

Co-Administration of Vitamin E and Atorvastatin Improves Insulin Sensitivity and Peroxisome Proliferator-Activated Receptor- γ Expression in Type 2 Diabetic Patients: A Randomized Double-Blind Clinical Trial

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What's Known

- Statins are lipid-lowering medications often prescribed to prevent late complications of type 2 diabetes mellitus (T2DM). However, they have negative effects on serum glucose.
- Peroxisome proliferator-activated receptor gamma (*PPAR- γ*), as an upstream gene of cholesterol efflux and insulin sensitivity pathway, controls insulin sensitivity and lipid profile.

What's New

- Co-administration of vitamin E and atorvastatin improves insulin sensitivity in T2DM patients.
- Vitamin E upregulates *PPAR- γ* gene expression in the peripheral blood mononuclear cells of hyperlipidemic patients, which is one of the probable mechanisms for improving insulin sensitivity.

Abstract

Background: Negative effects of statins on glucose metabolism have been reported. The present study aimed to investigate the effects of co-administration of vitamin E and atorvastatin on glycemic control in hyperlipidemic patients with type 2 diabetes mellitus (T2DM).

Methods: A randomized double-blind clinical trial was conducted at Vali-e-Asr Teaching Hospital (Zanjan, Iran) from July 2017 to March 2018. A total of 30 T2DM female patients were allocated to two groups, namely atorvastatin with placebo (n=15) and atorvastatin with vitamin E (n=15). The patients received daily 20 mg atorvastatin and 400 IU vitamin E or placebo for 12 weeks. Anthropometric and biochemical measures were recorded pre-and post-intervention. Peroxisome proliferator-activated receptor- γ (*PPAR- γ*) expression was measured in peripheral blood mononuclear cells (PBMCs). Independent sample *t* test and paired *t* test were used to analyze between- and within-group variables, respectively. The analysis of covariance (ANCOVA) was used to adjust the effect of baseline variables on the outcomes. $P < 0.05$ was considered statistically significant.

Results: After baseline adjustment, there was a significant improvement in homeostatic model assessment for insulin resistance (HOMA-IR) ($P = 0.04$) and serum insulin ($P < 0.001$) in the atorvastatin with vitamin E group compared to the atorvastatin with the placebo group. In addition, co-administration of vitamin E with atorvastatin significantly upregulated *PPAR- γ* expression (OR=5.4, $P = 0.04$) in the PBMCs of T2DM patients.

Conclusion: Co-administration of vitamin E and atorvastatin reduced insulin resistance and improved *PPAR- γ* mRNA expression. Further studies are required to substantiate our findings.

Trial registration number: IRCT 20170918036256N1

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Keywords • Atorvastatin • Diabetes mellitus • Insulin resistance • Peroxisome proliferator-activated receptors • Vitamin E

Introduction

Asia is a hotspot for the type 2 diabetes mellitus (T2DM) global epidemic.¹ Approximately 60% of T2DM patients have

hyperlipidemia.² Dyslipidemia and insulin resistance result in micro-and macro-vascular complications, which in turn lead to morbidity and mortality in these patients.³ Primary approaches to control these risk factors are lifestyle modification, dietary compounds, and designing a new drug delivery systems.⁴ Statin therapy is shown to be effective in preventing late complications in patients with T2DM.⁶ Statins activate the peroxisome proliferator-activated receptor gamma (*PPAR-γ*) that regulates the expression of several genes involved in lipid metabolism.^{7, 8} *PPAR-γ* upregulates genes involved in cholesterol efflux, anticoagulants, and antioxidants. In addition, it improves insulin sensitivity and leads to reduced serum insulin and glucose levels.⁹ However, some studies have reported that atorvastatin leads to poor glycemic control in diabetic patients.^{11, 12} Therefore, recognition and detailed understanding of molecular events that control metabolic pathways will facilitate the development of drugs targeting specific therapeutic factors.¹³

There are reports of the beneficial effects of combined vitamin E and atorvastatin therapy on glycemic control.¹⁴⁻¹⁷ This therapy may also reduce the negative effect of atorvastatin on blood glucose. Herein, we studied the effects of atorvastatin therapy, with and without vitamin E, on insulin sensitivity and lipid profile in T2DM patients with hyperlipidemia. Additionally, the *PPAR-γ* mRNA expression was assessed to identify one of the possible involved pathways. The effect of atorvastatin on serum Low-density lipoprotein cholesterol (LDL-C) level was also determined.

Materials and Methods

A randomized double-blind clinical trial was conducted at Vali-e-Asr Teaching Hospital (Zanjan, Iran) from July 2017 to March 2018. The study was approved by the Ethics Committee of Zanjan University of Medical Sciences (IR.ZUMS.REC.1395.268) and registered in the Iranian Registry of Clinical Trials (IRCT 20170918036256N1). The participants were informed about the goals of the research and written informed consent was obtained from the patients.

In accordance with a previous study on the effect of atorvastatin on LDL-C, the sample size was calculated using the below formula.¹⁸ In line with this study, we assumed 42% (P1) and 4% (P2) reduction of the effect of atorvastatin in the intervention group compared to the placebo group, a power of 80% in a two-sided test, and

$\alpha=0.05$ (type I error). Accordingly, a sample size of 15 patients per group was determined.

