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EZETIMIBE REDUCES α-TOCOPHEROL LEVELS IN TYPE 2 DIABETES

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ABSTRACT – *Objective:* α-tocopherol has a protective role against free radical-induced damage. The intestinal absorption of α-tocopherol is in part carried out by Nieman-Pick1-like1 protein, which is the target of ezetimibe, a drug used for the treatment of hypercholesterolemia. Ezetimibe is frequently prescribed to patients with type 2 diabetes in combination with statins. Therefore, the aim of the present study was to investigate the impact of ezetimibe treatment on the blood level of α-tocopherol.

Materials and Methods: Two hundred patients affected by type 2 diabetes, regularly attending the diabetes clinic, were recruited. All patients should not have received vitamin supplements in the six months preceding the study. α-tocopherol was measured by reverse-phase high-performance liquid chromatography with UV detection. Other laboratory parameters were determined by standard laboratory procedures. Patients were analyzed according to ezetimibe treatment. A linear multivariate regression analysis was used to estimate the factors associated with α-tocopherol level.

Results: The majority of patients were on ezetimibe/statin treatment. Patients taking ezetimibe showed a significantly lower α-tocopherol level, 29.9±5.7 *vs.* 32.9±7.0 μmol/L (p=0.006) compared to those not taking the drug. Ezetimibe was significantly and negatively associated with α-tocopherol level in the multivariate analysis (β standardized coefficient = -.217, p = .002).

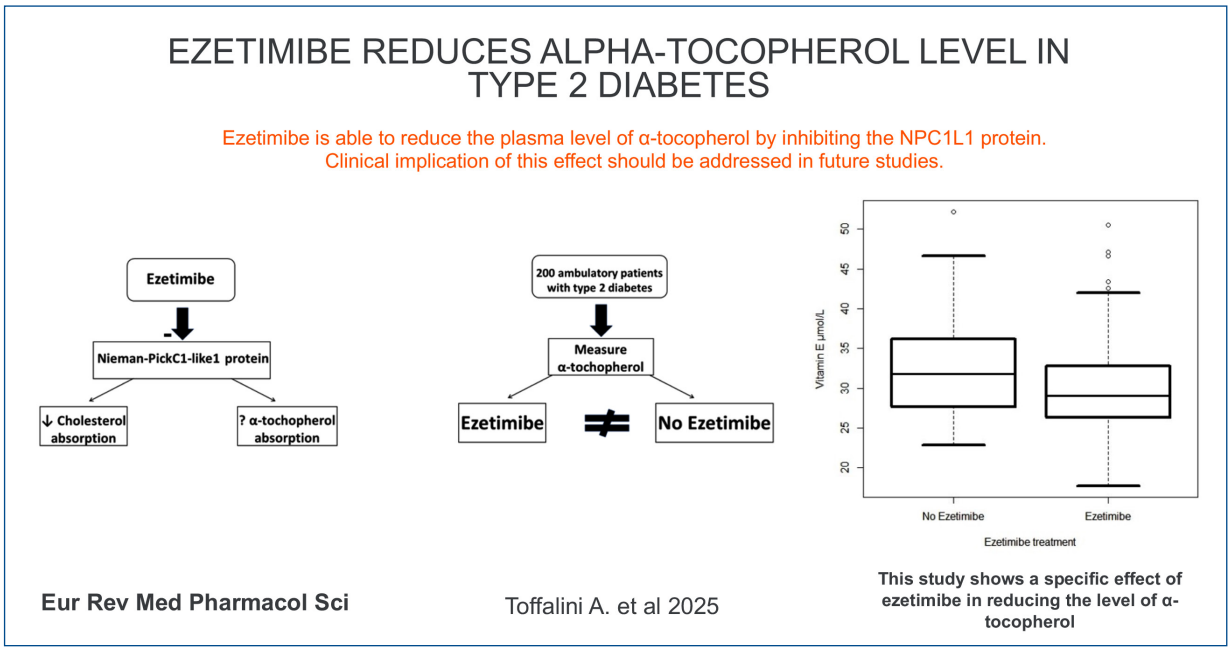
Conclusions: Treatment with ezetimibe can reduce α-tocopherol plasma levels by inhibiting the Niemann-Pick C1-like protein. Future studies are required to address the clinical implications of this finding.

