The effect of vitamin D supplementation on blood sugar and different indices of insulin resistance in patients with non-alcoholic fatty liver disease (NAFLD)

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ABSTRACT
Background: Vitamin D supplementation has been shown to decrease insulin resistance through which it might cause fatty liver. Fatty liver increasingly results in type 2 diabetes mellitus (T2DM). Insulin resistance and fatty liver are particularly closely related. The aim of present study is to examine the effect of vitamin D supplementation on blood sugar and different indices of insulin resistance in patients with non-alcoholic fatty liver disease (NAFLD).

Materials and Methods: This randomized placebo-controlled clinical trial was conducted on 60 patients with NAFLD, who were divided equally into intervention and control groups. Patients in the intervention group received vitamin D3 (50,000 IU) and patients in the control group received placebo capsules every week for 10 weeks. Blood sugar, homeostatic model assessment-insulin resistance (HOMA-IR), and homeostatic model assessment-beta cell (HOMA-B) were checked at baseline and after 10 weeks of the intervention. Adjustment for variables was performed by analysis of covariance (ANCOVA).

Results: Vitamin D supplementation resulted in increased serum 25-hydroxy vitamin D [25(OH) D] concentration in the intervention group compared to the control group [+68 (12) vs. −1.9 (2.44); \( P = 0.001 \)]. Intake of vitamin D supplements led to a marginally significant decrease in fasting blood glucose [FBS: −12 (4) in the intervention group compared to − 3 (2) in the control group; \( P = 0.055 \)]. Also, HOMA-IR decreased in the intervention group compared to the control group [−1.75 (0.23) vs. 0.12 (0.41); \( P = 0.066 \)].

Conclusions: Vitamin D supplementation resulted in decreased HOMA-IR and FBS concentration in patients with NAFLD; however, it did not affect the insulin level and HOMA-B significantly.

Key words: Blood sugar, insulin resistance, non-alcoholic fatty liver, vitamin D

INTRODUCTION
Non-alcoholic fatty liver disease (NAFLD) is a public health problem worldwide, with an incidence of 20-30% in European countries.1 The spectrum of NAFLD ranges from usual steatosis to non-alcoholic steatohepatitis (NASH) and eventually carcinoma. Almost 5% of people with liver steatosis progress to NASH.2 NAFLD is tightly correlated with overweight and is considered as a predictor of the metabolic syndrome. Incidence of NAFLD among patients with diabetes is considered to be 65%, which is much more than its incidence among healthy individuals.3-5 NAFLD is a multi-factorial disease with intricate pathogenesis. Clinical features of NAFLD encompass overweight, insulin resistance (IR), and dyslipidemia.6 Liver steatosis is associated with IR. IR increases the amount of fat tissue lipolysis and the flow of free fatty acids to the liver cell.7 Hyperglycemia induces lipid reposition in the hepatocytes by increasing lipogenesis while blocking fatty acid oxidation (FAO) and lipid transfer in the liver.8,9

Several studies have shown a significant relationship between vitamin D level and chronic heart disease (CHD), diabetes,
metabolic syndrome, and IR. Vitamin D plays a vital role in glucose intolerance and IR.\(^\text{[10-13]}\) It has been shown that colon, pancreas, and also immune cells have vitamin D receptors.\(^\text{[14,15]}\) Vitamin D has been suggested as having a main role in IR and pathology of type 2 diabetes mellitus or \(\beta\)-cell functioning.\(^\text{[16,17]}\) Vitamin D deficiency increases serum parathyroid hormone (PTH) level and this, in turn, increases IR in the peripheral tissue. Several studies demonstrated that IR plays an important role in NAFLD.\(^\text{[10,13]}\)

Therefore, the aim of this study was to determine the effect of vitamin D supplementation on IR in patients with NAFLD.

**Materials and Methods**

A randomized controlled trial (IRCT registration code: IRCT2013060411763N8) was conducted on 60 patients with NAFLD. This study was conducted in Metabolic Liver Disease Research Center in Isfahan University of Medical Sciences. The study was performed with the approval of the local ethics committee of Isfahan University of Medical Sciences. Written informed consent was obtained from the participants. NAFLD was confirmed by ultrasound. Exclusion criteria of our study were: having acute illness, hepatitis C, B, or Wilson’s disease; history of chronic liver disease or other conditions that affect the gallbladder and bile ducts; pregnancy; history of taking any drugs affecting the level of alanine aminotransferase (ALT), such as valproic acid, tamoxifen, 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) inhibitors, metformin, angiotensin-converting enzyme 1 (ACE1), and angiotensin-converting enzyme receptor 1 (ACER 1); and any kinds of medication addiction.

