Effect of Short-term Vitamin D Supplementation on the Alterations of Glycemic Variables in Response to Exhaustive Eccentric Exercise in Patients with Non-alcoholic Fatty Liver

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ABSTRACT

BACKGROUND:
Exhaustive eccentric exercise (EEE), along with a positive role in weight loss and physiological adaptation, increases liver enzymes and disturbs glucose homeostasis. Many studies have been considered to neutralize the adverse effects of EEE, including vitamin D (Vit D) supplementation. The present study aimed to investigate the effect of short-term Vit D supplementation on the alteration of glycemic variables in response to EEE in patients with non-alcoholic fatty liver disease (NAFLD).

METHODS:
In this clinical trial, 22 overweight women with NAFLD were randomly assigned to control (C; n=11) and experimental (Exp; n=11) groups. C group received a lactose placebo daily with the same color, shape, and warmth percentage; Exp group received 2000 IU of Vit D daily for 6 weeks (42 days). Blood samples were taken to measure the liver enzymes, lipid profile, and Vit D levels alteration at four stages: Pre1(before the first EEE session), post 1 (after the first EEE session), pre 2 (before the second EEE session), and post 2 (after the second EEE session). Repeated measures ANOVA and independent *t* test were used to analyze the data using SPSS software (version 26) at a significance level of $P < 0.05$.

RESULTS:
The results show a significant increase in glucose, insulin, and homeostatic model assessment for insulin resistance (HOMA-IR) levels in both C and Exp groups following the EEE (comparing pre 1 and post 1). Also, after 6 weeks of Vit D supplementation, glucose, insulin, and HOMA-IR increased significantly in both C ($P = 0.001$, $P = 0.001$, and $P = 0.001$, respectively) and Exp ($P = 0.001$, $P = 0.001$, and $P = 0.001$, respectively) groups following EEE (comparison of pre 2 and post 2). However, these increases were significantly lower in Exp group compared with the C group (comparing post 2).

CONCLUSION:
Short-term Vit D supplementation downregulates the increased glucose, insulin, and insulin resistance induced by EEE in patients with NAFLD.

KEYWORDS:
Vitamin D, HOMA-IR, Exercise, Non-alcoholic fatty liver disease

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, defined as fat accumulation, especially triglycerides, in hepatocytes, which are associated with metabolic syndrome, insulin resistance, type 2 diabetes, and cardiovascular disease.[1,2] Sedentary lifestyles and poor eating habits are among the main causes of increased insulin resistance and obesity, the two important risk factors for NAFLD.[2,3] Different exercise and physical activity cause diverse alterations in insulin resistance and recognizing these changes might affect the interpretation of exercise-induced mechanisms in patients with NAFLD.[4,5] It has been reported that exhaustive eccentric exercise (EEE), along with a positive role in weight loss and physiological adaptation, might lead to cell damage.[6] Many studies have reported liver damage and increased insulin resistance following EEE due to the reduced hepatic circulation and subsequent mitochondrial swelling in hepatocytes.[7,8] Different approaches (e.g. supplementation) have been considered to neutralize the adverse effects of EEE including increased liver enzymes and muscle damage.[9] Among which vitamin D (Vit D) supplementation plays a significant role in the prevention of NAFLD.[10] According to studies, Vit D with anti-fibrosis and anti-inflammatory properties is effective to induce insulin sensitivity by improving liver enzymes and reducing insulin resistance.[11,12]

To our knowledge, few studies have investigated the EEE-induced glycemic variables responses before and after a short-term Vit D supplementation in patients with NAFLD; thus, we aimed to investigate the effect of short-term Vit D supplementation on glycemic variables alterations in response to EEE in patients with NAFLD.

MATERIALS AND METHODS

Study Design and Participants

This is a single-blind quasi-experimental study with pre-test and post-test design, with one experimental (Exp), and control (C) group. After sharing the participation announcement in the social networks, 40 overweight women were volunteered to participate in the study. Inclusion criteria were being diagnosed as having NAFLD, body mass index (BMI) ≥ 25 kg/m^2, and ages between 20-40 years. Exclusion criteria were smoking, history of heart or kidney disease, taking lipid-lowering drugs, being infected with coronavirus disease 2019 (COVID-19), Vit D supplementation and regular exercise six months before the start of the study, fundamental diet changes, and failure to follow the study protocol. 26 individuals who met the inclusion criteria were then selected as subjects that were randomly assigned into Exp (n=13) and C (n=13) groups. Then, written informed consent and related questionnaires were completed by the subjects. Also, height was calculated using a wall tape measure, with a minimum of 0.1 cm. Body weight (BW), BMI, body fat percentage (BFP), and waist–hip ratio (WHR) were assessed using a bioelectric impedance device (InBody, Korea). It should be noted that two individuals in each group refused to continue the study protocol. Figure 1 shows the CONSORT flow diagram for the study.

