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

Banafsheh Sadat Tabaei1,2, MSc; Seyyedeh Neda Mousavi1,3, PhD; Aliasghar Rahimian4, MSc; Hadi Rostamkhani5, MSc; Ali Awsat Mellati1,2, PhD; Maryam Jameshorani1, MD

1Zanjan Metabolic Diseases Research Center, Zanjan University of Medical Sciences, Zanjan, Iran; 2Department of Clinical Biochemistry, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran; 3Department of Nutrition, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran; 4Department of Clinical Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Correspondence Authors:
Seyedeh Neda Mousavi, PhD; Mahdavi Blvd., 13th St., School of Medicine, Zanjan University of Medical Sciences, Postal code: 45139-56184, Zanjan, Iran
Tel: +98 24 33052584
Fax: +98 24 33052477
Email: neda.mousavi@zums.ac.ir
Ali Awsat Mellati, PhD; Mahdavi Blvd., 13th St., School of Medicine, Zanjan University of Medical Sciences, Postal code: 45139-56184, Zanjan, Iran
Tel/Fax: +98 24 3344301
Email: mellati@zums.ac.ir
Received: 28 November 2020
Revised: 20 February 2021
Accepted: 26 May 2021

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


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

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.

Introduction

Asia is a hotspot for the type 2 diabetes mellitus (T2DM) global epidemic.1 Approximately 60% of T2DM patients have
hyperlipidemia. Dyslipidemia and insulin resistance result in micro- and macro-vascular complications, which in turn lead to morbidity and mortality in these patients. Primary approaches to control these risk factors are lifestyle modification, dietary compounds, and designing a new drug delivery systems. Statin therapy is shown to be effective in preventing late complications in patients with T2DM. Statins activate the peroxisome proliferator-activated receptor gamma (PPAR-γ) that regulates the expression of several genes involved in lipid metabolism. 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. However, some studies have reported that atorvastatin leads to poor glycemic control in diabetic patients. Therefore, recognition and detailed understanding of molecular events that control metabolic pathways will facilitate the development of drugs targeting specific therapeutic factors.

There are reports of the beneficial effects of combined vitamin E and atorvastatin therapy on glycemic control. 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. 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.

\[
\begin{align*}
\alpha &= 0.05 \\
n &= \frac{\left(Z_1 - \frac{\alpha}{2} + Z_1 - \beta\right)^2 \times P_1 (1 - P_1) + P_2 (1 - P_2)}{(P_1 - P_2)^2}
\end{align*}
\]

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 \( \mu \)g/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.
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/m²) 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)×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 (FBS; 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 μL 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 μL 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, 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 μL alcohol (200 μL of ethanol and 200 μL of methanol) was added to polypropylene tubes, each containing 200 μL of the serum sample, and the mixture was vortexed for 10 sec. Then, 500 μL 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 μL) was added to the dried hexane phase (45–50 °C), and 150 μL 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 than the A+P group (P=0.001). Serum vitamin E level was significantly higher in the A+E than the A+P group (P<0.001).

After baseline adjustment, serum insulin level and HOMA-IR decreased significantly in the A+E group than 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 than the A+P group (4.33±0.55 vs. 1.94±0.27, P=0.001). Serum HbA1c, 2hPG, TG, and TC
decreased more in the A+E group that 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 that 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 that the A+P group. However, the reductions were not statistically significant.

Previous studies have reported the negative effects of atorvastatin on glycemic control.\textsuperscript{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.\textsuperscript{23} Hence, new drugs in the form of combination therapy may reduce these adverse
Co-administration of vitamin E and atorvastatin

In line with previous studies on combination therapy, 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. On the other hand, 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. They found that vitamin E supplements did not improve FBS, lipid profile, and serum insulin. In contrast with our study, their patients did not receive any types 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 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.

