

Co-administration of vitamin E and atorvastatin improves insulin sensitivity via peroxisome proliferator-activated receptor γ in hyperlipidemic type 2 diabetic patients: a randomized double-blinded clinical trial

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Abstract

Background: Contrary to the reports about the useful effects of atorvastatin on blood lipids and insulin sensitivity by up-regulation of peroxisome proliferative-activated receptor gamma (PPAR- γ) expression, to our knowledge, there is inconclusive results about vitamin E. Also, there is no study to assess co-administration of vitamin E and atorvastatin on PPAR- γ mRNA expression, insulin sensitivity and lipid profile in diabetic patients. We compared this effect in hyperlipidemic subjects with type 2 diabetes mellitus (T2DM).

Methods: At the present randomized clinical trial (RCT), 30 T2DM women with hyperlipidemia were categorized into the treated group with 20 mg atorvastatin plus 400 IU vitamin E supplement (n = 15) or atorvastatin plus placebo (n = 15) per day for 12 weeks. Anthropometric and biochemical measures were done at the baseline and after the 12-week intervention. PPAR- γ mRNA expression was measured in the peripheral blood mononuclear cells (PBMCs) of all patients.

Results: After adjusting for the baseline measures, vitamin E resulted in significant improvements in insulin sensitivity in terms of HOMA-IR (-1.01 ± 0.52 vs. -2.56 ± 0.54 , $P = 0.04$) and serum insulin (-0.55 ± 0.35 vs. -6.5 ± 1.3 , $P < 0.001$), compared with the atorvastatin plus placebo. Adjusted for the baseline variables, compared with the atorvastatin plus placebo, vitamin E supplementation could up-regulate PPAR- γ mRNA expression (OR=5.4, 95% CI=0.8-36.9, $P=0.04$) in PBMC of T2DM women.

Conclusions: Vitamin E supplementation along with atorvastatin may improve insulin sensitivity through up-regulation of PPAR- γ gene. More RCTs are needed to reach conclusive results.

Trial registration: The present study is registered under ClinicalTrials.gov Identifier no. IRCT20170918036256N1.

Background

Prevalence of type 2 diabetes Mellitus (T2DM) is quadrupled from 1980 to 2014 ⁽¹⁾. Approximately 60% of T2DM patients have hyperlipidemia ⁽²⁾. Dyslipidemia and insulin resistance lead to micro- and macro-vascular complications, which lead to morbidity and mortality in these patients ⁽³⁾. The major primary approaches to control these risk factors are lifestyle manifestations and medications ^(4, 5). Statin therapy is considered as the cornerstone of clinician's efforts toward prevention of late complications in patients with T2DM ⁽⁶⁾. Recently, recognition and detailed conception of the molecular events that control metabolic pathways help to the development of drugs targeting the responsible elements in therapeutic events ^(7, 8).

Peroxisome proliferator-activated receptors (PPARs) are one of the transcriptional factors, which have been identified as the regulators for the expression of several genes involved in metabolism ⁽⁹⁾. Recently, the PPAR family ligands have drawn great notice in the management of risk factors of T2DM ⁽⁴⁾. PPAR- γ expresses at the highest level in adipocytes and regulates their differentiation, as well as fatty acid

uptake and accumulation^(10, 11). PPAR- γ upregulates the genes involved in cholesterol efflux, anticoagulants, and antioxidants. Also, it improves insulin sensitivity and leads to reduced insulin and glucose plasma levels^(12, 13). Synthetic PPAR- γ ligands are currently used to treat hyperlipidemia and T2DM⁽¹⁴⁾. Statins are PPAR- γ activators, which have anti-inflammatory effects by suppression of pro-inflammatory cytokines and prevent the accumulation of cholesterol⁽¹⁵⁾. Vitamin E is a fat soluble antioxidant which includes tocopherols and tocotrienes⁽¹⁶⁾. Alpha tocopherol is the major form of vitamin E in the plasma and tissue⁽¹⁷⁾. It is reported that α -tocopherol activates expression of PPAR- γ gene in the cancerous cells⁽¹⁸⁾. Despite some evidence, although scarce, no study, to our knowledge, has evaluated whether vitamin E supplementation with atorvastatin results in a greater useful effect on insulin sensitivity and lipid profile in T2DM patients with hyperlipidemia or not. Then, we designed the present study to assess the effects of atorvastatin therapy with and/or without vitamin E with considering the PPAR- γ mRNA expression as a primary outcome, as well as insulin sensitivity and lipid profile as the secondary outcomes, in T2DM patients with hyperlipidemia. We hypothesized that vitamin E supplementation has synergistic effect with atorvastatin on lipid profile and insulin sensitivity via PPAR pathway.