Vitamin D Supplementation and EEE Training Protocol

In this study, Exp group received 2000 IU/day of Vit D (Zahravi Pharmaceutical Company) for 6 weeks (42 days). C group received a daily dose of lactose placebo with the same color, shape, and warmth percentage as the Vit D. The subjects completed a 3-day food frequency questionnaire before the intervention. The subjects were asked to consume the same foods as well as the same amount of calories one day before the blood sampling, in both pre and post-test. The subject's diet consisted of an average of 55% carbohydrates, 30% fat, and 15% protein.

Two sessions (one session before supplementation and one session after) of EEE training were performed on the treadmill with a negative slope. After 5 minutes of warm-up with a zero slope and a speed of 3 km/h on the treadmill, the test was started at a speed of 4 km/h and a slope of -2°. Every 3 minutes, -2° was added to the slope and 1 km/h to the speed, then was continued to the exhaustion. Finally, 5 minutes of cooling down at a speed of 3 km/h and a zero slope were performed. The exercise intensity was controlled using a polar heart rate monitor and Borg index. All training sessions were performed between 8 and 11 in the morning, at the fasting state, and a temperature of 20-25°C in the Sports Sciences Faculty laboratory, Razi University, Kermanshah.

Measurement of Blood Samples

Blood samples were taken at 4 stages: before the first

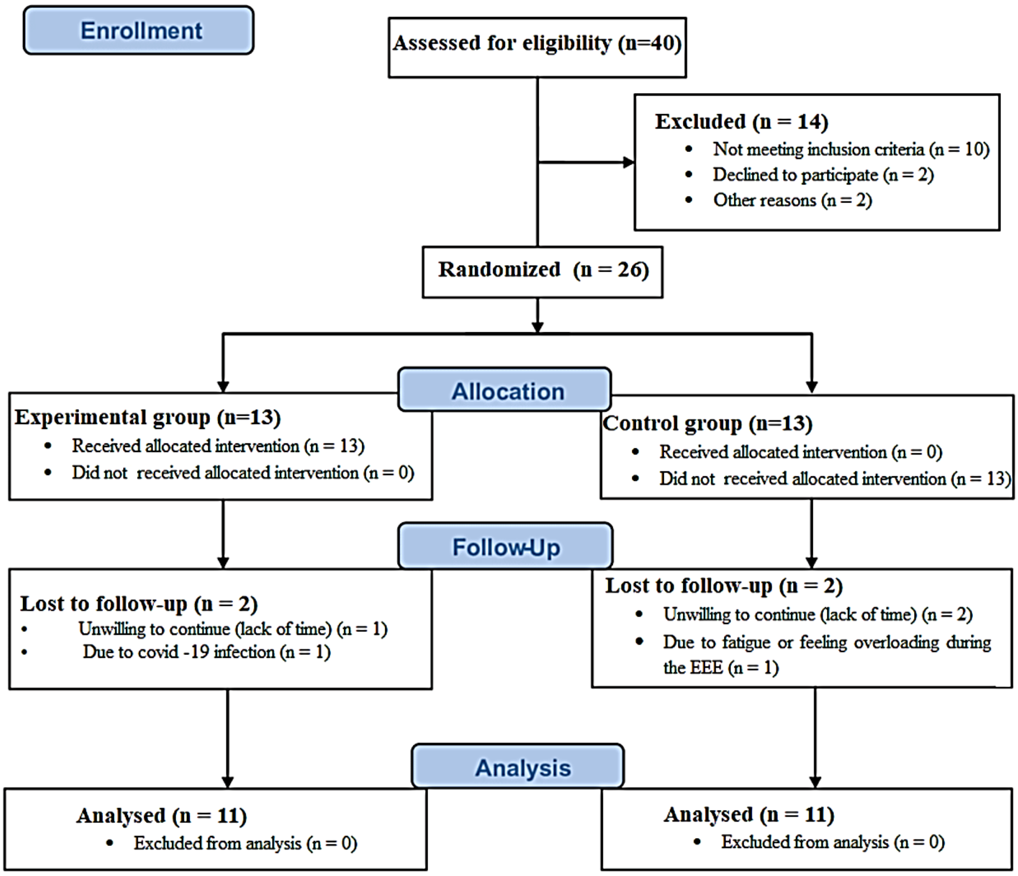

Fig. 1: CONSORT flow diagram of the study.

training protocol (pre 1), immediately after the first training protocol (post 1), before the second training protocol (pre 2), and immediately after the second training protocol (post 2).