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta} \right)^2 \times P_1(1-P_1) + P_2(1-P_2)}{(p_1 - p_2)^2}$$

The inclusion criteria were patients aged 18-65 years, body mass index (BMI) of 25-35 kg/m², hemoglobin A1C (HbA1c) level from 7% to 9%, and consumption of 20 mg atorvastatin per day to control low-density lipoprotein (LDL). The patients used comparable medications to control their blood glucose. The exclusion criteria were intake of thiazolidinedione, vitamin E, or other dietary supplements within the previous three months, pregnancy, breastfeeding, weight loss >10% during the previous six months, hypothyroidism, or hyperthyroidism, using weight-loss drugs, smoking, and diagnosis of any chronic disease.

A total of 43 T2DM female patients were assessed for eligibility, out of which 13 did not fulfill the inclusion criteria (figure 1). The remaining patients were randomly allocated to two groups, namely the atorvastatin with placebo (A+P) group (n=15) and atorvastatin with vitamin E (A+E) group (n=15). Block randomization was used for the allocation of the participants with a block size of six (five participants in each block). Selection bias was reduced using randomly coded boxes of the same weight, shape, and color. These were numbered according to a random sequence to conceal the selection process from the clinical care team and the researcher.

The A+P group received 20 mg of atorvastatin and 25 µg/d lactose as a placebo. The A+E group received 20 mg atorvastatin and 400 IU vitamin E (in tocopherol form). Both groups received the medications for 12 weeks. All medications were purchased from Jalinus Arya Co., Iran. The participants were given a weekly supply of either vitamin E, or placebo in the same shape, color, and packaging. Medication adherence was assessed by monitoring the unused part of the weekly supply.

Lifestyle

All participants were requested not to change their habitual diet. An expert dietitian monitored and evaluated their diet. Dietary intake over the last three days of each month was assessed. Consumption of supplements was monitored weekly via telephone interviews and double-checked using a food frequency questionnaire. Physical activity level was assessed using the International Physical Activity Questionnaire (IPAQ) at the beginning of the study.¹⁹

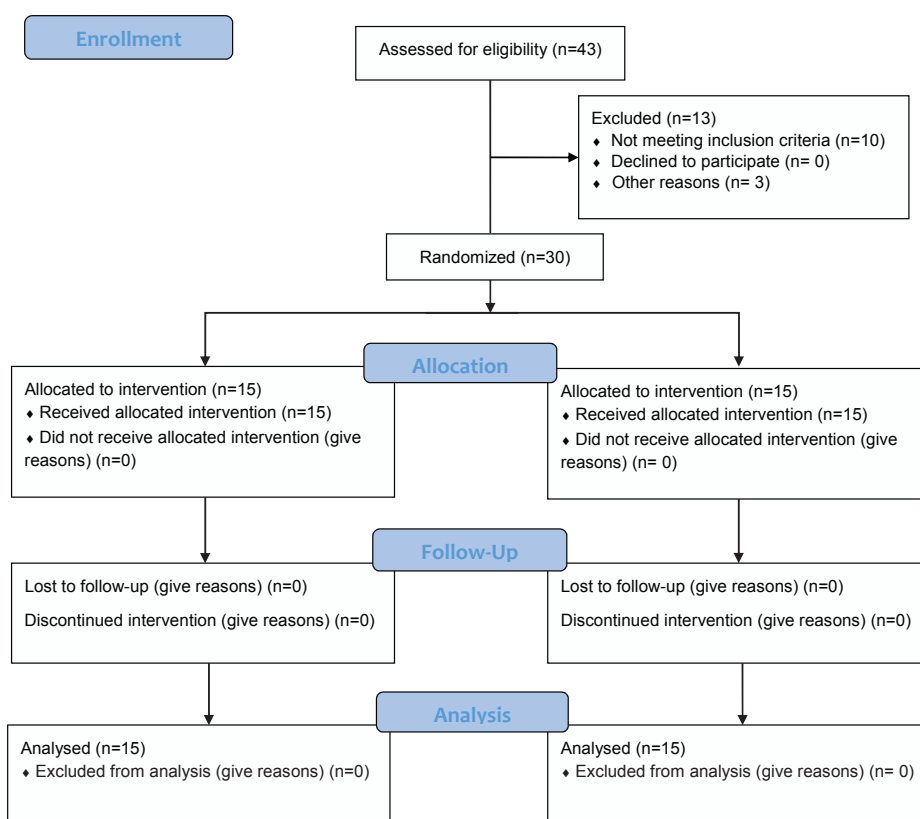


Figure 1: The CONSORT diagram shows the allocation process throughout the trial.

Measurements

The objective of the measurements was to evaluate the effect of co-administration of atorvastatin and vitamin E on the anthropometric data, fasting blood sugar (FBS) levels, serum insulin concentrations and sensitivity, lipid profile, and PPAR- γ mRNA expression.

Anthropometric Measurements

Bodyweight (kg) and height (m) were measured using standard scales, based on which the BMI (kg/m^2) of each participant was calculated. Fat distribution was measured using the waist-to-hip (WHR) ratio. Waist circumference was measured midway between the lower ribs and the iliac crest, and hip circumference was measured at the widest diameter of the buttocks. All measurements (except height) were taken at baseline and after 12 weeks.