Patients were randomly assigned to consume vitamin D supplements (n = 30) or placebo (n = 30) for 6 weeks. Random assignment was performed using computer-generated random numbers. Placebo capsules were of the same appearance, color, odor, and taste as vitamin D3 capsules. Intervention period was 10 weeks, and patients received vitamin D supplements or placebo every week (1 vitamin D or placebo/week). The level of serum 25-hydroxy vitamin D [25(OH) D] was measured at the beginning and end of the study. Dietary records were collected every 2 weeks and intake was determined based on the estimated values in household measurements. To obtain the nutrient intake of the participants on the basis of these 5-d food diaries, we used NUTRITIONIST IV Software (Version 7.0; N-squared Computing, Salam, OR, USA), which was modified for Iranian food items. Five-day physical activity records were collected using 24-h physical activity record questionnaire (one per 2 weeks). Physical activity level was estimated as metabolic equivalent minutes per week (MET-hours/week). In order to calculate MET-hours/week, we used the following formula: days per week multiplied by hours of exercise each time × MET equivalent of exercise, and summed up all MET-hours/week values to estimate the total MET-hours/week for each person.\(^\text{[14]}\)

**Biochemical measurements**

Fasting blood samples were taken and 25(OH) D was assessed using direct competitive immune assay kit (Diaserine Italian Company, Monza, Italy) at the beginning and end of the study. Blood glucose level was measured using Hitachi auto-analyzer, and serum insulin levels were measured with radioimmunoassay kit Pars Azmoon, Tehran, Iran (Tehran Pars test; Tehran, Iran). IR was measured on the basis of the homeostasis model assessment of IR (HOMA-IR), and the percentage of \(\beta\)-cell function was assessed on the basis of the homeostasis model assessment of \(\beta\) (HOMA-\(\beta\)) using the following formulae:

\[
\text{HOMA-IR} = [\text{fasting insulin (mU/l)} \times \text{fasting glucose (mg/l)}]/405
\]

\[
\%\text{HOMA-}\beta = [\text{fasting insulin (mU/l)} \times 360/\text{fasting glucose (mg/l)}] - 63
\]

**Degree of fat accumulation in liver**

Level of liver steatosis was measured using ultrasonography with Esaote medical ultrasound machine (convex 3.5 MHz) at the beginning and end of the study. Hepatic ultrasonography was done by someone who was blinded to the objectives of the study. For ultrasound, patients were required to fast for 8 h. Ultrasonography was performed in supine position. Right and left lobes of the upper and lower surfaces were studied. Echogenicity of the liver, presence or absence of bulky tumors, and cystic or solid calcification also were assessed. Intrahepatic bile ducts, portal vein, and hepatic artery were evaluated. Liver steatosis was scored semi-quantitatively on a scale of 0-3, where 0 denoted absent, 1 was given for mild, 2 for moderate, and 3 for severe steatosis. Steatosis was graded according to Saverymuttu et al.\(^\text{[18]}\)

**Statistical analysis**

The normal distribution of variables was confirmed by the Kolmogorov-Smirnov test. Log transformation was used for non-normally distributed variables. Independent-sample Student’s t-test was used to detect differences in general characteristics and dietary intake between the two groups, and paired t-test was used to assess differences within groups. These adjustments were performed using analysis of covariance (ANCOVA). Changes of NAFLD grades were analyzed by ordinal regression (adjustment for age and sex). Chi-square test was used to detect differences in fatty liver grades in the two groups at baseline. \(P < 0.05\) was considered as the level of significance. All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS), version 16(SPPS Inc.).
Ethical considerations
This study is approved by Ethical Committee of Isfahan University of Medical Sciences and Helsinki’s guideline is followed, completely and addicts to any kinds of drug.

Results
The study flowchart shows the screening, randomization, and follow-up of the participants [Figure 1]. In this study, 29 men and 31 women participated. Mean age of the participants was 48.5 years.

Glycemic indicator of 60 patients is given in Table 1. Compliance with the treatments was good in both groups and no side effects were reported. On the basis of 5-d dietary intake and physical activity records, no significant differences were seen between the two groups.

When the analyses were adjusted for baseline characteristics, vitamin D supplementation resulted in increase of serum 25(OH) D concentrations compared with placebo [+68 (12) compared to −1.9 (2.44) nmol/ml; P = 0.001] [Table 2]. Intake of vitamin D supplements led to a marginally significant reduction in fasting blood glucose (FBS) and HOMA-IR level (FBS: −12 (4) compared to −3 (2) mg/dl in the intervention and control groups, respectively; P = 0.055 and HOMA-IR: −1.75 (0.23) compared to 0.12 (0.41) in the intervention and control groups, respectively; P = 0.066). Moreover, serum calcium was increased in the intervention group compared to the control group [4 (0.4) compared vs. 3.2 (1) mg/dl; P = 0.032].

Discussion
The aim of this study was to assess the effect of vitamin D supplementation on blood sugar and different indices of IR in patients with NAFLD.

In this study, vitamin D supplementation caused a marginally significant decrease in FBS level and HOMA-IR, however, had no significant effect on insulin level and HOMA-B.

