Journal of Advanced Research 28 (2021) 27–33

Contents lists available at ScienceDirect

Journal of Advanced Research

journal homepage: www.elsevier.com/locate/jare

Combined effect of high-dose vitamin A, vitamin E supplementation, and zinc on adult patients with diabetes: A randomized trial

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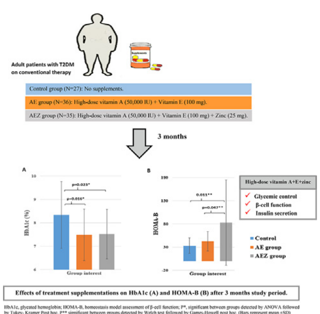
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 May 2020
Revised 16 June 2020
Accepted 17 June 2020
Available online 21 June 2020

Keywords:
Type 2 diabetes mellitus
Vitamin A
Vitamin E
Zinc
Insulin

ABSTRACT

In type 2 diabetes mellitus (T2DM), hyperglycemia leads to oxidative insult. Vitamins A and E have antioxidant potentials and may help in managing diabetes. The combined effect of high-dose vitamin A plus E supplementation with and without zinc on T2DM, has never been examined. Thus, this study aimed to evaluate and compare the effect of high-dose vitamin A plus E supplementation (AE) versus high-dose vitamin A plus E with zinc (AEZ), on different diabetic parameters. Ninety-eight patients with T2DM were randomized to receive either: 50,000 IU vitamin A and 100 mg vitamin E (AE group, N = 36), an equivalent dose of vitamin A and E combined with 25 mg zinc (AEZ group, N = 35), or no supplements (control group, N = 27) for three months. Compared to control, AEZ group showed significant reductions in fasting blood glucose, 2 h postprandial blood glucose, and glycated hemoglobin (HbA1c) with significant increases in homeostasis model assessment of beta-cell function and difference value of fasting insulin. Two hair loss cases were recorded in both treated groups. Although vitamin A needs dose moderation, these results suggest that, high-dose vitamin A plus E supplementation combined with zinc may improve glycemic control, β-cell function, and insulin secretion in adults with T2DM.

Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous and progressive disorder with variable degrees of insulin resistance and pancreatic β-cell dysfunction characterized by chronic hyperglycemia [1]. Hyperglycemia leads to oxidative insult through different pathways, causing tissue damage and injury [2]. Studies have elucidated a relationship between increased oxidative stress

Peer review under responsibility of Cairo University.

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https://doi.org/10.1016/j.jare.2020.06.013
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and decreased insulin sensitivity, highlighting the putative role of antioxidant therapy in diabetes control [3,4].

Vitamin A is an essential fat-soluble micronutrient with the highest antioxidant potential among all vitamins [5]. Besides its role as an antioxidant, vitamin A possesses pleiotropic roles in cell regulation, endocrine development, and even pancreatic function [6]. Vitamin A deficiency is prevalent in developing countries due to poor nutrition [7]. Studies showed a lower concentration of serum vitamin A in patients with diabetes than in normal subjects [8]. Moreover, vitamin A deficiency was reported in all the malnourished diabetic patients as compared to the malnourished control population [9]. Evidence has suggested that, daily intake of vitamin A improves pancreatic β-cell function and prevents or delays the transition from pre-diabetes to T2DM [10]. Meerza et al. showed in a study conducted on mice that, vitamin A has both antioxidant and antihyperglycemic potentials and, therefore can be considered a dietary intervention in patients with T2DM [2]. However, given the complexity associated with vitamin A supplementation, more studies are needed to affirm the beneficial effects of vitamin A in patients with diabetes.

Zinc (Zn) is a ubiquitous trace element involved in a multitude of biological functions [11]. Zn functions as a complex antioxidant through participation in superoxide dismutase (SOD), a key antioxidant enzyme, which is vital for intra-and extracellular antioxidant defense [12], stabilizes cell membranes and inhibits lipid peroxidation [13]. In addition, Zn plays an essential role in the synthesis, storage, and secretions of insulin by pancreatic tissue [14]. Zn deficiency is common in patients with diabetes [15]. A *meta*-analysis of 25 studies reported beneficial effects of zinc supplementation in diabetes [16].