Biochemical Parameters

Fasting blood samples (20 mL) were taken from the antecubital vein at baseline and after 12 weeks. These were collected using EDTA-coated sterile tubes (10 mL) and regular tubes (10 mL). To eliminate the effect of sex hormones on lipid profile, blood samples were not taken during the menstrual phase (days 1-5).²⁰

Blood samples in the regular tubes were centrifuged at 3000 g for 10 minutes, followed

by freezing the serum samples. Serum lipid profile, FBS, two-hour plasma glucose (2hPG), HbA1c and insulin levels were measured using an immunoassay kit (Pars Azmoon Co., Tehran, Iran) with Hitachi automated analyzer (Hitachi High-Tech Co., Japan). Serum insulin was measured using an enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech Life Inc., USA). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as fasting insulin (mU/L) \times FBS (mmol/L)/405.²¹

Peripheral Blood Mononuclear Cells Isolation and Gene Expression

Blood samples in the EDTA-containing tubes were diluted with equal volumes of phosphate-buffered saline (PBS; Sigma Aldrich Co., Germany) in Falcon™ flasks. The flasks were centrifuged at 800 g for 40 min at 4 °C and the peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient separation. This layer was then washed with PBS and again centrifuged at 600 g for 10 min at the same temperature. Total mRNA was extracted using an RNX-plus kit (SinaClon Co., Iran) according to the manufacturer's instructions. The quantity and quality of the extracted RNA were assessed at 260 nm using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and agarose gel electrophoresis,

respectively. Finally, cDNA was synthesized using a synthesis kit (Takara Bio, Inc., Japan) in 20 μ L reaction volume. Gene expression was measured in duplicate using the real-time polymerase chain reaction (PCR) method in an ABI StepOne™ sequence detection system (Applied Biosystems, California, USA). The mixture contained 1 μ L of cDNA, 10 pmol of each forward and reverse primers, and the SYBR® Green I Master Mix (Roche). Primers were designed using the Gene Runner software, version 3.05 (Hastings Software Inc., USA). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as a housekeeping gene with 5'-ACCATGAGAAGTATGACAAC-3' and 5'-TGAGTCCTTCCACGATACC-3' sequences. *PPAR- γ* primers were 5'-GCCTTTTGGTGACTTTATGGAG-3' and 5'-CTTGTAGCAGGTTGTCTTGAATG-3'. A cycle threshold (Ct) with a standard deviation <1 was considered the stability for the *GAPDH* gene as a suitable control.²²

The amplification profile included one cycle at 95 °C for 10 min and 40 two-step cycles at 95 °C for 15 sec and 60 °C for 60 sec. The results were analyzed using the LinRegPCR 11.0 software (Heart Failure Research Center, The Netherlands).

Serum Vitamin E Measurement

Serum levels of vitamin E (alpha-tocopherol) were measured using a high-pressure liquid chromatography (HPLC) system (Knauer, Germany). Internal standards, including alpha-tocopherol and tocopherol acetate, were purchased from Sigma-Aldrich (Tokyo, Japan). A C18 column (250×4.6 mm) was used for alpha-tocopherol separation. Methanol was used as the mobile phase with 0.8 mL/min flow rate, 53 bar pressure, and 30 °C temperature. The scanned wavelength range was 190–540 nm. Isolated serum samples from FBS (10 min, 3500 g) were placed into microtubes. To prevent vitamin E oxidation, the microtubes were packed in foils and filled with nitrogen. A total of 400 μ L alcohol (200 μ L of ethanol and 200 μ L of methanol) was added to polypropylene tubes each containing 200 μ L of the serum sample and the mixture was vortexed for 10 sec. Then, 500 μ L of hexane was added to each tube and vortexed for 60 sec. Samples were centrifuged (five min, 4500 g) and the supernatants were collected from the microtubes (this procedure was repeated three times). Methanol (200 μ L) was added to the dried hexane phase (45-50 °C) and 150 μ L of dilution was injected into the HPLC system. The peak areas of tocopherols at 280 nm were integrated. After each sample analysis,

the column was washed with propan-2-ol (1 mL/min at 45 °C for 60 min) to ensure reproducibility between runs.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics software, version 16.0 (IBM Corp., UK). The Kolmogorov–Smirnov test was used to assess the normal distribution of the data. The independent-sample *t* test and paired *t* test were used for within- and between-group analysis, respectively. The analysis of covariance (ANCOVA) was used to adjust the effect of baseline variables on outcome variables. Fold change of *PPAR- γ* expression was assessed using the logistic regression analysis adjusted to the baseline measures and treatments. Data were expressed as mean±SE and *P*<0.05 was considered statistically significant.

Results

A total of 30 female patients (all married) with confirmed T2DM were randomly allocated to A+P and A+E groups. There was no significant difference in energy and nutrient intake and physical activity between and within the groups (*P*>0.05). Pre- and post-intervention dietary intake data for both groups are shown in table 1. Patient characteristics at baseline were not significantly different (table 2).

Anthropometric and biochemical measurements at baseline and after week 12 are shown in table 3. Serum LDL-C level in the A+P group decreased significantly after 12 weeks of intervention (*P*=0.006). Similarly, in the A+E group, serum insulin level (*P*=0.001), HbA1c (*P*=0.042), 2hPG (*P*=0.041), LDL-C (*P*=0.033), total cholesterol (TG) (*P*=0.026), triglyceride (TC) (*P*=0.011), and HOMA-IR (*P*=0.001) decreased significantly after 12 weeks compared to baseline values. *PPAR- γ* mRNA expression significantly increased after 12 weeks in both groups (*P*<0.001 and *P*<0.001, respectively). *PPAR- γ* gene expression was significantly upregulated in the A+E group compared to the A+P group (*P*=0.001). Serum vitamin E level was significantly higher in the A+E compared to the A+P group (*P*<0.001).

