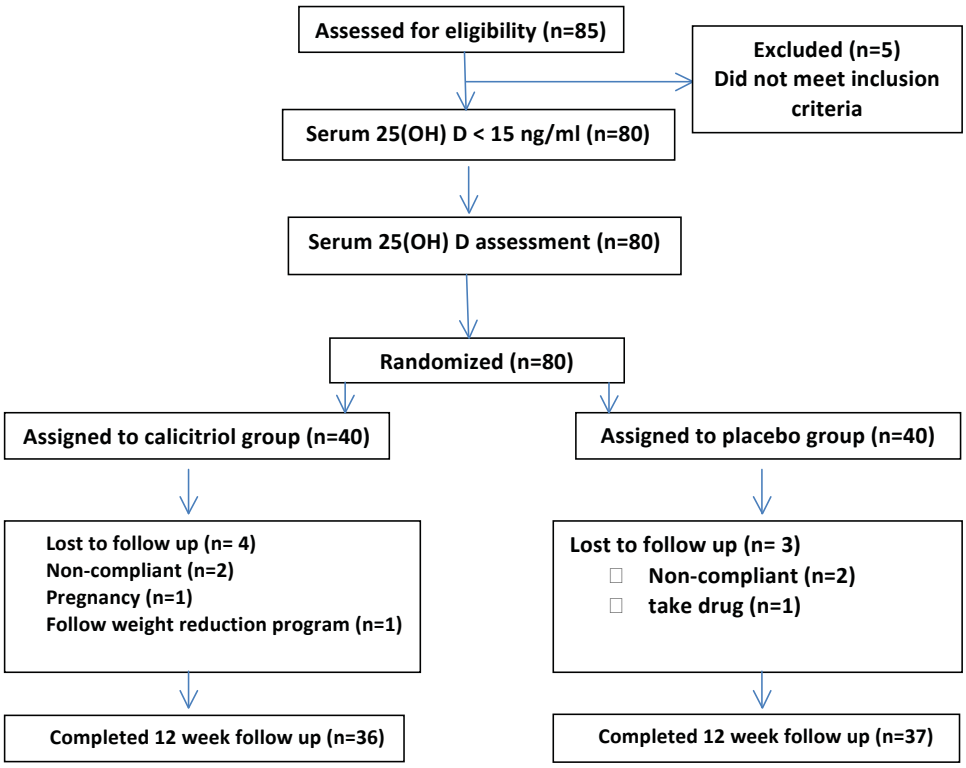

Figure1. Follow-up of participants through the intervention

Table 1. Daily dietary intake of energy and some nutrients of the two study groups

Dietary intake	Calcitriol group (n = 37)	placebo group (n=36)	*P*-value*
Total Energy (Kcal)			
Before intervention	2178.18 ± 385.17	2261.92 ± 437.07	0.37
After intervention	1743.92 ± 345.91	1803.81 ± 391.29	0.48
Total protein (g/day)			
Before intervention	79.85 ± 20	85.8 ± 19.93	0.102
After intervention	77.43 ± 22.71	86.11 ± 17.11	0.08
Total carbohydrate (g/day)			
Before intervention	282.72 ± 52.33	301.42 ± 72.6	0.24
After intervention	223.3 ± 53.21	238.9 ± 68.52	0.19
Total fat (g/day)			
Before intervention	82.81 ± 24.27	83.33 ± 20	0.82
After intervention	54.32 ± 24.7	57.91 ± 19.5	0.48
SFA[1] (g/day)			
Before intervention	21.99 ± 6.32	23.58 ± 6.51	0.34
After intervention	9.47 ± 6.64	10.35 ± 6.11	0.46
PUFA[2] (g/day)			
Before intervention	59.87 ± 17.89	60.4 ± 14.7	0.89
After intervention	45.14 ± 21.39	40.27 ± 19.19	0.31
Fiber (g/day)			
Before intervention	14.79 ± 5.83	16.74 ± 7.59	0.21
After intervention	13.8 ± 5.49	13.92 ± 9.76	0.63
Vitamin C (mg/day)			
Before intervention	107.34 ± 75.86	127.83 ± 85.77	0.27
After intervention	110.34 ± 74.76	130.94 ± 83.32	0.26
Vitamin E (mg/day)			
Before intervention	13.04 ± 7.54	10.68 ± 8.13	0.19
After intervention	12.7 ± 7.04	10.58 ± 7.95	0.21
Calcium (mg/day)			
Before intervention	873 ± 586	677 ± 386	0.08
After intervention	829 ± 533	625 ± 454	0.18
Vitamin D (µg/day)			
Before intervention	0.53 ± 0.6	0.39 ± 0.37	0.22
After intervention	0.4 ± 0.47	0.37 ± 0.35	0.7

Data are expressed as means ± SD; *Differences between groups were evaluated by independent *t*- test; 1SFA: Saturated Fatty Acid; 2PUFA: Polyunsaturated Fatty Acid

in the calcitriol group compared with the placebo group after 12 weeks (-7.5 ± 7.3 µmol/L *vs.* -2.8 ± 8.5 µmol/L, $P = 0.01$). AST level was decreased in the calcitriol group (-4.2 ± 4.3 µmol/L, $P < 0.001$), but it increased in the placebo group (12.6 ± 6.1 µmol/L, $P = 0.02$) after 12 weeks.