KEYWORDS: Diabetes, Antioxidant, α-tocopherol, NPC1L1, Ezetimibe.

INTRODUCTION

α-tocopherol, a fat-soluble antioxidant and an essential nutrient for human beings, participates in the protective reactions against damage from free radicals[1-3]. Dietary uptake is an indispensable source of α-tocopherol due to the inability of human cells to synthesize this molecule[4]. α-tocopherol appears to be a promising candidate for cardiovascular disease (CVD) prevention[5]. Type 2 diabetes is an increasing chronic disease, and CVD are the major cause of morbidity and mortality for patients affected by this condition[6]. In this context, some observational studies have shown an association between α-tocopherol intake and a lower rate of overall and cardiovascular mortality[7-10].

At the intestinal level, Nieman-PickC1-like 1 protein (NPC1L1) contributes to the absorption of α-tocopherol as well as of coenzyme Q10 and of vitamin K[4]. However, other proteins, such as CD36, are also involved in the absorption of α-tocopherol[4].



Graphical Abstract. Ezetimibe is known to reduce the absorption of cholesterol, no information is available on its impact on the absorption of vitamin E. The comparison between subjects treated with ezetimibe and those not treated, showed that the vitamin E levels were significantly lowers in treated subjects. Future studies are needed to address the clinical implications of this finding.

It should be emphasized that the main function of NPC1L1 protein is the intestinal absorption of cholesterol[11]. Therefore, NPC1L1 inhibition is a crucial therapeutic target for reducing plasma cholesterol levels.

The intestinal uptake of α-tocopherol is essential for the maintenance of whole-body homeostasis[12]. However, how α-tocopherol is transported across the membrane by NPC1L1 protein remains unknown.

NPC1L1 protein is the therapeutic target of ezetimibe. This drug inhibits its transport activity, thereby reducing the absorption of cholesterol, as well as the absorption of α-tocopherol[11]. Ezetimibe in rats reduced the absorption of α-tocopherol almost completely when both substances were administered orally together[13].

Patients with type 2 diabetes are frequently treated with ezetimibe, usually in combination with statins. The combination of statin and ezetimibe is the cornerstone of hypercholesterolemia treatment in diabetes to achieve the specific target of LDL-cholesterol.

Therefore, the aim of the present study was to estimate the impact of ezetimibe treatment on the blood level of α-tocopherol in patients with type 2 diabetes.

MATERIALS AND METHODS

In this cross-sectional observational study, 200 patients regularly attending our diabetic clinic at the University of Verona were consecutively recruited. The inclusion criteria were age between 18 and 80 years, both sexes, and T2D diagnosed with the standard criteria at least 3 months before enrollment. The exclusion criteria were a history of pernicious anemia, autoimmune gastritis, pancreatitis, pregnancy, and vitamin supplementation within 6 months before the study.

The study was approved by the Local Ethics Committee of the Hospital Trust of Verona, with protocol number No. 3853CESC and date July 6, 2022. Informed consent was obtained from each participant. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki and its latest amendments. The patients were recruited between September 2022 and March 2023, and the study was conducted at the University of Verona.

Venous blood samples were drawn in the morning after an overnight fast. Serum creatinine (Roche enzymatic method) and other biochemical blood measurements were determined using standard laboratory procedures (Cobas 8000, Roche Diagnostics GmbH, Mannheim, Baden-Württemberg, Germany). Low-density lipoprotein (LDL) cholesterol was calculated using Friedewald's equation, except when triglycerides were above 400 mg/dl. Hemoglobin A1c (HbA1c) was measured by an automated high-performance liquid chromatography analyzer (HA-8140; Menarini Diagnostics, Florence, Italy). The glomerular filtration rate (GFR) was estimated us-

ing the CKD Epidemiology Collaboration (CKD-EPI) equation[14]. Albuminuria was measured by an immuno-nephelometric method on a morning spot urine sample and expressed as the albumin-to-creatinine ratio. Patients studied during the programmed blood test for medical control underwent an additional blood test for the α-tocopherol, which was measured by reverse-phase high-performance liquid chromatography (HPLC) with UV detection (HPLC Prominence, Shimadzu Corporation, Kyoto, Japan).