After baseline adjustment, serum insulin level and HOMA-IR decreased significantly in the A+E group compared to the A+P group (-6.51±1.32 vs. -0.55±0.35, *P*<0.001 and -2.53±0.45 vs. -1.01±0.52, *P*=0.042, respectively). *PPAR- γ* mRNA expression after baseline adjustment was significantly upregulated in the A+E group compared to the A+P group (4.33±0.55 vs. 1.94±0.27, *P*=0.001). Serum HbA1c, 2hPG,

Table 1: Dietary information of the atorvastatin with the placebo group in comparison with the atorvastatin with vitamin E supplemented group

| Dietary intake | A+P (mean±SE) | | P value [‡] | A+E (mean±SE) | | P value [‡] | P value [†] |
|----------------------------|------------------|-------------------|----------------------|------------------|-------------------|----------------------|----------------------|
| | Pre-intervention | Post-intervention | | Pre-intervention | Post-intervention | | |
| Total Energy (Kcal) | 1960.12±210.55 | 1870.14±206.26 | 0.320 | 1917.01±312.15 | 1887.04±428.62 | 0.601 | 0.743 |
| Total protein (g/day) | 65.54±10.12 | 66.50±9.82 | 0.651 | 64.53±13.85 | 66.86±12.71 | 0.750 | 0.502 |
| Total carbohydrate (g/day) | 265.34±25.82 | 250.21±27.83 | 0.584 | 269.91±23.90 | 260.31±19.64 | 0.454 | 0.691 |
| Total fat (g/day) | 70.33±11.54 | 66.72±12.65 | 0.420 | 64.72±12.54 | 63.93±16.38 | 0.414 | 0.203 |
| Cholesterol (mg/day) | 143.73±22.96 | 141.63±22.00 | 0.209 | 145.74±35.45 | 144.42±74.73 | 0.409 | 0.663 |
| SFA (g/day) | 17.54±5.30 | 16.92±4.14 | 0.509 | 16.63±6.60 | 16.12±5.44 | 0.801 | 0.200 |
| PUFA (g/day) | 31.33±8.80 | 32.53±4.11 | 0.811 | 34.24±8.73 | 35.65±7.61 | 0.332 | 0.927 |
| MUFA (g/day) | 23.81±5.00 | 23.11±3.42 | 0.211 | 23.36±5.34 | 24.00±4.62 | 0.001 | 0.005 |
| Fiber (g/day) | 11.32±3.00 | 12.41±2.51 | 0.760 | 12.82±2.11 | 12.54±2.35 | 0.601 | 0.805 |
| Vitamin E (mg/day) | 2.66±1.00 | 2.43±1.36 | 0.616 | 2.55±0.80 | 2.92±1.15 | 0.545 | <0.001 |

SFA: Saturated fatty acid, PUFA: Polyunsaturated fatty acid, MUFA: Monounsaturated fatty acid. [†]Independent sample *t* test to show between-group differences post-intervention; [‡]Paired sample *t* test to show within-group differences between pre-and post-intervention in each group; P<0.05 is considered statistically significant.

Table 2: Patient characteristics at baseline in the atorvastatin with placebo and atorvastatin with vitamin E groups

| Variable | Group | | P value |
|--------------------------|----------------|----------------|---------|
| | A+P (mean±SE) | A+E (mean±SE) | |
| Age (years) | 50.65±1.12 | 50.43±1.44 | 0.922 |
| Duration of T2DM (years) | 4.71±1.30 | 5.82±1.32 | 0.562 |
| Energy (kcal) | 1960.12±210.55 | 1917.01±312.15 | 0.591 |
| PA (%) | Low | 61.59% | 0.405 |
| | Moderate | 38.41% | |
| BMI (kg/m ²) | 27.11±0.90 | 26.83±0.50 | 0.601 |
| WHR | 0.97±0.01 | 0.96±0.01 | 0.644 |
| FBS (mg/dl) | 177.42±19.64 | 154.80±15.24 | 0.372 |
| 2hPG (mg/dl) | 254.58±31.95 | 263.72±31.25 | 0.840 |
| HbA1c (%) | 7.63±0.42 | 7.37±0.40 | 0.673 |
| HDL cholesterol (mg/dl) | 46.91±4.34 | 48.70±3.16 | 0.746 |
| LDL cholesterol (mg/dl) | 101.50±7.22 | 98.16±7.70 | 0.759 |
| TC (mg/dl) | 204.95±16.02 | 201.21±11.92 | 0.850 |
| TG (mg/dl) | 192.11±25.90 | 206.03±20.34 | 0.681 |
| Insulin (μU/L) | 10.55±2.71 | 12.56±2.54 | 0.602 |
| HOMA-IR | 4.91±1.62 | 4.55±0.93 | 0.822 |
| PPAR-γ | 1 | 1.02±0.11 | 0.855 |
| Vitamin E (mg/L) | 9.03±0.62 | 9.50±1.23 | 0.712 |

WHR: Waist-to-hip ratio, FBS: Fasting blood sugar, 2hPG: 2-hour plasma glucose, HbA1c: Glycosylated hemoglobin, TC: Total cholesterol, TG: Triglyceride, PPAR-γ: Peroxisome proliferator-activated receptor, A+P: Atorvastatin with placebo, A+E: Atorvastatin with vitamin E; Quantitative variables were compared using the independent sample *t* test. Categorical variables were assessed using the chi-square test. P<0.05 is considered statistically significant.

TG, and TC decreased more in the A+E group compared to the A+P group, but the reductions were not statistically significant (table 4).

The result of logistic regression analysis showed that *PPAR-γ* mRNA expression was significantly upregulated in the A+E group compared to the A+P group after adjusting for baseline covariate measures (OR: 5.4, 95% CI: 0.8-36.9, P=0.041).