Methods

Study enrolment

Totally, 30 T2DM women with hyperlipidemia were gathered between July 2017 and March 2018 from T2DM patients who were referring to the Vali-e-Asr Hospital, Zanjan, Iran. Women aged 18–65 y, BMI of 25–35 kg/m², 7% <HbA1c < 9%, who needed 20 mg atorvastatin per day to control LDL.C level were included. The exclusion criteria were as follows: intake of thiazolidinedione, vitamin E or any supplementation within the three month ago, pregnancy and/or breast-feeding, weight loss more than 10% during the past 6 month, hypo/hyper thyroids, intake of weight loss drugs, smoking, diagnosis of any chronic diseases.

Study planning

This was a randomized, double-blinded, controlled trial to study the effects of atorvastatin and vitamin E co-administration on the anthropometric measures, fasting blood sugar (FBS), serum insulin concentrations and sensitivity, lipid profiles, and PPAR- γ mRNA expression in T2DM patients. Thirty eligible women were randomly assigned by block randomization method to one of the 2 groups: atorvastatin and placebo (A + P) group (n = 15) received 20 mg of atorvastatin (Jalinus Arya Co, Iran) plus placebo (25 μ g/d as lactose; Jalinus Arya Co, Iran) and/or atorvastatin plus vitamin E (A + E) group (n = 15) received 20 mg atorvastatin (Jalinus Arya Co, Iran) plus 400 IU vitamin E (Jalinus Arya Co. Iran) for 12 weeks. The placebo was similar to vitamin E in shape, color and packaging. (Fig. 1)

Lifestyle

Participants requested don't change their dietary habits. Diets were checked and controlled by an expert dietitian. Dietary intake was assessed by 3 days food records at the end of each month. Supplement consumption was monitored once a week by telephone interviews and double checked by using the food record questionnaires. Physical activity level was assessed by International Physical Activity questionnaire (IPAQ) at the beginning of the study.

Measurements

Anthropometric measures

Body weight and height were measured by the standard scales. BMI (Body Mass Index) was calculated according to the weight/ height² (kg/m²). Fat distribution was measured by waist to hip ratio (WHR) in which waist and hip circumferences were gathered from the halfway between the lower rib-the iliac crest and hip from the maximum circumference over the buttocks. All measures (except height) were taken at baseline and after 12 week.

Biochemical parameters

20 ml of fasting blood samples from antecubital vein were collected at baseline and at 12 wk. 10 ml of samples were gathered in EDTA-coated sterile tubes and 10 ml in the regular tubes.

To eliminate the effect of sex hormones on lipid profile, sampling was not performed between days 1 and 5 of the menstrual cycle.

Blood samples in the regular tubes were centrifuged for 20 min (3000 *g*) and the serum samples were frozen. Serum lipid profile, FBS, 2-h plasma glucose (2hPG), HbA1c and insulin levels were measured by an enzymatic method (Pars Azmoon Co. kit, Tehran, Iran) using Hitachi autoanalyzer. Serum insulin was measured by ELISA (Immunotech Co. kit); and homeostasis model assessment for insulin resistance (HOMA-IR) was calculated by the below formula (fasting insulin (mU/L) × FBS (mmol/L)) / 405).