There are some evidences showing that vitamin D deficiency has a relationship with the risk factors of chronic diseases, including NAFLD and other metabolic risk factors. It has been suggested that low serum levels of vitamin D may increase IR and, in turn, the risk of diabetes mellitus type 2. NAFLD is associated with IR in both liver and muscle tissue. IR elevates the amount of fat tissue lipolysis and increases the flow of free fatty acids inside the liver cell.

Effects of vitamin D supplementation on the metabolism of glucose have been demonstrated in several studies. Our findings are similar to the results of other studies, and IR was found to decrease after vitamin D intake. Inzucchi et al. demonstrated in their study a 60% reduction in

![Study flow diagram]

**Table 1: Laboratory characteristics in intervention and control groups**

| Characteristic | Intervention group (n=30) | Control group (n=30) | P² | P³ |
|----------------|--------------------------|----------------------|----|----|
| FBS (mg/dl)    | Before*                  | 113.33 (22)          | 123.44 (8.12) | 0.65 | 0.43 |
|                | After*                   | 101 (18)             | 120.21 (6.12) | 0.055 |
|                | P                        | 0.042                | 0.077 |
| Insulin (mIU/ml) | Before*                  | 15.48 (3.7)          | 11.82 (3.82) | 0.77 | 0.31 |
|                | After*                   | 10.1 (0.2)           | 15.32 (1.3)  | 0.12 |
|                | P                        | 0.064                | 0.087 |
| HOMA-IR        | Before*                  | 3.1 (0.33)           | 3.12 (0.13)  | 0.62 | 0.12 |
|                | After*                   | 1.45 (0.1)           | 3.24 (0.2)   | 0.066 |
|                | P                        | 0.044                | 0.089 |
| HOMA-B         | Before*                  | 65.34 (11.23)        | 67.13 (23.36) | 0.75 | 0.21 |
|                | After*                   | 63.11 (8.12)         | 71.21 (21.32) | 0.14 |
|                | P                        | 0.087                | 0.097 |
| Calcium (mg/dl) | Before*                  | 9.5 (3)              | 12.9 (2)    | 0.76 | 0.43 |
|                | After*                   | 13 (1)               | 9.7 (1)     | 0.032 |
|                | P                        | 0.03                 | 0.055 |
| Vitamin D serum (nmol/l) | Before* | 49 (1) | 47 (2) | 0.32 | 0.32 |
|                | After*                   | 117 (13)             | 45.8 (0.44) | 0.001 |
|                | P                        | 0.001                | 0.65 |

1 All values are means (SD). ² Obtained from independent sample t-test. ³ Obtained from analysis of covariance (ANCOVA). ⁴ Paired t-test. Significance levels at <0.05. ① Before and after intervention. FBS: Fasting blood sugar, HOMA-IR: Homeostatic model assessment insulin resistance, HOMA-B: Homeostatic model assessment-beta cell function.
Table 2: Dietary intake and physical activity of NAFLD of intervention and control groups

|                      | Intervention group (n=30) | Control group (n=30) | P value 2 |
|----------------------|--------------------------|----------------------|-----------|
| Energy (kcal/day)    | 2217.2 (461)             | 2045.1 (461)         | 0.76      |
| Carbohydrate (% cal/day) | 61 (58)                 | 58 (62)              | 0.52      |
| Protein (% cal/day)  | 12 (12)                  | 12 (12)              | 0.91      |
| Fat (% cal/day)      | 27 (30)                  | 30 (34)              | 0.65      |
| Cholesterol (mg/day) | 225 (57)                 | 236 (111)            | 0.44      |
| Dietary fiber (g/day)| 19 (7)                   | 24 (5)               | 0.34      |
| Vitamin D (mg/day)   | 3 (0.4)                  | 4 (0.3)              | 0.18      |
| Physical activity score (MET-hours/week) | 32.3 (1.44) | 33.2 (1.22) | 0.54 |

1. All values are means (SD). 2. Obtained from independent samples t-test

Increasing these inflammatory parameters can even lead to the lipid profile disorders which can put patients with non-alcoholic fatty liver disorder in metabolic syndrome and cardiovascular dysfunction.\(^{33,34}\)

The strength of the present study is that it was a double-blind RCT conducted in a country with a high prevalence of NAFLD and hypovitaminosis vitamin D. In this study, we had several limitations. The first limitation was the use of ultrasound for the diagnosis of fatty liver disease, while for accurate diagnosis of fatty liver, liver biopsy is needed. The second limitation was the small sample size of participants, especially in grades one and two. A larger sample size with longer period of follow-up perhaps would have given more favorable results. More studies need to be conducted to demonstrate the effect of vitamin D supplementation on glycemic indicators.

**Conclusion**

Vitamin D supplementation was inversely associated with IR. Vitamin D supplementation may have beneficial effects on controlling the glycemic indicator.

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