Discussion

Co-administration of atorvastatin and vitamin E has beneficial effects on insulin sensitivity by

regulating serum insulin levels and HOMA-IR. In addition, serum HbA1c, 2hPG, TG, and TC decreased more in the A+E group compared to the A+P group, although the reductions were not statistically significant.

Previous studies have reported the negative effects of atorvastatin on glycemic control.^{11, 12, 23} It has been suggested that statins may cause adverse metabolic effects (i.e., reducing insulin secretion and exacerbating insulin resistance due to its effect on glucose transporter GLUT-4 expression in adipocytes) and impairs glucose tolerance.²³ Hence, new drugs in the form of combination therapy may reduce these adverse

Table 3: Anthropometric and biochemical measurements at baseline and week 12

| Variable | A+P (mean±SE) | | A+E (mean±SE) | | P value [‡] | P value [†] | P value [*] |
|--------------------------|----------------|----------------|----------------|----------------|----------------------|----------------------|----------------------|
| | Baseline | Week 12 | Baseline | Week 12 | | | |
| Energy (kcal) | 1960.12±210.55 | 1870.14±206.26 | 1917.01±312.15 | 1887.04±428.62 | 0.743 | 0.320 | 0.601 |
| BMI (kg/m ²) | 27.11±0.90 | 26.83±0.81 | 26.83±0.50 | 26.54±0.52 | 0.825 | 0.261 | 0.223 |
| WHR | 0.97±0.01 | 0.96±0.01 | 0.96±0.01 | 0.95±0.1 | 0.900 | 0.281 | 0.364 |
| FBS (mg/dl) | 177.42±19.64 | 152.00±11.61 | 154.80±15.24 | 134.37±8.15 | 0.233 | 0.081 | 0.202 |
| 2hPG (mg/dl) | 254.58±31.95 | 222.70±32.94 | 263.72±31.25 | 177.13±26.50 | 0.310 | 0.093 | 0.041 |
| HbA1c (%) | 7.63±0.42 | 7.35±0.44 | 7.37±0.40 | 6.76±0.26 | 0.234 | 0.161 | 0.043 |
| HDL-C (mg/dl) | 46.91±4.34 | 50.42±3.47 | 48.70±3.16 | 50.67±4.21 | 0.972 | 0.189 | 0.661 |
| LDL-C (mg/dl) | 101.50±7.22 | 78.56±5.14 | 98.16±7.70 | 80.23±3.84 | 0.804 | 0.006 | 0.033 |
| TC (mg/dl) | 204.95±16.02 | 174.15±16.90 | 201.21±11.92 | 168.09±4.81 | 0.742 | 0.058 | 0.011 |
| TG (mg/dl) | 192.11±25.90 | 170.33±3.35 | 206.03±20.34 | 151.04±20.90 | 0.543 | 0.354 | 0.026 |
| Insulin (μU/L) | 10.55±2.71 | 9.95±2.50 | 12.56±2.54 | 6.07±1.42 | 0.201 | 0.155 | 0.001 |
| HOMA-IR | 4.91±1.62 | 3.90±1.23 | 4.55±0.93 | 2.02±0.49 | 0.157 | 0.081 | 0.001 |
| PPAR-γ | 1 | 2.95±0.27 | 1.02±0.11 | 5.35±0.54 | 0.001 | <0.001 | <0.001 |
| Vitamin E (mg/L) | 9.03±0.62 | 7.89±1.02 | 9.50±1.23 | 15.90±1.36 | <0.001 | 0.312 | <0.001 |

WHR: Waist-to-hip ratio, FBS: Fasting blood sugar, 2hPG: 2-hour plasma glucose, HbA1c: Glycosylated hemoglobin, TC: Total cholesterol, TG: Triglyceride, PPAR-γ: Peroxisome proliferator-activated receptor, A+P: Atorvastatin with placebo, A+E: Atorvastatin with vitamin E; [‡]Independent sample *t* test values to show the differences between the two studied groups after 12 weeks; [†]Paired sample *t* test to show differences in the A+P group at baseline and after 12 weeks; ^{*}Paired sample *t* test to show differences in the A+E group at baseline and after 12 weeks; P<0.05 is considered statistically significant.

Table 4: Mean changes between treatment groups at baseline and after 12 weeks

| Variable | A+P (mean±SD) | A+E (mean±SD) | P value [†] |
|--------------------------|---------------|---------------|----------------------|
| BMI (kg/m ²) | -0.21±0.17 | -0.63±0.11 | 0.061 |
| WHR | -0.005±0.004 | 0.003±0.002 | 0.153 |
| FBS (mg/dl) | -25.41±13.12 | -20±15 | 0.810 |
| 2hPG (mg/dl) | -31.84±17.23 | -86.65±37 | 0.243 |
| HbA1c (%) | -0.24±0.16 | -0.64±0.27 | 0.230 |
| HDL cholesterol (mg/dl) | 3.57±2.41 | 1.96±4.14 | 0.730 |
| LDL cholesterol (mg/dl) | -23±6.42 | -17.96±7.14 | 0.612 |
| TC (mg/dl) | -30.94±14 | -33.21±10.61 | 0.889 |
| TG (mg/dl) | -21.84±22.13 | -55±19.85 | 0.315 |
| Insulin (μU/L) | -0.55±0.35 | -6.51±1.32 | <0.001 |
| HOMA-IR | -1.01±0.52 | -2.53±0.45 | 0.042 |
| PPAR-γ | 1.94±0.27 | 4.33±0.55 | 0.001 |
| Vitamin E (mg/L) | -1.15±1.05 | 6.46±1.13 | <0.001 |

WHR: Waist-to-hip ratio, FBS: Fasting blood sugar, 2hPG: 2-hour plasma glucose, HbA1c: Glycosylated hemoglobin, TC: Total cholesterol, TG: Triglyceride, PPAR-γ: Peroxisome proliferator-activated receptor, A+P: Atorvastatin with placebo, A+E: Atorvastatin with vitamin E; [†]ANCOVA with baseline values as a covariate for the A+E group relative to the A+P group; P<0.05 is considered statistically significant.

effects and could help T2DM patients. In line with previous studies on combination therapy,¹⁴⁻¹⁷ we used vitamin E for its beneficial effects on glycemic control together with atorvastatin.