Body mass index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared. Height and weight were measured using a calibrated stadiometer and balance-beam scale, respectively, during the scheduled visit. Height was recorded to the nearest 0.1 cm and weight to the nearest 0.1 kg with minimal clothing. A physician measured the blood pressure of patients using a mercury sphygmomanometer after they had been seated quietly for at least 5 minutes. Patients were considered to have hypertension if their blood pressure was ≥140/90 mmHg or if they were taking any anti-hypertensive drugs. Information on smoking status and the use of medications was obtained from all patients *via* interviews during medical examinations. The duration of diabetes was calculated from the date of diabetes diagnosis. A single ophthalmologist diagnosed diabetic retinopathy using fundoscopy after pupillary dilation according to a clinical disease severity scale (no retinopathy, non-proliferative, proliferative, or laser-treated retinopathy). The presence of proliferative retinopathy was confirmed by fundus fluorescein angiography. Nephropathy was defined as the presence of eGFR <60 ml/min/1.73 m^2 and/or abnormal albuminuria (i.e., an albumin-to-creatinine ratio ≥30 mg/g creatinine). A confirmed history of myocardial infarction, angina, coronary revascularization, stroke, transitory ischemic attack, carotid revascularization, non-traumatic amputation, gangrene, and/or lower limb revascularization was considered a valid proxy for prior clinical cardiovascular disease (CVD). Ultrasonography scanning of common and internal carotid arteries was performed (Esaote Wall Track System, Esaote S.p.A., Genova, Italy), and a cut-off of 60% was used to define a significant arterial stenosis. Ultrasonography scanning of lower limb arteries was performed, and any detected stenosis or moderate-to-severe reduction of blood flow at the proximal and/or distal level was considered as a marker of peripheral artery disease.

Statistical analysis

Data were summarized as mean ± standard deviation (SD) for continuous variables and absolute values or percentages for categorical variables. Differences in clinical/ biochemical characteristics were tested using the Student's *t*-test for normally distributed variables and the Mann-Whitney test for non-normally distributed variables. The χ^2 test was used to study differences in proportions or percentages between the two groups for categorical variables. A forced-entry linear regression model was performed adjusting for age, BMI, eGFR, ezetimibe (dummy variable), and gender. Covariates for the multivariate regression model were chosen as potential confounding based on their biological plausibility. A *p*-value of less than 0.05 was considered statistically significant. The Analyses were carried out with SPSS Statistics for Data Analysis v.20.0.1.1 (IBM Corp., Armonk, NY, USA).

RESULTS

Ezetimibe was taken by 79.5% of type 2 diabetes patients; of these, 62.0% were men and 37.1% were women (p=.033). A previous cardiovascular event was recorded in 18.2% of the patients.

In Table I, the main clinical and laboratory characteristics of the patients stratified by the use of ezetimibe are shown. Patients in the ezetimibe group were older, with a longer duration of diabetes. In this group, males were more represented than females. As expected, they showed a significantly lower concentration of total and LDL cholesterol (102.7 ± 28.3 *vs.* 64.0 ± 24.2, p <.001) and a higher prevalence of clinical cardiovascular diseases (2.5% *vs.* 18.2%, p=.007). The concentration of α-tocopherol was significantly lower in patients treated with ezetimibe, 29.9 ± 5.7 *vs.* 32.9 ± 7.0 µmol/L (p=.006), Figure 1. Men showed a lower level of α-tocopherol than women (29.6 ± 5.8 *vs.* 32.5 ± 6.3, p=.002). Ezetimibe was significantly associated with the level of α-tocopherol in the multivariate analysis, standardized β coefficient -.217, p=.002 (Table II). Other significant variables associated with α-tocopherol were sex and BMI.