PBMC isolation and gene expression

Samples in the EDTA-containing tubes were diluted with PBS (equal volume) in Ficolls. Ficolls were centrifuged for 40 min at 800 *g* at 4 °C and PBMCs were isolated according to the density gradient. Then, this layer washed with PBS and centrifuged again for 10 min at 600 *g* at the same temperature. Total mRNA was extracted using RNX-plus kits (Sinaclon, EX6101) according to the kit's guideline. Quantity and quality of the extracted RNA was assessed at 260 nm with a NanoDrop (Wilmington, DE, USA), and agarose gel electrophoresis, respectively. Finally, cDNA was synthesized according to the Takara kit (Takara Bio, Inc., Japan) in 20 µl volumes. Gene expression was measured in duplicated manner by the RT-PCR method performed in an ABI StepOne sequence detection system (Applied Biosystems, California, USA). 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. GAPDH was considered as housekeeping gene at this sequence: 5'-ACCATGAGAAGTATGACAAC-3' and 3'-TGAGTCCTTCCACGATACC-

5'. PPAR- γ primers were as follow: 5'-GCCTTTTGGTGACTTTATGGAG-3' and 3'-CTTGTAGCAGGTTGTCTTGAATG-5'.

A Ct standard deviation < 1 was considered the stability for the GAPDH gene as an appropriate control⁽¹⁹⁾. The amplification profile included one cycle at 95 °C for 5 min and 40 two-step cycles at 93 °C for 30 s and 40 one-step cycles at 72 °C for 45 s. The results were analyzed by the LinRegPCR software (version 11.0).

Serum vitamin E measurement

Serum vitamin E levels as α -tocopherol was measured by HPLC method (Knauer, Germany). Standards including alpha tocopherol and tocopherol acetate were purchased from Sigma-Aldrich (Tokyo- Japan). A C₁₈ column (250 mm \times 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 wavelength-range scanned was 190–540 nm.

Isolated serum samples from FBS (10 min, 3500 gr) were allocated into micro tubes. To prevent oxidation of vitamin E, micro tubes were packed in foils and filled by nitrogen. 400 μ l of alcohols (200 μ l of ethanol and 200 μ l of methanol) were added to 200 μ l of each serum sample in the polypropylene tubes. The mixture was vortexed for 10 s. Then, 500 μ l of hexane was added to each tube and vortexed for 60 s. Samples were centrifuged (5 min, 4500 g) and supernatants were collected at the other micro tubes (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 to the HPLC system, finally. Peak areas of tocopherols were integrated at 280 nm. After each sample analysis, the column was washed with propan-2-ol (1 ml/min for 60 min at 45 °C) to ensure reproducible retention on the column.

Statistics

According to the previous study⁽²⁰⁾, with the standard deviation (SD) difference of 40.9 mg/dl for LDL.C, power of 80% in a two-sided test and $\alpha = 0.05$ (type I error), the sample size computed 12 patient in each group. With 10% dropout, we set the participant target at 15 patients.

IBM SPSS Statistics software (version 16; IBM Corp) was used for analysis. Data were reported as means \pm SE. $P < 0.05$ was considered significant. At the first, normal distribution of data was assessed by the K-S test. Between-group analysis was performed by the independent sample t-test. Within-group differences before and after the study was analyzed by the paired t-tests. To adjust the effect of baseline variables on the outcomes, ANCOVA test was used. Fold change of PPAR γ expression was assessed by the Logistic regression model adjusted to the baseline measures and treatments.

Results

Thirty T2DM-proven women followed the study up to the end. All participants were married. Energy, nutrient intake and physical activity level had not significant difference between the two groups and

within the groups ($p > 0.05$).

Variables had not significantly difference at the baseline (Table 1). At the A + P group, serum LDL.C level was significantly decreased after 12 week of intervention ($p = 0.006$). At the A + E group, serum insulin level ($p = 0.001$), HbA1c ($p = 0.04$), 2hPG ($p = 0.04$), LDL.C ($P = 0.03$), TG ($P = 0.02$), TC ($P = 0.01$), and HOMA-IR ($P = 0.001$) were significantly decreased at the end compared to the baseline. PPAR- γ mRNA expression was significantly increased after 12 week in the both treated groups ($p < 0.001$ and $p < 0.001$, respectively) (Table 2).