Daily intake of vitamin E and atorvastatin reduced serum insulin levels and improved HOMA-IR. However, this combination therapy had no statistically significant effect on blood glucose and lipid profile compared to atorvastatin alone. The 2hPG level was reduced by 80 mg/dL in the A+E group. Although this reduction is not statistically significant, it is clinically valuable. A recent study reported a beneficial effect of a daily dose of 40 mg atorvastatin on serum insulin and insulin resistance, however, the effect on FBS was not significant.²⁴ But, the reported effect

of vitamin E on blood glucose and lipid profile has been inconsistent. Khabaz and colleagues assessed the effect of 12 weeks of 800 IU vitamin E supplements on blood sugar and lipid profile in T2DM patients.²⁵ They showed that vitamin E supplements did not improve FBS, lipid profile, and serum insulin. In contrast with our study, their patients did not receive any type of drugs for lipid control.

The results of the present study showed that a daily dose of 400 IU vitamin E supplements for 12 weeks resulted in a significant reduction of HbA1c in the A+E group. However, the reduction in the A+P groups was not clinically nor statistically significant. In line with our results, despite using a different dosage, a previous

study reported that a daily dose of 1600 IU α -tocopherol reduced HbA1c levels.¹⁴ A study on type 1 diabetic patients showed a similar effect with 100 IU vitamin E supplements.¹⁵ Other studies also reported reductions in HbA1c, serum insulin, and HOMA-IR with a daily dose of 600 or 900 IU vitamin E supplements.^{16, 17}

PPAR- γ gene expression was significantly upregulated in the PBMCs of the A+E group, which may have a beneficial effect on insulin sensitivity. *PPAR- γ* is generally anti-inflammatory and improves insulin sensitivity.²⁵ *PPAR- γ* upregulates the expression of genes involved in cholesterol efflux, anticoagulants, and antioxidants. It also improves whole-body insulin sensitivity, which leads to reduced insulin and glucose plasma levels.^{6, 26} Synthetic *PPAR- γ* ligands are currently used to treat hyperlipidemia and T2DM.⁷ A previous study on the effect of vitamin E on a rabbit model reported that daily intramuscular injections of vitamin E (50 mg/kg) increased *PPAR γ* expression levels in the aortae of rabbits fed a cholesterol-rich diet.²⁷ Another study reported that vitamin E supplementation decreased *PPAR- γ* expression in the liver, which was associated with increased insulin sensitivity.²⁸ Kim and colleagues reported that soybean oil (vegetable oil containing vitamin E) increased *PPAR- γ* expression in the bone, which was associated with higher adiposity and insulin resistance.²⁹ Tocotrienols enhance the interaction of the ligand-binding domains of *PPAR α* with the receptor-interacting motif of *PPAR- γ* coactivator-1 α (*PGC-1 α*). They also improve whole-body glucose utilization and insulin sensitivity by upregulating *PPAR- γ* target genes.^{10, 30} Two previous studies on cell culture showed that vitamin E (both α - and γ -tocopherol) upregulates adiponectin expression at mRNA and protein levels via a mechanism that increases *PPAR- γ* mRNA through an increase in its endogenous ligand 15-Deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂).^{31, 32} Another study investigated the effect of a three-day pretreatment with oral atorvastatin (10 mg/kg per day) compared to oral pioglitazone (10 mg/kg per day) on *PPAR- γ* gene expression in rat heart. They showed that both medications upregulated *PPAR- γ* gene expression and increased myocardial 15d-PGJ₂ levels.³³

The main limitation of the present study was the participation of female patients only, which prevents the generalization of the results to the male population. Moreover, all participants were treated with atorvastatin and the study lacked a dedicated group taking vitamin E only. To comprehensively assess insulin sensitivity, it is recommended to study *PPAR- γ* expression

in response to vitamin E and atorvastatin (individually and in combination) in other tissues such as muscles. It is also recommended to measure serum adiponectin levels to accurately identify the involved pathways. Identification of other pathways such as the production of reactive oxygen species (ROS), and the function of beta cells is suggested. Assessing the expression of the target genes related to *PPAR- γ* in lipid and glucose metabolism is required to obtain a comprehensive set of results.

Conclusion

Co-administration of vitamin E and atorvastatin reduced insulin resistance and improved *PPAR- γ* mRNA expression in comparison with atorvastatin alone. Further studies with larger sample sizes are required to substantiate our findings.