DISCUSSION

The primary finding of the present study is the significantly lower level of α-tocopherol in patients with type 2 diabetes who are treated with ezetimibe compared to those not receiving it. The effect of this drug on α-tocopherol levels was maintained in the multivariate analysis. α-tocopherol is considered an important protective factor against oxidative stress[15], particularly by limiting free radical-induced damage to

Table I. Clinical characteristics of ambulatory patients with type 2 diabetes according to the treatment of dyslipidemia.

	No Ezetimibe (n=41)	Ezetimibe (n=159)	*p*
Age, yr	64.8 ± 9.4	67.6 ± 8.4	.067
Duration of diabetes, yr	9.9 ± 8.2	12.7 ± 8.2	.053
BMI, Kg/m^2	27.6 ± 4.0	28.2 ± 4.7	.688
Sex, M %	80.0	62.3	.025
Systolic blood pressure, mmHg	135.5 ± 18.3	133.9 ± 18.1	.730
Diastolic blood pressure, mmHg	82.3 ± 7.0	79.4 ± 11.1	.268
Glycated hemoglobin, mmol/mol Hb	51.3 ± 13.2	53.2 ± 10.7	.329
Total cholesterol, mg/dl	175.3 ± 27.7	136.1 ± 29.4	<.001
HDL-cholesterol, mg/dl	49.3 ± 11.6	49.6 ± 16.7	.916
LDL-cholestrol, mg/dl	102.7 ± 28.3	64.0 ± 24.2	<.001
Triglycerides, mg/dl	117.0 ± 50.7	113.5 ± 45.5	.679°
AST, U/L	26.6 ± 21.6	24.2 ± 9.3	.505
ACR, mg/gr	59.9 ± 218.4	184.6 ± 468.9	.060°
eGFR, ml/min 1.73 m^2	83.9 ± 17.8	78.9 ± 20.1	.172
Clinical cardiovascular disease, %	2.5	18.2	.007
Nephropathy, %	25.0	28.6	.407
Retinopathy, %	10.5	15.8	.299

°Test Mann-Whitney, otherwise *t*-test or χ^2 test. Data are presented as mean±standard deviation. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate transaminase; ACR, albumin-creatinine ratio; eGFR, estimated glomerular filtration rate.

cell membranes and plasma high-density (HDL) and low-density (LDL) lipoprotein[16]. Moreover, α-tocopherol decreases the cytotoxic effect of oxidized lipoproteins, smooth muscle cell proliferation, platelet aggregation, and systemic inflammation[17].

To the best of our knowledge, this is the first report of lower vitamin E levels in subjects treated with ezetimibe.

It has been claimed that vitamin E levels, as a lipid-soluble antioxidant, play a crucial role in mitigating lipid peroxidation reactions[18], and low