Table 1
Patient characteristics before the intervention

Variables	Groups		P value
	A+P	A+E	
Age, yr	50.6 ± 1.1 ¹	50.4 ± 1.4	0.9
Time of T2DM, yr	4.7 ± 1.3	5.8 ± 1.3	0.56
Energy, kcal	1960.1 ± 210.5	1917 ± 312.1	0.5
PA, %	Low	59.9%	0.4
	Moderate	40.1%	
	High	-	
BMI, kg/m ²	27.1 ± 0.9	26.8 ± 0.5	0.6
WHR	0.97 ± 0.01	0.96 ± 0.01	0.64
FBS, mg/dl	177.4 ± 19.6	154.8 ± 15.2	0.37
2hPG, mg/dl	254.5 ± 31.9	263.7 ± 31.2	0.84
HbA1c, %	7.6 ± 0.4	7.3 ± 0.4	0.67
HDL cholesterol, mg/dl	46.9 ± 4.3	48.7 ± 3.1	0.74
LDL cholesterol, mg/dl	101.5 ± 7.2	98.1 ± 7.7	0.75
TC, mg/dl	204.9 ± 16.02	201.2 ± 11.9	0.85
TG, mg/dl	192.1 ± 25.9	206 ± 20.3	0.68
Insulin, µU/L	10.5 ± 2.7	12.5 ± 2.5	0.6
HOMA-IR	4.9 ± 1.6	4.5 ± 0.93	0.82
PPAR-γ	1	1.02 ± 0.1	0.85
Vitamin E, mg/L	9.03 ± 0.6	9.5 ± 0.2	0.71
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; P, placebo; A: atorvastatin			
¹ Means ± SE (all such values)			

Table 2
Anthropometric and biochemical measurements at baseline and week 12

Variables	A + P		A + E		p value [‡]
	Baseline	Week 12	Baseline	Week 12	
Energy, kcal	1960.1 ± 210.5	1870.1 ± 206.2	1917 ± 312.1	1887 ± 428.6	0.7
p value [†]	0.32		0.6		
BMI, kg/m ²	27.1 ± 0.9	26.8 ± 0.8	26.8 ± 0.5	26.5 ± 0.5	0.8
p value	0.26		0.2		
WHR	0.97 ± 0.01	0.96 ± 0.01	0.96 ± 0.01	0.95 ± 0.1	0.9
p value	0.28		0.36		
FBS, mg/dl	177.4 ± 19.6	152 ± 11.6	154.8 ± 15.2	134.3 ± 8.1	0.2
p value	0.08		0.2		
2hPG, mg/dl	254.5 ± 31.9	222.7 ± 32.9	263.7 ± 31.2	177.1 ± 26.5	0.3
p value	0.09		0.04		
HbA1c, %	7.6 ± 0.42	7.3 ± 0.4	7.3 ± 0.4	6.7 ± 0.26	0.2
p value	0.16		0.04		
HDL.C, mg/dl	46.9 ± 4.3	50.4 ± 3.4	48.7 ± 3.1	50.6 ± 4.2	0.9
p value	0.18		0.66		
LDL.C, mg/dl	101.5 ± 7.2	78.5 ± 5.1	98.1 ± 7.7	80.2 ± 3.8	0.8
p value	0.006		0.03		
TC, mg/dl	204.9 ± 16	174 ± 16.9	201.2 ± 11.9	168 ± 4.8	0.7
p value	0.05		0.01		
TG, mg/dl	192.1 ± 25.9	170.3 ± 3.3	206 ± 20.3	151 ± 20.9	0.5
p value	0.3		0.02		
Insulin, µU/L	10.5 ± 2.7	9.9 ± 2.5	12.5 ± 2.5	6 ± 1.4	0.2

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; P, placebo

[†] p values are related to the differences in each group from baseline up to the end; evaluated by paired sample t-test.

[‡] P values are related to the differences between the two studied groups after 12 week, evaluated by independent sample t-test.

p value	0.15		0.001		
HOMA-IR	4.9 ± 1.6	3.9 ± 1.2	4.5 ± 0.93	2 ± 0.49	0.1
p value	0.08		0.001		
PPAR-γ	1	2.95 ± 0.27	1.02 ± 0.1	5.3 ± 0.54	0.001
p value	< 0.001		< 0.001		
Vitamin E, mg/L	9.03 ± 0.6	7.89 ± 1.02	9.5 ± 1.2	15.9 ± 1.3	< 0.001
p value	0.31		< 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; P, placebo					
† p values are related to the differences in each group from baseline up to the end; evaluated by paired sample t-test					
‡ P values are related to the differences between the two studied groups after 12 week, evaluated by independent sample t-test					

After 12 week, BMI in the A + E group was significantly reduced, compared to the A + P group ($p = 0.02$). PPAR- γ gene expression was significantly up-regulated at the A + E, compared to the A + P group ($p = 0.001$). Serum vitamin E levels was significantly higher in the A + E than A + P group ($p < 0.001$) (Table 2).