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>A+P (mean±SE)</th>
<th>A+E (mean±SE)</th>
<th>P value†</th>
<th>P value‡</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Week 12 Baseline Week 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>1960.12±210.55</td>
<td>1870.14±206.26</td>
<td>0.743</td>
<td>0.320</td>
<td>0.601</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>27.11±0.90</td>
<td>26.83±0.81</td>
<td>0.981</td>
<td>0.281</td>
<td>0.364</td>
</tr>
<tr>
<td>WHR</td>
<td>0.97±0.01</td>
<td>0.96±0.01</td>
<td>0.825</td>
<td>0.261</td>
<td>0.223</td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>177.42±19.64</td>
<td>152.00±11.61</td>
<td>0.233</td>
<td>0.081</td>
<td>0.202</td>
</tr>
<tr>
<td>2hPG (mg/dL)</td>
<td>254.58±31.95</td>
<td>222.70±32.94</td>
<td>0.310</td>
<td>0.093</td>
<td>0.041</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.63±0.42</td>
<td>7.35±0.44</td>
<td>0.972</td>
<td>0.189</td>
<td>0.661</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>46.91±4.34</td>
<td>50.42±4.47</td>
<td>0.543</td>
<td>0.354</td>
<td>0.026</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>101.50±7.02</td>
<td>78.56±5.14</td>
<td>0.804</td>
<td>0.006</td>
<td>0.033</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>204.95±16.02</td>
<td>174.15±16.90</td>
<td>0.742</td>
<td>0.058</td>
<td>0.011</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>192.11±25.90</td>
<td>170.33±33.35</td>
<td>0.534</td>
<td>0.354</td>
<td>0.026</td>
</tr>
<tr>
<td>Insulin (µU/L)</td>
<td>10.5±2.71</td>
<td>9.5±2.50</td>
<td>0.201</td>
<td>0.155</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.9±1.23</td>
<td>4.5±0.93</td>
<td>0.157</td>
<td>0.081</td>
<td>0.001</td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>1</td>
<td>2.9±0.27</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin E (mg/L)</td>
<td>9.03±0.62</td>
<td>7.89±1.02</td>
<td>&lt;0.001</td>
<td>0.312</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>A+P (mean±SD)</th>
<th>A+E (mean±SD)</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Week 12 Baseline Week 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>-0.21±0.17</td>
<td>-0.63±0.11</td>
<td>0.061</td>
</tr>
<tr>
<td>WHR</td>
<td>-0.005±0.004</td>
<td>0.003±0.002</td>
<td>0.153</td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>-25.41±13.12</td>
<td>-20±15</td>
<td>0.810</td>
</tr>
<tr>
<td>2hPG (mg/dL)</td>
<td>-31.8±17.23</td>
<td>-86.65±37</td>
<td>0.243</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>-0.24±0.16</td>
<td>-0.64±0.27</td>
<td>0.230</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>3.57±2.41</td>
<td>1.96±4.14</td>
<td>0.730</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>-23±6.42</td>
<td>-17.96±7.14</td>
<td>0.612</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>-30.94±14</td>
<td>-33.21±10.61</td>
<td>0.889</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>-21.84±22.13</td>
<td>-55±19.85</td>
<td>0.315</td>
</tr>
<tr>
<td>Insulin (µU/L)</td>
<td>-0.55±0.35</td>
<td>-6.51±1.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>-1.01±0.52</td>
<td>-2.53±0.45</td>
<td>0.042</td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>1.94±0.27</td>
<td>4.33±0.55</td>
<td>0.001</td>
</tr>
<tr>
<td>Vitamin E (mg/L)</td>
<td>-1.15±1.05</td>
<td>6.46±1.13</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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

*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. *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. Synthetic *PPAR-γ* ligands are currently used to treat hyperlipidemia and T2DM. 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 *PPARγ* expression in the bone, which was associated with increased adiposity and insulin resistance. Tocotrienols enhance the interaction of the ligand-binding domains of *PPARα* with the receptor-interacting motif of *PPAR-γ* coactivator-1α (*PGC-1α*). They also improve whole-body glucose utilization and insulin sensitivity by upregulating *PPAR-γ* target genes. 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-Δ12,14-prostaglandin J2 (15d-PGJ2). 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 the rats’ hearts. They showed that both medications upregulated *PPAR-γ* gene expression and increased myocardial 15d-PGJ2 levels.

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.

**Acknowledgment**

The present manuscript was extracted from a Master’s thesis by B.S. Tabaei. The study was financially supported by Zanjan Metabolic Diseases Center, Zanjan University of Medical Sciences, Zanjan, Iran (A-12-130-15). The authors would like to thank the patients for their participation and collaboration.

**Authors’ Contribution**

AAM designed the study and critically revised the manuscript; SNM designed the study, contributed to data analysis and drafting the manuscript; MJ contributed in data acquisition, and drafting the manuscript; AR contributed to data analysis and drafting the manuscript; BT contributed in data acquisition, and drafting the manuscript; HR contributed in data acquisition, and drafting the manuscript; MJ contributed in data acquisition, and drafting the manuscript; AR contributed to data analysis and drafting the manuscript; All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Conflict of Interest:** None declared.

**References**

2. Long AN, Dagogo-Jack S. Comorbidities of