Vitamin E is a hydrophobic antioxidant in which alpha-tocopherol is the most abundant, and biologically active [17]. Vitamin E reduces both oxidative stress and oxidative stress-associated damage in T2DM [18]. Observational studies have revealed that, vitamin E supplementation is associated with beneficial effects on glycemic control in T2DM [19,20]. However, a *meta*-analysis of 14 randomized controlled trials (RCTs) on vitamin E supplementation failed to support this finding due to insufficient evidence [21].

In light of the deleterious consequence of oxidative stress in diabetes, specific antioxidant treatment should be recommended, along with usual diabetes medications [22]. Vitamins A and E have antioxidant potentials and may be of interest in control diabetes. There is little evidence on the potential role of vitamin A and E supplementation in managing patients with T2DM. To the authors' best knowledge, the combined effect of high-dose vitamin A plus E supplementation, with and without zinc in managing diabetes, has never been examined. Accordingly, the present study was conducted to evaluate and compare the effect of high-dose vitamin A plus E supplementation (AE) versus combined high-dose vitamin A plus E with the zinc (AEZ) versus control, on parameters associated with diabetes control including blood glucose, glycated hemoglobin (HbA1c), lipid profile, as well as β-cell function, insulin secretion and resistance in adult patients with T2DM.

Materials and methods

Study design and patients

This was an interventional open-labeled randomized trial where adult outpatients of both sexes, aged between 20 and 64 years with an established diagnosis of T2DM, body mass index (BMI) < 40 kg/m^2, on fixed oral hypoglycemic dosage for at least 3 months, with normal renal and hepatic functions, were recruited. Key exclusion criteria included: patients with type 1 diabetes, taking multivitamin or mineral supplements in the previous three months, those take hormone replacement therapy, chelating therapy, anticonvulsants, warfarin, medications containing retinoid, or corticosteroids, any clinical evidence suggesting kidney or liver disease, a history of surgery in the last month, or with concurrent acute illness, or those receiving insulin preparations as a part of diabetes management. Furthermore, pregnant, lactating women and those trying to conceive were also excluded.

All patients were enrolled from outpatient diabetic clinics of Kasr Alainy Hospital, Cairo University, Egypt during the period between November 2015 and February 2017. The study protocol was approved by the Research Ethics Committee, Faculty of Pharmacy, Cairo University, Egypt with serial number CL (1461). The trial was registered at clinicaltrail.gov with a registration identifier (NCT03112382). Informed consent was obtained from all patients priori the study.

Recruited patients were randomly assigned to one of three groups (control, AE, or AEZ group). Patients in AE group received high-dose of 50,000 I.U. vitamin A one capsule daily (A-viton ®, Kahira Pharm. & Chem. Ind. Co. Cairo, Egypt) and 100 mg vitamin E one capsule daily (E-viton®, Kahira Pharm. & Chem. Ind. Co. Cairo, Egypt). Combined AEZ group received one capsule daily containing an equivalent dose of vitamin A and E combined with 75 mg zinc gluconate equivalent to 25 mg zinc (Vitazinc®, Egyptian Int. pharmaceutical industries Co. Cairo, Egypt). Patients in the control group received no supplements or vitamins during study period. All capsules were taken after breakfast with glass of water for three months.