Liver enzymes (Alanine transaminase [ALT], Aspartate transaminase [AST], and gamma-glutamyl transferase [GGT]) were measured with the enzyme-linked immunosorbent assay (ELISA) method (Greiner Bio-One kit, made in Germany), fasting insulin levels with ELISA method (Mercodia kit, made in Sweden), glucose with enzymatic method (Pars Azmun kit, made in Iran), and the insulin resistance index was also performed using the homeostatic model assessment for insulin resistance (HOMA-IR) (Homeostasis Model Assessment of Insulin Resistance) equation. The resistance can be assessed from the fasting glucose and insulin concentrations by the formula: resistance (HOMA) = [glucose (mg/dL) × insulin (μU/mL)]/405. Vit D levels were assessed by the direct competitive immunoassay method.

Statistical Analysis

The Shapiro–Wilk test was used to examine the normality of the distribution. ANOVA with repeated measures group was used to compare within-group changes, and an independent *t* test was used for between-group comparisons. All analyses were performed with SPSS software (version 26) at a significance level of $P < 0.05$.

RESULTS

The mean and standard deviation of the anthropometric characteristics are presented in Table 1. Based on these results, no significant difference was observed between the Exp and C groups.

Mean and standard deviations of liver enzymes and Vit D levels are presented in Table 2. Based on these results, no significant differences in liver enzymes and Vit D levels were observed between the Exp and C groups. The results also showed increased liver enzymes and Vit D deficiency.

Table 1. Mean ± SD of anthropometric indices among the groups

Variables	Exp (n=11)	C (n=11)	*P* value [a]
Age (y)	26.90±4.18	26.18±4.42	0.561
Height (cm)	165.45±4.98	164.01±6.01	0.440
BW (kg)	76.68±4.08	74.12±3.23	0.056
BMI (kg/m^2)	28.03±1.87	27.63±2.01	0.084
BFP (%)	29.60±3.44	28.60±2.57	0.332

Abbreviations: BW, body weight; BMI, body mass index; PFB, body fat percentage

P values were calculated using an independent *t* test for between-groups comparison

Table 2. Liver enzymes and Vit D levels among the groups

Variables	Exp (n=11)	C (n=11)	*P* value
ALT (U/L)	35.01±1.67	34.34±1.44	0.261
AST (U/L)	39.04±4.01	37.09±3.16	0.053
GGT (U/L)	36.20±2.10	35.21±1.23	0.169
Vit D (ng/mL)	21.08±2.14	22.90±1.22	0.057

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase.

P values were calculated using an independent *t* test for between-groups comparison.

Figures 2, 3, and 4 show a significant increase in glucose, insulin, and HOMA-IR levels in both C and Exp groups following the EEE (comparing pre 1 and post 1). Also, after 6 weeks of Vit D supplementation, glucose, insulin, and HOMA-IR increased significantly in both C and Exp groups following EEE (comparison of pre2 and post 2). However, in Exp group, the increase in glucose, insulin, and HOMA-IR in post 2 was significantly lower than in post 1.

The results of the independent *t* test showed significantly lower glucose, insulin, and HOMA-IR in Exp group compared with the C group in both pre 2 and post 2, while no such differences were observed between the Exp and C groups in the aforementioned variables in pre 1 and post 1.

DISCUSSION

This study aimed to investigate the effect of short-term Vit D supplementation on glycemic variables (insulin, glucose, and insulin resistance) alteration following EEE in overweight women with NAFLD. In the present study, a significant increase in glycemic variables was observed following EEE. Studies have shown glucose to be the exclusive muscle fuel in intense exercise

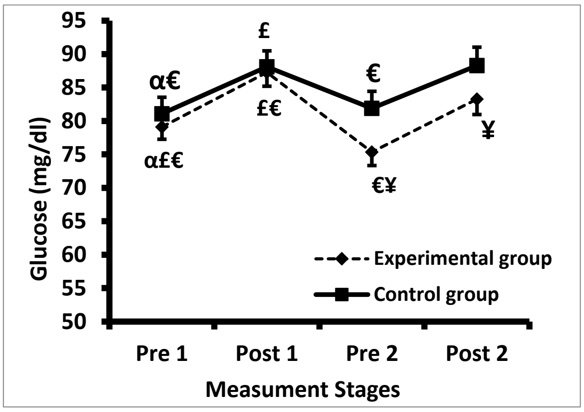

Fig. 2: Glucose levels at different stages in the Exp and C groups. α: Significantly different compared with post1. £: Significantly different compared with pre 2. €: Significantly different compared with post 2. ¥: Significantly different comparing Exp and C groups.