After adjusting for the baseline measures by ANCOVA test, there was no significant difference in serum insulin and HOMA-IR reduction in the calcitriol group compared with the placebo group after 12 weeks of the study. Reductions in serum levels of FPG and insulin, as well as HOMA were seen over the 12 weeks of intervention in the calcitriol group ($P = 0.001$, $P < 0.001$ and $P = 0.001$, respectively). Adjusted for age of participants, reductions in serum level of insulin and HOMA-IR were seen over 12 weeks of intervention in the calcitriol group ($P = 0.004$ and $P = 0.01$, respectively), but HDL.C level was increased ($P = 0.025$) compared with the placebo.

Adjusted for the differences in body size including weight, BMI, body fat, waist and hip circumferences, calcitriol had significant effects on total cholesterol ($P = 0.005$) and FPG ($P = 0.03$) in NAFLD patients.

Table 2. patient characteristics before intervention[1]

Variables	Placebo group (n = 36)	Calcitriol group (n = 37)
Age, y	44 ± 10.8[2]	39.8 ± 11
Sex		
Female, %	35.3%	40.5%
Male, %	64.7%	59.5%
Education		
Illiterate, %	7%	5%
Diploma, %	32.5%	30%
College, %	60.5%	65%
Job		
Employee	25%	32.5%
Self-employed	75%	67.5%
Body weight, Kg	91.2 ± 13.7	90.9 ± 13.8
BMI, Kg/m²	30.3 ± 3.5	30.3 ± 3.9
WC, cm	109.6 ± 9.9	106.4 ± 10.8
HC, cm	112.6 ± 9.4	109.4 ± 6.7
Body fat, %	34.9 ± 7.7	35.3 ± 7.4
Married, %	86.7%	87.5%
FPG, mg/d L	90.5 ± 6.6	92.2 ± 5.1
Insulin (µU/L)	15.8 ± 5.6	19.04 ± 6.4
HOMA-IR	3.55 ± 1.3	4.3 ± 1.5
TC, mg/dL	187.2 ± 29.5	185 ± 31.5
LDL cholesterol, mg/dL	112.5 ± 25.1	108.5 ± 25.7
HDL cholesterol, mg/dL	38.6 ± 9.9	36.3 ± 6.6
TG, mg/dL	198.6 ± 90.6	197.6 ± 79.2
ALT, µmol/L	46.9 ± 18.1	45.9 ± 14.5
AST, µmol/L	31.5 ± 18.1	30.5 ± 7.7
25 (OH) D, ng/mL	10 ± 3.8	9.9 ± 3.9

[1]Group difference, $P > 0.05$. WC: waist circumference; HC: hip circumference; FPG: fasting plasma glucose; TC: total cholesterol; TG: triglyceride; ALT: alanine amino transferase; AST: aspartate amino transferase; [2]Means ± SD (all such values).

Table 3. Grade of fatty liver in the calcitriol supplemented and placebo groups

Group	Grade of fatty liver	Before, N (%)	After, N (%)	Changes†; N	*P*-value*
Calcitriol	Normal	0	15 (40.5)		
	Stage 1	19 (51.3)	20 (54)	Without change: 4	
	Stage 2	16 (43.2)	2 (5.4)	1 degree reduction: 33	
	Stage 3	2 (5.4)	0		
Placebo	Normal	0	3 (8.3)		< 0.001
	Stage 1	22 (61.1)	19 (52.7)	Without change: 31	
	Stage 2	11 (30.5)	12 (33.3)	1 degree reduction: 5	
	Stage 3	3 (8.3)	2 (5.5)		

†Reduction in the grade of NAFLD after 12 week of the study; **P*-value is for the calcitriol group relative to the placebo group by using an ANCOVA with BMI changes as covariate

Grade of NAFLD

At baseline, there was no significant difference between patients in the grade of NAFLD, but after 12 weeks of the study, the grade of NAFLD improved significantly in the calcitriol group compared with the placebo group ($P = 0.002$). Decrease in the grade of NAFLD was significantly higher in the calcitriol compared with the placebo group, adjusted for BMI changes ($P < 0.001$) (Table 3)