The clinical and laboratory parameters were measured at baseline and repeated 12 weeks at the end of the study. The difference value (DV) was calculated for each parameter as the 12 weeks value minus the baseline. Dietary intake was assessed for each subject using the 24-hour dietary recall questionnaire at the entry of the study [23]. Patients were asked not to alter their usual diets or physical activity throughout the study period, and any changes in their medication were avoided whenever possible. Patient's recruitment and follow-up visits were adjusted in a way not contradict with a great change in diet habits during the Holy Ramadan month. Patient's compliance was assessed on a monthly basis by counting the remaining number of capsules and confirmed by comparing serum zinc concentration at baseline and three months end of the study. Patients who completed the study had drug compliance of at least 98%. Side effects from trial medications were also noted. Serum calcium (Ca) and alanine transaminase (ALT) were recorded to detect osteoporosis and liver damage respectively that may occur with vitamin A ingestion. The homeostasis model assessment (HOMA) index was used to evaluate the insulin resistance (IR) and β-cell function, with the following equations: HOMA-IR = fasting insulin (mU/l) $\times$ fasting glucose (mmol/L)/22.5.

HOMA of beta-cell function (HOMA-B) = 20 $\times$ fasting insulin/(fasting plasma glucose mmol/L -3.5) [24].

Blood sampling and assay

After an overnight fasting period of approximately 10 h, two venous blood samples each of 5 mLs were drawn aseptically from the subjects with metal-free stainless steel needles and divided into a sterile plain vial for serum zinc, insulin and lipid profile (total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL)), oxalate/fluoride vial for fasting blood glucose (FBG) and an Ethylenediaminetetraacetic acid (EDTA) vial for HbA1c. Serum was separated within 1 h. Analysis was done on the same day. Another two mLs of blood were collected in the post-prandial state in one oxalate/fluoride vial for 2 h postprandial blood glucose (2hrPP). The same sample treatment was repeated 3 months at the end of the study. Serum zinc was estimated with a Pye Unicam Model SP1900 atomic absorption spectrophotometer, UK, at 213.7 nm per acetylene-air-flame atomization. The analysis for the rest of the parameters was performed with the AU680 Beckman Coulter auto- chemistry analyzer, USA, at the chemical pathology department, Kasr Alainy hospital, Cairo, Egypt.

Sample size and statistical analysis

Sample size estimation was calculated a priori using G*Power 3.1.9.2 software (University of Düsseldorf, Düsseldorf, Germany). Based on predicted HbA1c % in adult Egyptian patients with T2DM [25], a total sample size of 81 was required to detect an effect size of 0.353 with an 80% power at a significance value of 0.05. Data was expressed as mean ± standard deviation (SD). For multiple comparisons, in case of continuous data analysis such as means, one-way analysis of variance (ANOVA) followed by Tukey-Kramer Post hoc; was performed. When ANOVA homogeneity of variance assumption; is not met, the Welch test followed by Games-Howell post hoc was applied. For nominal data such as numbers, percent, or sex, fisher's exact test; was used. A p-value < 0.05, was considered as significant for all statistical purposes [26]. IBM Statistical Package for the Social Sciences (SPSS) version 22 was used for statistical analysis.

Results

One hundred, six patients were eligibly recruited in the present study. Ninety eight of them completed the study. Four patients were lost to follow-up, two changed diabetes treatment regimen and two started lipid-lowering agents by the 2nd or 3rd month. At the beginning of the study, demographics and clinical characteristics of study patients were comparable between the three groups (Table 1).

Efficacy of trial medications

Effects of supplementations on glycemic variables:

As shown in Table 2, baseline levels of FBG, 2hrPP, and HbA1c were not significantly different. Significant differences were noted three months after supplementation with high-dose vitamin A plus E with and without zinc. In the AEZ group, a statistically significant decrease in finals (reading after 3 months) and DV of FBG, 2hrPP, HbA1c were recorded compared to the control group (p < 0.05). On the other hand, high-dose vitamin A plus E supplementation showed, a statistically significant decrease in finals and DV of FBG, HbA1c when compared to the control group (p < 0.05).

Effects of supplementations on β-cell function, insulin secretion and resistance:

Although baseline FINS, HOMA-IR, HOMA-B were comparable, after three months of supplementation, ANOVA detected a significant change in DV of FINS in the AEZ group, where post hoc test showed higher insulin secretion in the AEZ group compared to AE and control groups (p < 0.05). In addition, Welch followed by post hoc test, showed a statistically significant improvement in finals and DV of HOMA-B in AEZ group compared to vitamin AE group, and control group (p < 0.05) as shown in Fig. 1.