Adjusting to the baseline measures, serum insulin level and HOMA-IR were significantly decreased at the A + E compared with A + P group, adjusted to the baseline variables (-6.5 ± 1.3 vs. -0.55 ± 0.35 , $p < 0.001$ and -2.5 ± 0.54 vs. -1.01 ± 0.52 , $p = 0.04$, respectively). PPAR- γ mRNA expression was significantly up-regulated at the A + E than A + P group (4.3 ± 0.5 vs. 1.9 ± 0.27 , $p = 0.001$), adjusted for baseline measures. Serum HbA1c, 2hPG, TG, and TC more decreased in the A + E than A + P group, but these changes were not significant (Table 3).

Table 3
Mean changes from baseline up to the end of intervention between treatment groups

Variables	A+P	A+E	p value [†]
BMI, kg/m ²	-0.21 ± 0.17	-0.63 ± 0.11	0.06
WHR	-0.005 ± 0.004	0.003 ± 0.002	0.15
FBS, mg/dl	-25.4 ± 13.1	-20 ± 15	0.8
2hPG, mg/dl	-31.8 ± 17.2	-86.6 ± 37	0.2
HbA1c, %	-0.24 ± 0.16	-0.64 ± 0.27	0.23
HDL cholesterol, mg/dl	3.5 ± 2.4	1.9 ± 4.1	0.7
LDL cholesterol, mg/dl	-23 ± 6.4	-17.9 ± 7.1	0.6
TC, mg/dl	-30.9 ± 14	-33.2 ± 10.6	0.89
TG, mg/dl	-21.8 ± 22.1	-55 ± 19.8	0.3
Insulin, µU/L	-0.55 ± 0.35	-6.5 ± 1.3	< 0.001
HOMA-IR	-1.01 ± 0.52	-2.5 ± 0.4	0.04
PPAR-γ	1.9 ± 0.27	4.3 ± 0.5	0.001
Vitamin E, mg/L	-1.1 ± 1.05	6.4 ± 1.1	< 0.001
WHR, waist to hip ratio; FBS, fasting blood sugar; 2hPG: 2 hour plasma glucose; HbA1c: glycosylated hemoglobin; TC, total cholesterol; TG, triglyceride; PPARγ: peroxisome proliferator-activated receptor; P, placebo			
[†] P values are for the atorvastatin plus vitamin Supplemented group relative to the atorvastatin plus placebo by using an ANCOVA with baseline values as covariate.			

According to the result of logistic regression model, PPAR-γ mRNA expression was significantly up-regulated at the A + E compared with the A + P group after adjusting for the baseline measure as covariate (OR = 5.4, 95% CI = 0.8–36.9, p = 0.04).

Discussion

To our knowledge, this is the first RCT performed to assess the effects of co-administration of vitamin E with atorvastatin on insulin sensitivity, blood glucose and lipids in the T2DM patients with hyperlipidemia. PPAR-γ mRNA expression was assessed as the involved pathway in this effect. Our results suggest that atorvastatin along with vitamin E supplementation compared with atorvastatin alone may have beneficial effect on insulin sensitivity by regulation of serum insulin and HOMA-IR through PPAR-γ mRNA up-regulation. Serum HbA1c, 2hPG, TG, and TC more decreased in the A + E than A + P

group, but these changes were not statistically significant. We think this may have been due to not enough sample size which make the present study as a pilot one and a larger sample is needed in the future studies.

PPARs belong to the superfamily of nuclear receptors (NRs), which bind to the ligand and then heterodimerize with a retinoid-X-receptor, finally bind to peroxisome proliferative-response element (PPRE) to initiate signaling pathways^(9, 21). PPARs have three isotypes including γ , α and β/δ which have tissue-specific distribution with different biological activity⁽²²⁾. PPAR- γ is an isotype which highly expresses at the adipose tissue and regulates gene expression of lipoprotein lipase, glucose transporters such as GLUT4 and adiponectin⁽²³⁾. At the present study, we showed that vitamin E supplementation along with atorvastatin increases PPAR- γ gene expression which is associated with lower insulin resistance. Our result is in agreement with the previous animal study which vitamin E supplementation (50 mg/kg) increased PPAR- γ level in aortae of rabbits fed a cholesterol-rich diet⁽²⁴⁾. They concluded that the protection effect of vitamin E against atherosclerosis is related to the increase in ATP-binding cassette transporter A1 (ABCA1) gene expression which is a target gene for PPAR- γ .