Discussion

Epidemiological studies have shown that low serum levels of vitamin D are associated with NAFLD.[42] as Also, a meta-analysis has reported that NAFLD patients are 26% more likely to be vitamin D deficient compared to controls.[43] Although the cross-sectional design of these studies[44] does not allow establishment of

Table 4. Anthropometric and biochemical characteristics in the placebo and calcitriol supplemented groups before and after the 12-wk interventions[1]

Variables	Placebo group (n = 36)		Mean difference	Calcitriol group (n=37)		Mean difference	*P*-value[2]
	Baseline	Week 12		Baseline	Week 12		
Weight, kg	91.2 ± 13.7	89.4 ± 13.9	-1.84 ± 1.75	90.9 ± 13.8	89.1 ± 13.9	-1.8 ± 2.01	0.06
BMI, kg/m^2	30.3 ± 3.5	29.9 ± 2.8	-0.44 ± 1.78	30.3 ± 3.9	29.2 ± 4.1	-1.09 ± 0.77	0.07
WC, cm	109.6 ± 9.9	107.1 ± 9.6	-2.5 ± 2.8	106.4 ± 10.8	103.3 ± 11.4	-3.1 ± 3.5	0.32
HC, cm	112.6 ± 9.4	111.2 ± 9.8	-1.4 ± 2.4	109.4 ± 6.7	106.9 ± 7.4	-2.4 ± 2.04	0.23
BF, %	34.9 ± 7.7	32.6 ± 7.9	-2.35 ± 2.9	35.3 ± 7.4	33 ± 7.3	-2.25 ± 2.2	0.83
FPG, mg/dL	90.5 ± 6.6	89.4 ± 7.8	-1.05 ± 6.2	92.2 ± 5.1	89.9 ± 5.4	-2.27 ± 3.7	0.38
Insulin, µU/L	15.8 ± 5.6	14.8 ± 4.2	-1.01 ± 3.3	19.04 ± 6.4	15.7 ± 5.5	-3.29 ± 3.6	0.08
HOMA	3.55 ± 1.3	3.3 ± 0.99	-0.28 ± 0.89	4.3 ± 1.5	3.5 ± 1.3	-0.81 ± 0.87	0.2
TC, mg/dL	187.2 ± 29.5	193.2 ± 26.4	5.9 ± 17.8	185 ± 31.5	186.3 ± 31.5	1.08 ± 16.8	0.23
HDL.C, mg/dL	38.6 ± 9.9	38.2 ± 8.6	-0.43 ± 4.1	36.3 ± 6.6	38.1 ± 6.2	1.75 ± 3.5	0.04
LDL.C, mg/dLl	112.5 ± 25.1	115.6 ± 19.4	3.1 ± 14.4	108.5 ± 25.7	112.2 ± 26	3.59 ± 14.3	0.45
TG, mg/dL	198.6 ± 90.6	195.8 ± 77.9	-2.8 ± 37.5	197.6 ± 79.2	169.2 ± 64.9	-28.5 ± 50.8	0.006
ALT, µmol/L	46.9 ± 18.1	44.1 ± 20.3	-2.82 ± 8.5	45.9 ± 14.5	38.3 ± 13.9	-7.5 ± 7.3	0.35
AST, µmol/L	31.5 ± 18.1	29.5 ± 14.3	-2 ± 3.8	30.5 ± 7.7	26.3 ± 9.3	-4.16 ± 4.3	0.27
25 (OH) D[a], ng/mL	10.06 ± 3.8	11 ± 4.7	0.94 ± 2.1	9.9 ± 3.9	27.1 ± 7.2	17.24 ± 6.3	<0.001

[1]Values are means ± SDs; WC: waist circumference; HC, hip circumference; BF: body fat; FPG: fasting plasma glucose; TC: total cholesterol; TG: triglyceride; ALT: alanine amino-transferase; AST: aspartate amino-transferase; [2]*P*-values are for the calcitriol group relative to the placebo group by using an ANCOVA with baseline values as covariate a To convert 25(OH) D values to ng/mL, divide by 2.5.

a causative nature for the associations between hypovitaminosis of vitamin D and NAFLD. There is a linear inverse correlation between serum 25(OH) D levels and the degree of NAFLD, which suggests that vitamin D may exert a dose-dependent effect on fat accumulation in hepatocytes. To our knowledge, this is the first randomized controlled trial (RCT) conducted to assess the effects of calcitriol supplementation in combination with weight reduction diet on liver lipid accumulation, serum lipid profile and insulin sensitivity in patients with NAFLD.

In the present study, reduction in anthropometric measures was observed over 12 weeks in both groups. However, no significant differences were seen between groups after intervention. Adjusted for the baseline parameters, TG level decreased and HDL.C increased in the supplemented compared to the placebo group. AST level was decreased in the calcitriol group, but it increased in the placebo group after 12 weeks. Reductions in mean difference of ALT, insulin and HOMA were higher in the calcitriol than the placebo group.