Effects of supplementations on lipid profile:

Table 3 shows the levels of lipid profile (TC, TG, HDL-c, LDL-c, VLDL-c, HDL risk factor) in all three groups. Following 3 months of supplementation, ANOVA failed to detect any significant difference between the three groups.

Safety of trial medications

As shown in table 2**,** after three months supplementation, serum Ca, and ALT remained comparable among all three groups. Two females, one in the AE group and one in the AEZ group experienced annoying scalp hair loss by the end of the 3rd month. No other side effects were detected during the study period.

Discussion

It is believed that, oxidative stress plays an important role in the pathogenesis of T2DM [4]. The increased production of reactive oxygen species (ROS) causes cellular damage in a progressive manner and results in mitochondrial dysfunction which inconsequently affects energy homeostasis and glucose-stimulated insulin release [8]. The results of the present study describe for the first time, the combined effect of high-dose vitamin A, vitamin E, and zinc on adults with diabetes.

In this clinical trial, A dose of 50,000 IU used to treat vitamin A deficiency [27], was consumed daily for three months in both AE and AEZ groups. In Gunasekara et al. study, a 5000 IU vitamin A dose was supplemented with other multivitamins/minerals daily for four months to assess effects of multi-mineral/vitamin supplementation with and without zinc, on diabetes control [28].

Table 1
Demographics and clinical characteristics of study subjects.

Parameter	Control N = 27	AE group N = 36	AEZ group N = 35	p-value
Age (year)	50.2 ± 9.2	50.2 ± 9.5	52.4 ± 6.8	0.493#
Gender (No. of males (%))	4 (14.8%)	8 (22.2%)	14 (40%)	0.067¥
Duration of diabetes (years)	5.9 ± 5.0	4.6 ± 3.3	4.6 ± 4.6	0.416#
BMI (kg/m^2)	31.9 ± 3.7	33.9 ± 3.7	31.9 ± 4.4	0.068#
WHR	0.93 ± 0.06	0.93 ± 0.06	0.93 ± 0.05	0.855#
Hemoglobin A1C (%)	8.08 ± 1.37	7.66 ± 1.13	7.77 ± 1.12	0.364#
ALT (U/L)	24.2 ± 8.7	24.0 ± 14.2	21.5 ± 11.5	0.583#
Creatinine (mg/dL)	0.67 ± 0.17	0.69 ± 0.18	0.76 ± 0.22	0.148#
Albumin (g/dL)	4.29 ± 0.31	4.23 ± 0.39	4.12 ± 0.37	0.172#
Type of diabetes treatment (No. of patients taking (%))				
Metformin	1 (3.7%)	3 (8.3%)	3 (8.6%)	0.788¥
Glibenclamide	2 (7.4%)	5 (13.9%)	2 (5.7%)	0.564¥
Gliclazide	1 (3.7%)	1 (2.7%)	5 (14.3%)	0.146¥
Metformin plus glibenclamide	18 (66.7%)	23 (63.9%)	22 (62.9%)	0.965¥
Metformin plus gliclazide	5 (18.5%)	4 (11.1%)	3 (8.6%)	0.464¥

Data are presented as mean ± SD unless otherwise mentioned. BMI: body mass index; WHR: waist to hip ratio; ALT: alanine transaminase enzyme, P#: Non-significant by ANOVA; P¥: Non-significant by Fisher's Exact test.

Table 2
Effects of high-dose vitamin A plus E (AE) supplementation versus high-dose vitamin A plus E supplementation combined with zinc (AEZ) versus control on the glycemic control parameters, insulin secretion, and resistance of patients with T2DM at baseline and after three months study period.