The effect of 10 mg/kg d oral atorvastatin was compared with the 10 mg/kg d pioglitazone on PPAR- γ gene expression in the heart of rats after a 3-day pretreatment⁽²⁵⁾. Results showed that both of them up-regulated PPAR- γ gene expression via increase in myocardial 15-deoxy-prostaglandin J2 (15DPGJ2) levels in the rat myocardium. Tocotrienols enhanced the ligand-binding domains of PPAR α with the receptor-interacting motif of PPAR- γ coactivator-1 α (PGC-1 α) interaction. Also, they improved whole body glucose usage and insulin sensitivity by up-regulation of PPAR- γ target genes (14). One cell culture study showed that vitamin E, both α and γ -tocopherol, up-regulates adiponectin expression at mRNA and protein level via a mechanism that increases PPAR- γ mRNA through increase in 15DPGJ, as an endogenous ligand⁽²⁶⁾.

The combination effect of atorvastatin plus vitamin E was studied in dialysis patients⁽²⁷⁾. Results showed that atorvastatin is effective in the plasma TC, TG, LDL, apo-lipoprotein B and oxidized LDL reduction in these patients. Adding the alpha-tocopherol to atorvastatin had additional value on in vitro LDL oxidization. Our results are in contrast with this study. At our study, mean changes of TG and TC were higher in the atorvastatin plus vitamin E group than atorvastatin alone, but these changes were not significant. This inconclusive result may be due to various diseases. In one study, the effects of pitavastatin and atorvastatin compared on glycemic control and insulin sensitivity in type 2 diabetic patients with hypercholesterolemia⁽²⁸⁾. Results showed that with the similar effects on lipid profile, mean changes in HbA1c was significantly higher in the pitavastatin than atorvastatin-treated group. Also, pitavastatin lowered the glycoalbumin, FBS and HOMA-IR, significantly. The present study had the same results. Atorvastatin plus vitamin E had more effect in serum insulin and HOMA-IR than atorvastatin treatment, alone.

Another human study assessed the effect of daily supplementation of 800 IU vitamin E in type 2 diabetic patients for 12 week⁽²⁹⁾. They concluded that vitamin E supplementation can't improve FBG, lipid profile, HbA1c, serum insulin, and blood pressure in these patients. Patients didn't receive any drug for lipid control. Vitamin E effect on blood glucose control is controversial. One study showed that consumption of 1600 IU/day α -tocopherol reduced HbA1c levels, significantly⁽³⁰⁾. Another study on type 1 diabetic patients has shown this reduction by 100 IU vitamin E supplements⁽³¹⁾. Other studies with 600 and 900 IU/day vitamin E decreased oxidative stress, HbA1c, serum insulin and HOMA-IR^(32, 33).

Conclusions

Co-administration of vitamin E with atorvastatin reduced insulin resistance and increased PPAR- γ mRNA expression than atorvastatin, alone. Although more studies with larger samples are needed. Also, recognition of other signaling pathways and target genes related to PPAR- γ in lipid and glucose metabolism are needed to reach concise results.

Declarations

Ethics approval and consent to participate: The ethic committee of Zanjan University of Medical Sciences, Zanjan, Iran, ethically approved the study. Patients signed the consent form before participation (ZUMS.REC.1395.268).

Consent for publication: Not applicable.

Availability of data and materials: Not applicable.

Competing interests: Not applicable.

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Author's contribution: The authors' responsibilities were as follows: Banafsheh Sadat Tabaei, Seyedeh Neda Mousavi, Maryam Jamehshorani and Ali Awsat Mellati: conceived and designed the study protocol and provided administrative, technical and material support. Banafsheh Sadat Tabaei, Aliasghar Rahimian and Hadi Rostamkhani participated in the development of the project. Seyedeh Neda Mousavi contributed to the final content, analyzed and interpreted the data, wrote the first draft of the manuscript. Seyedeh Neda Mousavi and Ali Awsat Mellati provided critical revision and important intellectual content. All authors have read and approved the final version of the manuscript.