One cross-sectional study showed that the presence of NAFLD was significantly associated with recent osteoporotic fracture in middle-aged and elderly Chinese men.[45] The causative effect of vitamin D was not evaluated in this study, but findings suggested the need for screening men with NAFLD for the presence of osteoporosis in fracture prevention. A review article conclude that NAFLD may act as a risk factor for reduced bone mineral density due to increase in inflammatory markers.[46] Increasing evidence shows that vitamin D3 has roles in fat and lean body mass regulation.[47–50] There are few clinical trials on the effects of vitamin D3 supplementation on body fat.[42] These studies have inconsistent results due to different doses, duration, sample size and type of participants. Also, vitamin D status is different among populations and also in patients in comparison to healthy subjects. Numerous studies have proposed that low levels of 25(OH) D are strongly associated with features of the metabolic syndrome.[51,52] In some human studies, vitamin D has improved insulin resistance and glucose intolerance.[53] In addition, the findings of animal studies have shown that lack of vitamin D receptor (VDR) or vitamin D deficiency impair insulin secretion from pancreatic beta cells.[54] In our study, both groups had vitamin D deficiency before the beginning of the intervention. Anthropometric measures decreased in both groups, because all patients were on weight loss diet, but reductions in anthropometric measures were not significantly different between the two groups. Salehpour, et al.[42] investigated the effects of 12-week vitamin D3 supplementation on the anthropometric indices in healthy overweight and obese women. They showed that fat mass was reduced in the supplemented group, but weight and waist circumference did not change. In our study, fat mass and waist circumference were reduced in the placebo and intervention groups. All studies on the effects of vitamin D supplementation on body composition were done with calcium, and also no study has been done in NAFLD patients. In our study, calcitriol supplementation was accompanied by weight loss diet. The other results may be due to this difference.

In the current study, calcitriol supplementation reduced serum levels of insulin and insulin resistance (HOMA-IR) at the end of the study. These results are different from a recent meta-analysis of 35 RCTs, in which a total of 43407 patients were assessed.

Vitamin D had no significant effect on prevention of diabetes in non-diabetic subjects, or reduction of insulin resistance in pre-diabetic or diabetic patients. These studies were heterogeneous and different in follow-up.[55]

In one study, Targher, et al.[56] assessed the association between biopsy-proven NAFLD and vitamin D levels. In this study, 25(OH)D levels predicted the histological severity of NAFLD. Four studies confirmed this result in adults and children.[57–60] These results are consistent with our study in which calcitriol supplementation improved the grade of fatty liver, confirmed by sonographic echogenicity, in comparison to the placebo group. Vitamin D is involved in glucose and lipid metabolism regulation, cell differentiation and proliferation, immune system modulation, inflammation and apoptosis.[61,62] Insulin resistance is a key marker of NAFLD pathogenesis which is associated with oxidative stress and lipotoxicity. Then, lipid accumulation in NAFLD creates a chronic hepatic inflammation. Vitamin D is involved in the regulation of adipogenesis and inhibition of NF-κB transcription. Thus, it inhibits the expression of inflammatory cytokines such as IL-6, TNF-α and IL-1β. It also increases adiponectin secretion from adipoycytes and enhances GLUT-4 receptor expression in myocytes, both of which improve insulin resistance. Vitamin D downregulates the expression of toll like receptors (TLRs) known as TLR-2, TLR-4 and TLR-9 and ameliorates inflammation.[63] The active form of vitamin D, directly through its receptors or through regulation of intracellular calcium, helps to secrete insulin.[64] Vitamin D increases insulin sensitivity through the effect on its muscle cell receptors by increasing insulin receptor or increasing the sensitivity of insulin receptor to insulin and the effect on peroxisome proliferator-activated receptor (PPAR) δ and the influence on regulation of extracellular calcium.[65] In the current study, vitamin D supplementation improved HOMA-IR as a marker of insulin resistance. Our results are consistent with the previous studies on the effects of oral vitamin D on insulin resistance in pre-diabetic patients.[66] To our knowledge, this is the first RCT conducted to assess the effect of calcitriol supplementation in combination with weight loss diet on liver lipid accumulation, serum lipid profile and insulin sensitivity in patients with NAFLD. We used weight loss diet because it is the first step in clinical management of NAFLD. Calcitriol supplementation simultaneous with weight loss diet has greater benefits for lipid accumulation in the liver, insulin sensitivity and grade of fatty liver than weight loss diet alone. However, a larger sample size and longer duration are needed before reaching conclusive results. We should note that the levels of ALT and AST were almost in the normal range. Therefore, the grade of fatty accumulation was not great in the majority of patients. Another study with higher levels of these enzymes is needed to assess the effects of calcitriol on NAFLD progression.

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