Parameter	Control N = 27	AE group N = 36	AEZ group N = 35	P-value
FBG (mg/dL)				
Before	162.37 ± 47.28	157.66 ± 50.30	148.74 ± 46.79	0.524
After	**188.07 ± 49.39^{a,b}**	**155.50 ± 42.50^{b}**	**145.54 ± 44.37^{a}**	**0.001***
DV	**25.70 ± 29.75^{a,b}**	**−2.16 ± 35.13^{b}**	**−3.20 ± 35.73^{a}**	**0.002***
2hr PP (mg/dL)				
Before	240.14 ± 86.33	224.19 ± 69.00	234.00 ± 67.51	0.685
After	**262.37 ± 86.81^{a}**	228.22 ± 58.33	**211.48 ± 65.89^{a}**	**0.019***
DV	**22.22 ± 45.63^{a}**	4.02 ± 50.06	**−22.51 ± 50.91^{a}**	**0.002***
HbA1c (%)				
Before	8.08 ± 1.37	7.66 ± 1.13	7.77 ± 1.11	0.364
After	**8.34 ± 1.43^{a,b}**	**7.49 ± 1.10^{b}**	**7.52 ± 1.06^{a}**	**0.010***
DV	**0.26 ± 0.64^{a,b}**	**−0.16 ± 0.48^{b}**	**−0.24 ± 0.72^{a}**	**0.005***
FINS (μU/mL)				
Before	13.69 ± 7.90	11.46 ± 4.90	10.46 ± 6.12	0.137
After	10.09 ± 3.53	9.99 ± 4.70	12.04 ± 6.08	0.242$^{¥}$
DV	**−3.60 ± 5.72^{a}**	**−1.46 ± 4.09^{b}**	**1.63 ± 5.72^{a,b}**	**0.001***
HOMA-IR				
Before	5.65 ± 4.29	4.61 ± 2.54	4.00 ± 2.77	0.138
After	4.67 ± 2.08	4.21 ± 2.84	4.14 ± 2.19	0.679
DV	−0.98 ± 3.01	−0.39 ± 1.85	0.19 ± 2.4	0.182
HOMA-B				
Before	60.01 ± 42.24	56.51 ± 32.18	58.50 ± 59.61	0.957
After	**33.22 ± 16.63^{a}**	**42.85 ± 20.85^{b}**	**82.79 ± 92.08^{a,b}**	**0.004*¥**
DV	**−26.78 ± 35.23^{a}**	**−13.65 ± 28.73^{b}**	**23.79 ± 78.87^{a,b}**	**0.007*¥**
Zinc (μg/dL)				
Before	75.63 ± 15.45	77.85 ± 16.57	74.15 ± 18.13	0.672
After	**77.59 ± 16.54^{a}**	**79.20 ± 18.17^{b}**	**106.27 ± 26.32^{a,b}**	**<0.001*¥**
DV	**1.95 ± 16.18^{a}**	**1.35 ± 10.36^{b}**	**31.84 ± 19.42^{a,b}**	**<0.001*¥**
Ca (mg/dL)				
Before	9.08 ± 0.63	9.18 ± 0.63	8.98 ± 0.60	0.441
After	9.04 ± 0.54	9.16 ± 0.53	9.31 ± 0.55	0.231
DV	−0.008 ± 0.71	−0.04 ± 0.55	0.31 ± 0.71	0.09
ALT (U/L)				
Before	24.27 ± 8.7	24.02 ± 14.24	21.51 ± 11.5	0.583
After	23.42 ± 10.8	23.14 ± 15.30	20.06 ± 9.11	0.516
DV	−1.90 ± 7.32	−0.74 ± 8.49	−1.87 ± 13.0	0.875

Data are presented as mean ± SD. P$^{¥}$ values are determined by Welch test, otherwise P values are determined by ANOVA test. *P values statistically significant between groups. Groups with the same superscripted letters, either: (a, a) or (b, b) measuring the same parameter, showed statistically significant means after the post hoc tests at $p < 0.05$. DV, differential values (the difference between the post- and pre-administration values); FBG: fasting blood glucose; 2hr PP: 2 h postprandial glucose; HbA1c: glycated hemoglobin; FINS: fasting serum insulin; HOMA-IR: homeostasis model assessment for insulin resistance; HOMA-B: homeostasis model assessment for B-cell function; serum Ca: serum calcium; ALT: alanine transaminase enzyme.