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References

- Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol.* 2018;14:88-98. doi: 10.1038/nrendo.2017.151. PubMed PMID: 29219149.
- Long AN, Dagogo-Jack S. Comorbidities of diabetes and hypertension: mechanisms and approach to target organ protection. *J Clin Hypertens (Greenwich).* 2011;13:244-51. doi: 10.1111/j.1751-7176.2011.00434.x. PubMed PMID: 21466619; PubMed Central PMCID: PMC3746062.
- Parikh NH, Parikh PK, Kothari C. Indigenous plant medicines for health care: treatment of Diabetes mellitus and hyperlipidemia. *Chin J Nat Med.* 2014;12:335-44. doi: 10.1016/S1875-5364(14)60041-8. PubMed PMID: 24856756.
- Doosti M, Seyed Dorraji MS, Mousavi SN, Rasoulifard MH, Hosseini SH. Enhancing quercetin bioavailability by super paramagnetic starch-based hydrogel grafted with fumaric acid: An in vitro and in vivo study. *Colloids*

- Surf B *Biointerfaces*. 2019;183:110487. doi: 10.1016/j.colsurf.2019.110487. PubMed PMID: 31518957.
- 5 Shidfar F, Mousavi SN, Lorvand Amiri H, Agah S, Hoseini S, Hajmiresmail SJ. Reduction of Some Atherogenic Indices in Patients with Non-Alcoholic Fatty Liver by Vitamin D and Calcium Co-Supplementation: A Double Blind Randomized Controlled Clinical Trial. *Iran J Pharm Res*. 2019;18:496-505. PubMed PMID: 31089384; PubMed Central PMCID: PMC6487410.
 - 6 Constantinou C, Papas A, Constantinou AI. Vitamin E and cancer: An insight into the anticancer activities of vitamin E isomers and analogs. *Int J Cancer*. 2008;123:739-52. doi: 10.1002/ijc.23689. PubMed PMID: 18512238.
 - 7 Grygiel-Gorniak B. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications--a review. *Nutr J*. 2014;13:17. doi: 10.1186/1475-2891-13-17. PubMed PMID: 24524207; PubMed Central PMCID: PMC3943808.
 - 8 Lee J, Hong EM, Koh DH, Choi MH, Jang HJ, Kae SH, et al. HMG-CoA reductase inhibitors (statins) activate expression of PPARalpha/PPARgamma and ABCA1 in cultured gallbladder epithelial cells. *Dig Dis Sci*. 2010;55:292-9. doi: 10.1007/s10620-009-0734-3. PubMed PMID: 19225884.
 - 9 Rubic T, Lorenz RL. Downregulated CD36 and oxLDL uptake and stimulated ABCA1/G1 and cholesterol efflux as anti-atherosclerotic mechanisms of interleukin-10. *Cardiovasc Res*. 2006;69:527-35. doi: 10.1016/j.cardiores.2005.10.018. PubMed PMID: 16336952.
 - 10 Mousavi SN, Koohdani F, Eslaminejad MB, Izadi P, Eshraghian M, Sayahpour FA, et al. Extra virgin olive oil in maternal diet increases osteogenic genes expression, but high amounts have deleterious effects on bones in mice offspring at adolescence. *Iran J Basic Med Sci*. 2016;19:1299-307. doi: 10.22038/ijbms.2016.7915. PubMed PMID: 28096962; PubMed Central PMCID: PMC5220235.
 - 11 Cederberg H, Stancakova A, Yaluri N, Modi S, Kuusisto J, Laakso M. Increased risk of diabetes with statin treatment is associated with impaired insulin sensitivity and insulin secretion: a 6 year follow-up study of the METSIM cohort. *Diabetologia*. 2015;58:1109-17. doi: 10.1007/s00125-015-3528-5. PubMed PMID: 25754552.
 - 12 Ogawa H, Matsui K, Saito Y, Sugiyama S, Jinnouchi H, Sugawara M, et al. Differences between rosuvastatin and atorvastatin in lipid-lowering action and effect on glucose metabolism in Japanese hypercholesterolemic patients with concurrent diabetes. Lipid-lowering with highly potent statins in hyperlipidemia with type 2 diabetes patients (LISTEN) study. *Circ J*. 2014;78:2512-5. doi: 10.1253/circj.cj-14-0810. PubMed PMID: 25186922.
 - 13 Leonardini A, Laviola L, Perrini S, Natalicchio A, Giorgino F. Cross-Talk between PPARgamma and Insulin Signaling and Modulation of Insulin Sensitivity. *PPAR Res*. 2009;2009:818945. doi: 10.1155/2009/818945. PubMed PMID: 20182551.
 - 14 Dalan R, Goh LL, Lim CJ, Seneviratna A, Liew H, Seow CJ, et al. Impact of Vitamin E supplementation on vascular function in haptoglobin genotype stratified diabetes patients (EVAS Trial): a randomised controlled trial. *Nutr Diabetes*. 2020;10:13. doi: 10.1038/s41387-020-0116-7. PubMed PMID: 32341356; PubMed Central PMCID: PMC7186220.
 - 15 Suksomboon N, Poolsup N, Sinprasert S. Effects of vitamin E supplementation on glycaemic control in type 2 diabetes: systematic review of randomized controlled trials. *J Clin Pharm Ther*. 2011;36:53-63. doi: 10.1111/j.1365-2710.2009.01154.x. PubMed PMID: 21198720.
 - 16 Xu R, Zhang S, Tao A, Chen G, Zhang M. Influence of vitamin E supplementation on glycaemic control: a meta-analysis of randomised controlled trials. *PLoS One*. 2014;9:e95008. doi: 10.1371/journal.pone.0095008. PubMed PMID: 24740143; PubMed Central PMCID: PMC3989270.
 - 17 Ziegler M, Wallert M, Lorkowski S, Peter K. Cardiovascular and Metabolic Protection by Vitamin E: A Matter of Treatment Strategy? *Antioxidants (Basel)*. 2020;9. doi: 10.3390/antiox9100935. PubMed PMID: 33003543; PubMed Central PMCID: PMC7600583.
 - 18 Adams SP, Tsang M, Wright JM. Lipid-lowering efficacy of atorvastatin. *Cochrane Database Syst Rev*. 2015:CD008226. doi: 10.1002/14651858.CD008226.pub3. PubMed PMID: 25760954; PubMed Central PMCID: PMC6464917.
 - 19 Vasheghani-Farahani A, Tahmasbi M, Asheri H, Ashraf H, Nedjat S, Kordi R. The Persian, last 7-day, long form of the International Physical Activity Questionnaire: translation and validation study. *Asian J Sports Med*. 2011;2:106-16. doi: 10.5812/asjasm.34781. PubMed PMID: 22375226; PubMed Central PMCID: PMC3289200.