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Figures

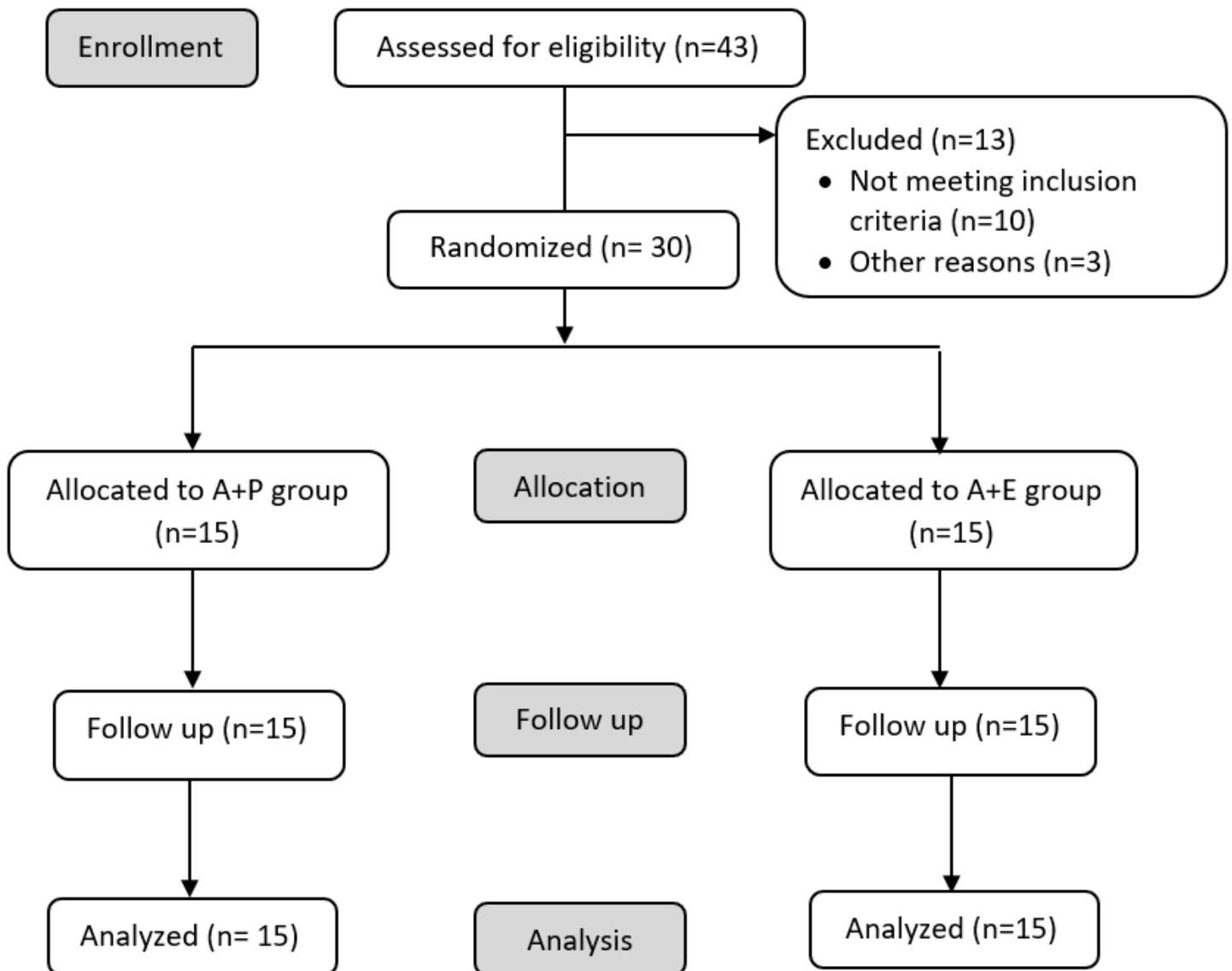


Figure 1

Screening, enrollment, random assignment, and follow-up of study participants