Although the selected dose in this trial was not associated with liver damage or reduced serum calcium induced osteoporosis, scalp hair loss cases recorded by the end of study period in both treated groups may indicate chronic toxicity [29]. Further research is warranted to moderate dose of vitamin A supplementation for maximization of benefit to risk ratio.

The present study showed a beneficial effect on glycemic control in adult patients with T2DM who received 12 weeks daily high-dose vitamin A plus E supplementation with and without the zinc (see graphical abstract). High-dose vitamin A plus E supplementation were significantly capable of reducing FBG and glycated HbA1c compared to control. Zinc supplementation along with high-dose vitamin A and E, in addition to the beneficial effect of the two vitamins, lead to significant reduction of 2hrPP, and increased HOMA-B, and DV of fasting insulin. These outcomes highlight the potential role of vitamins A, E and zinc as a therapeutic intervention in controlling patients with T2DM. The worsening in the control glycemic parameters' reflects the ineffectiveness of diabetes conventional therapy. Metformin and sulfonylurea, the blood glucose-lowering agents prescribed alone in the control group, do not provide a durable glycemic control as they do not improve the continuous decline in β-cell function [30]. Such therapies, focus on only insulin-centric targets, should also include glucose-centric agents such as β-cell antioxidant agents [31].

Vitamin A can impact T2DM pathogenesis through several potential molecular mechanisms including; chelation of oxide radicals, insulin sensitivity, obesity and beta cell regeneration [8]. The improved glycemic control with vitamin A and E supplementation supports recommendation suggested by Meerza et al., and Iqbal et al., to implement vitamin A as a dietary intervention in type 2 diabetes [2,8]. Moreover, the present results are in concordance with case-control trials which demonstrated favorable effects of vitamin E supplementation on glycemic control, including serum HbA1c [20], and fasting glucose [19]. Also, our results support the hypothesis of two *meta*-analysis of RCTs that, HbA1c was reduced significantly with vitamin E supplementation in patients with inadequate glycemic control [21,32]. A suggested mechanism is that, vitamin E prevents the glycosylation of hemoglobin by interrupting glycosylation at an early step in the Maillard reaction [33] or by partially inhibiting the formation of advanced glycosylation end products (AGEs) [34]. Moreover, vitamin E suppresses ROS generation in the pancreas and reduces the long-term pancreatic β-cell dysfunction and apoptosis caused by oxidative stress in T2DM [18,35].

The addition of zinc resulted in further improvement in glycemic control. This goes in parallel with *meta*-analysis results, showing a significant reduction in FBS, 2hrPP, and HbA1c following zinc supplementation in patients with T2DM [16]. Different