- 20 Vashishta S, Gahlot S, Goyal R. Effect of Menstrual Cycle Phases on Plasma Lipid and Lipoprotein Levels in Regularly Menstruating Women. *J Clin Diagn Res.* 2017;11:CC05-CC7. doi: 10.7860/JCDR/2017/26031.9799. PubMed PMID: 28658753; PubMed Central PMCID: PMC5483655.
- 21 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28:412-9. doi: 10.1007/BF00280883. PubMed PMID: 3899825.
- 22 Rebouças EdL, Costa JdN, Passos MJ, Passos JRdS, Hurk Rvd, Silva JRV. Real time PCR and importance of housekeeping genes for normalization and quantification of mRNA expression in different tissues. *Brazilian Archives of Biology and Technology.* 2013;56:143-54. doi: 10.1590/S1516-89132013000100019.
- 23 Nakata M, Nagasaka S, Kusaka I, Matsuoka H, Ishibashi S, Yada T. Effects of statins on the adipocyte maturation and expression of glucose transporter 4 (SLC2A4): implications in glycaemic control. *Diabetologia.* 2006;49:1881-92. doi: 10.1007/s00125-006-0269-5. PubMed PMID: 16685502.
- 24 Talaei A, Mahmudpour M, Shahdost M. The Effect of Atorvastatin on Insulin Resistance in Patients with Type Two Diabetes. *Journal of Arak University of Medical Sciences.* 2019;22:67-74.
- 25 Khabaz M, Rashidi M, Kaseb F, Afkhami-Ardekani M. Effect of vitamin E on blood glucose, lipid profile and blood pressure in type 2 diabetic patients. *Iranian Journal of Diabetes and Obesity.* 2009;1:11-5.
- 26 Derosa G, Maffioli P. Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) agonists on glycemic control, lipid profile and cardiovascular risk. *Curr Mol Pharmacol.* 2012;5:272-81. doi: 10.2174/1874467211205020272. PubMed PMID: 22122457.
- 27 Ren D, Collingwood TN, Rebar EJ, Wolffe AP, Camp HS. PPARgamma knockdown by engineered transcription factors: exogenous PPARgamma2 but not PPARgamma1 reactivates adipogenesis. *Genes Dev.* 2002;16:27-32. doi: 10.1101/gad.953802. PubMed PMID: 11782442; PubMed Central PMCID: PMC5155307.
- 28 Bozaykut P, Karademir B, Yazgan B, Sozen E, Siow RC, Mann GE, et al. Effects of vitamin E on peroxisome proliferator-activated receptor gamma and nuclear factor-erythroid 2-related factor 2 in hypercholesterolemia-induced atherosclerosis. *Free Radic Biol Med.* 2014;70:174-81. doi: 10.1016/j.freeradbiomed.2014.02.017. PubMed PMID: 24583459.
- 29 Kim DY, Kim J, Ham HJ, Choue R. Effects of d-alpha-tocopherol supplements on lipid metabolism in a high-fat diet-fed animal model. *Nutr Res Pract.* 2013;7:481-7. doi: 10.4162/nrp.2013.7.6.481. PubMed PMID: 24353834; PubMed Central PMCID: PMC3865271.
- 30 Fang F, Kang Z, Wong C. Vitamin E tocotrienols improve insulin sensitivity through activating peroxisome proliferator-activated receptors. *Mol Nutr Food Res.* 2010;54:345-52. doi: 10.1002/mnfr.200900119. PubMed PMID: 19866471.
- 31 Landrier JF, Gouranton E, El Yazidi C, Malezet C, Balaguer P, Borel P, et al. Adiponectin expression is induced by vitamin E via a peroxisome proliferator-activated receptor gamma-dependent mechanism. *Endocrinology.* 2009;150:5318-25. doi: 10.1210/en.2009-0506. PubMed PMID: 19833717.
- 32 Ye Y, Nishi SP, Manickavasagam S, Lin Y, Huang MH, Perez-Polo JR, et al. Activation of peroxisome proliferator-activated receptor-gamma (PPAR-gamma) by atorvastatin is mediated by 15-deoxy-delta-12,14-PGJ2. *Prostaglandins Other Lipid Mediat.* 2007;84:43-53. doi: 10.1016/j.prostaglandins.2007.04.001. PubMed PMID: 17643887.
- 33 Ye Y, Lin Y, Atar S, Huang MH, Perez-Polo JR, Uretsky BF, et al. Myocardial protection by pioglitazone, atorvastatin, and their combination: mechanisms and possible interactions. *Am J Physiol Heart Circ Physiol.* 2006;291:H1158-69. doi: 10.1152/ajpheart.00096.2006. PubMed PMID: 16603698.