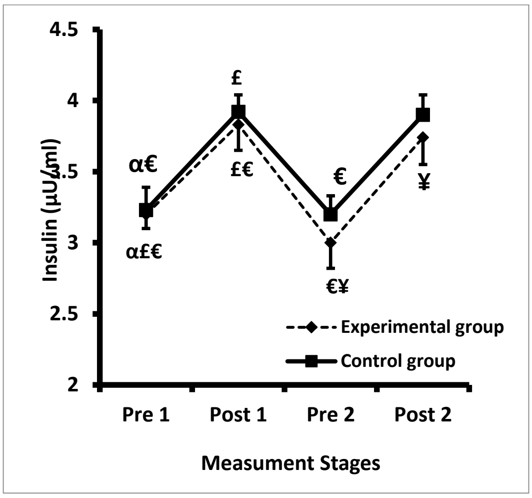

Fig. 3: Insulin levels at different stages in Exp and C. α: Significantly different compared with post 1. £: Significantly different compared with pre 2. €: Significantly different compared with post 2. ¥: Significantly different comparing Exp and C groups.

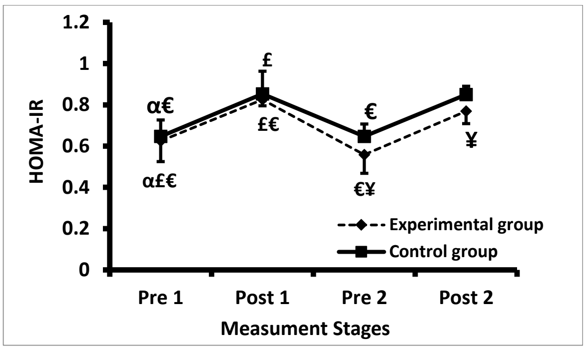

Fig. 4: HOMA-IR levels at different stages in Exp and C. α: Significantly different compared with post 1. £: Significantly different compared with pre 2. €: Significantly different compared with post 2. ¥: Significantly different comparing Exp and C groups.

(>80% VO_{2max}), unlike lesser intensities.[13,14] The blood glucose increases lightly during EEE and increases further immediately at exhaustion that persists for up to 1 hour.[15] Studies have shown that catecholamine levels also rise markedly following an EEE, increasing the glucose production by 7-8 fold while glucose utilization is only increased 3-4 fold.[16,17] Since the participants of the present study showed normal blood glucose, increased insulin following EEE is intelligible to correct the glucose level and restore muscle glycogen; A physiological response that might not happen in patients with diabetes. Interestingly, the results of the present study showed that short-term Vit D supplementation significantly reduced glycemic variables following EEE.[18] Previous studies have investigated the beneficial effects of long-term Vit D on glycemic variables,[19,20] however, the short-term effects of Vit D following EEE in patients with NAFLD have not been studied to date. The results of the present study showed that Vit D supplementation improved insulin resistance in women with NAFLD. Several potential mechanisms have been reported for Vit D to improve insulin action, including[20, 21]: prevention of hepatic glucose production by down-regulating gluconeogenesis key enzymes (e.g. phosphoenolpyruvate, carboxykinase, and glucose 6-phosphatase) and stimulation of glucose uptake and fatty acid oxidation by activating the adenosine monophosphate-activated protein kinase in muscle.[22,23] Therefore, Vit D might increase insulin sensitivity and decrease basal blood glucose levels in patients with NAFLD via regulating the glucose production from the liver and other cells.[24,25] Convincing evidence also shows that Vit D supplementation might ameliorate and prevent insulin resistance by regulating insulin production and secretion from beta-cells.[26]

The strengths of the present study included focusing on the novel questions using a randomized, single-blind, placebo-controlled trial with a low dropout rate. The limitation of the present study was the small sample size due to the COVID-19 and no financial support that obliged us not to measure some of the blood indicators and related gene expression, which can be a subject for further research.

CONCLUSION

In general, short-term Vit D supplementation plays a key role in maintaining glucose homeostasis and improving insulin resistance induced by EEE in patients with NAFLD. However, further research is needed to reach more definite conclusions.

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ETHICAL APPROVAL

The trial (IR.RAZI.REC.1399.079) was approved by the Ethics Committee of the Kermanshah Razi University and registered in the Iranian Clinical Trial Registration Center under the code IRCT20201130049538N1. Written informed consent was obtained from all participants, including agreement of the patients to participate as volunteers and possibility to leave the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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