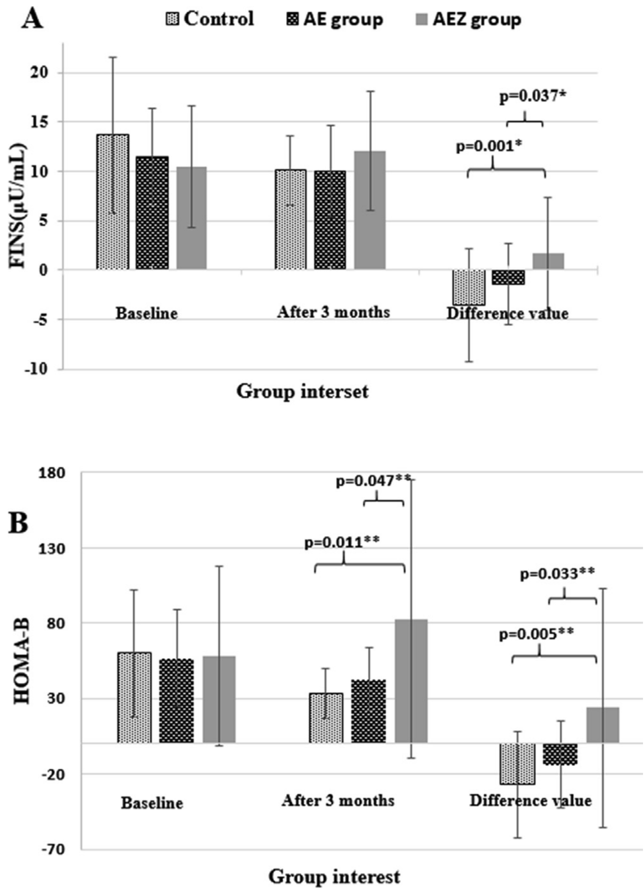

Fig. 1. Effects of treatment supplementations on FISN (A) and HOMA-B (B) at baseline and after 3 months study period. FISN, Fasting insulin; HOMA-B, homeostasis model assessment of β-cell function; AE group, high dose vitamin A plus E group; AEZ group, combined high-dose vitamin A plus E with zinc group; P*, significant detected by ANOVA followed by Tukey- Kramer Post hoc, P** significant detected by Welch test followed by Games Howell- post hoc. (Bars represent mean ± SD).

molecular mechanisms have explained the role of zinc supplementation in regulating blood glucose level. Zinc complexes has shown insulin mimetic and hypoglycemic properties [36]. Zinc improves the peripheral insulin sensitivity, as it can potentiate insulin stimulated glucose transport [37]. Furthermore, protein tyrosine phosphatase 1B which is a key regulator of the phosphorylation state of insulin receptor, is known to be a target of zinc ions [38].

Many nutrition studies have focused on the role of zinc on insulin secretion and resistance [39,40,41]. High-dose vitamin A plus E combined with zinc in the present trial showed a significant improvement in fasting serum insulin. A similar effect was revealed by Hegazi et.al., [42]. However, this effect on insulin secretion has been contradicted by other studies [28,43,44].

Elevated levels of blood glucose, observed at baseline for the three group, augment generation of ROS [30] which, induces oxidative stress in pancreatic β-cell due to its very low content of antioxidant enzymes [45]. Following three months study period, the control group, lack of antioxidant therapy, showed a marked decrease in HOMA-B which indicates a progressive decline in β-cell function due to oxidative insult [30,46]. Antioxidants, high-dose vitamin A and E in the AE group, resulted in less reduction in HOMA-B. However, zinc combined with high-dose vitamin A and E in the AEZ group served as a potent antioxidant scavenger, which was efficiently capable of improving the β-cell function, noted with a significant increase in HOMA-B. This outcome emphasizes the regenerative antioxidant role of zinc on the pancreatic tissue [47,48]. It is worth noting that the three groups had baseline zinc concentration within physiological levels (the normal zinc reference range for age > 10 years is (66–110 μg/dL) [49]). Zinc supplementation in the AEZ group increased zinc level towards the upper limit of the range which, counted for the favorable effect on fasting insulin and HOMA-B.

Several clinical trials have failed to report beneficial effects following consuming antioxidants in patients with T2DM [50,51,52,53]. The possible provided explanations were: small sample size, short trial duration which, was insufficient to detect a true HbA1c change, a small selected antioxidant dose which failed to elevate its level, and in turn, failed to exert a significant

Table 3
Effects of high-dose vitamin A plus E (AE) supplementation versus high-dose vitamin A plus E supplementation combined with zinc (AEZ) versus control on the lipid profile parameters of patients withT2DM at baseline and after three months study period.

Parameter	Control N = 27	AE group N = 36	AEZ group N = 35	P-value
TC (mg/dL)				
Before	208.69 ± 30.68	194.52 ± 34.37	189.79 ± 40.41	0.121
After	211.63 ± 35.55	198.38 ± 36.48	200.12 ± 39.09	0.340
DV	3.23 ± 17.73	5.75 ± 26.88	8.53 ± 23.07	0.687
TG (mg/dL)				
Before	146.51 ± 47.73	136.00 ± 63.69	137.79 ± 47.93	0.730
After	149.22 ± 43.04	148.17 ± 65.02	158.08 ± 57.35	0.734
DV	2.70 ± 38.29	17.63 ± 40.04	21.26 ± 35.31	0.146
HDL-C (mg/dL)				
Before	44.74 ± 8.35	46.51 ± 12.50	41.82 ± 9.86	0.183
After	47.77 ± 8.91	45.77 ± 10.74	43.09 ± 9.26	0.179
DV	3.03 ± 5.56	−0.45 ± 8.36	0.438 ± 6.50	0.105¥
LDL-C (mg/dL)				
Before	134.54 ± 28.53	119.85 ± 27.46	120.79 ± 38.45	0.153
After	135.29 ± 30.10	123.08 ± 32.37	123.09 ± 36.12	0.276
DV	0.75 ± 16.66	3.66 ± 27.06	0.468 ± 23.75	0.831
VLDL (mg/dL)				
Before	29.30 ± 9.50	27.25 ± 12.70	27.53 ± 9.56	0.734
After	29.48 ± 8.39	29.62 ± 12.97	32.04 ± 11.30	0.583
DV	0.17 ± 8.01	3.47 ± 8.02	4.71 ± 6.53	0.063
HDL-Risk factor				
Before	4.74 ± 0.82	4.55 ± 1.83	4.78 ± 1.50	0.834¥
After	4.53 ± 1.01	4.45 ± 0.96	4.78 ± 1.05	0.384
DV	−0.25 ± 0.62	−0.10 ± 1.46	0.06 ± 1.00	0.565

Data are given as mean ± SD. P¥ values are determined by Welch test, otherwise P values are determined by ANOVA test. DV, differential values (the difference between the pre- and post-administration values); TC, total cholesterol; TG, triglyceride; HDL-c, high density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; VLDL-c, very low-density lipoprotein cholesterol; HDL-Risk factor was calculated as TC/HDL-c clinical effect [21,54]. Moreover, the outcome parameters may have been influenced by; ethnic group, baseline HbA1c concentration, and fasting glucose control status [21].

It was concluded in the present study that, high-dose vitamin A plus E alone or combined with zinc have no positive impact on lipid profile. This is in accordance with the results of a *meta*-analysis on vitamin E supplementation which also failed to show a significant effect on lipid metabolism [21]. In contrast, an up to date *meta*-analysis of 32 studies has shown favorable effects with zinc supplementation on plasma lipid parameters as it significantly reduced TC, LDL-C, and TG [55].

The present study showed promising results after three months of high-dose vitamin A plus E supplementation combined with zinc, so we encourage future researches with a longer follow-up period where more significant findings might be shown. Furthermore, in future studies, more attention should be paid to levels of vitamin A and E. Baseline serum concentrations of vitamin A and E have to be assessed and monitored through the study period. This was not applicable in the present study due to financial constraints.

Conclusion

The present study sheds light on the future potential role of antioxidants to control hyperglycemia in patients with diabetes. Although vitamin A supplementation requires dose moderation, the results of this trial suggest that 12 weeks of high-dose vitamin A plus E supplementation combined with zinc may improve glycemic control, β-cell function, and insulin secretion in adult patients with T2DM. We recommend the use of antioxidant supplements such as vitamin A and E alone or combined with zinc as adjuncts to the standard therapy in patients with T2DM. More studies are needed to tailor vitamin A dose to maximize benefits, reduce possible adverse effects, and confirm these promising results.

Acknowledgement

The authors acknowledge the effort made by all staff members of Kasr Alainy Hospital during the patients' recruitment. Funding was received from Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Declaration of Competing Interest:

None.

Appendix A. Supplementary Data

Results of post hoc tests associated with this article; can be found in Table S1. Two-way ANOVA; was performed to rule out gender effect. Table S2 showed no significant effects observed in this trial due to gender. Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2020.06.013.

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