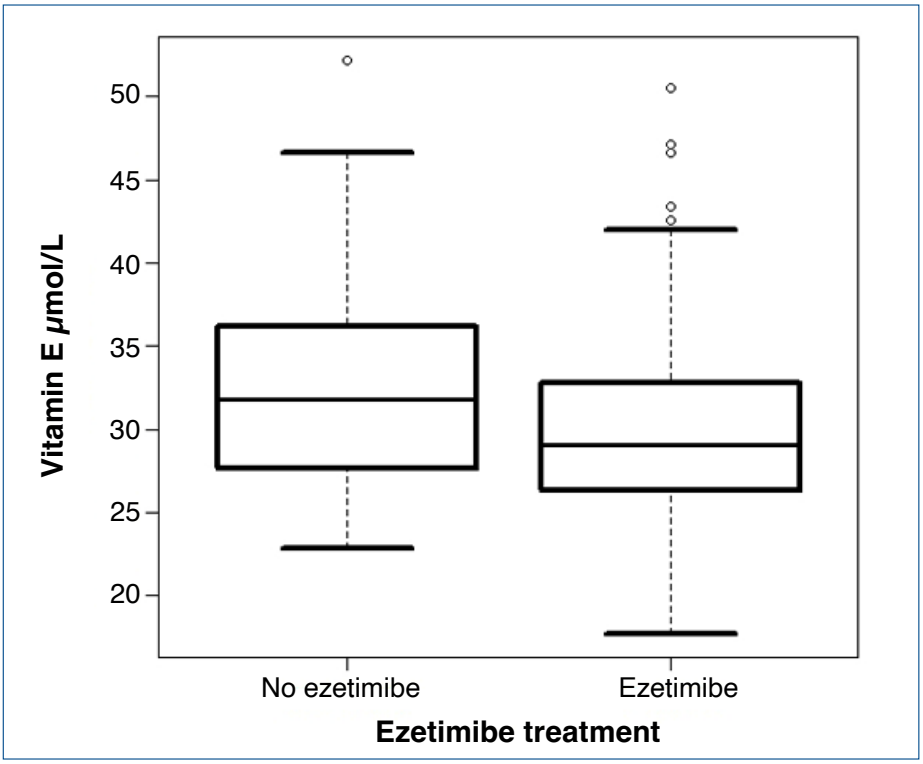


Figure 1. The figure shows the box plot of α-tocopherol concentrations according to the treatment with or without ezetimibe.

Table II. Multivariate linear regression analysis with vitamin E as dependent variable.

Variables	β standardized coefficient	*p*
Age, yrs	.012	.894
BMI, Kg/m^2	-.169	.018
eGFR, ml/min 1.73 m^2	.123	.155
Ezetimibe, yes	-.217	.002
Sex	-.283	<.001

BMI: body mass index; eGFR: estimated glomerular filtration rate.

levels of vitamin E lead to an increased oxidative burden in the body[19,20].

Vitamin E, as part of micronutrients with antioxidant and anti-inflammatory properties, is considered an ingredient of a heart-healthy diet[21,22]. The absorption of dietary α-tocopherol is primarily mediated by the NPC1L1 protein, and other proteins may also be involved in the transport of α-tocopherol across intestinal cells. NPC1L1 is also a therapeutic target of ezetimibe[11,23,24]. The aim of inhibiting NPC1L1 activity is primarily to reduce the absorption of dietary cholesterol[25].

Even though ezetimibe reduced the VE concentrations in our patients, none of them showed vitamin E levels compatible with deficiency or a very low level. Two possible explanations may put forward to explain why α-tocopherol was not completely inhibited by ezetimibe. The first is that NPC1L1 is not the only protein that transports α-tocopherol in the intestine, other proteins, such as CD36[23], are implicated. The second explanation, as shown in an animal model[13], is that only the simultaneous administration of ezetimibe and vitamin E was able to reduce blood α-tocopherol to very low levels. The latter situations may occur rarely in humans. Thus, it is unclear whether the reduction of vitamin E induced by ezetimibe is clinically relevant. To address this point, a future study, investigating antioxidant activity, is needed. However, a recent review on micronutrients proposed an alternative cutoff for vitamin E based on levels associated with lower mortality, suggesting it is less than 30 µmol/L[26]. Thus, the latter is a blood level higher than that currently accepted for vitamin E deficiency.

Nevertheless, in light of the effect of ezetimibe on the vitamin E level, it should be clearly stated that the combination therapy "statins + ezetimibe" is the cornerstone of hypercholesterolemia treatment[27]. ESC/EAS guidelines[28] recommend adding ezetimibe to statin treatment when the LDL cholesterol target is not achieved, and increasing evidence supports combination therapy as the standard of care, as it reduces the likelihood of being off-target compared to statins alone[29,30].

Our study has limitations; it is cross-sectional, and thus it does not allow for conclusions about causality. Additionally, we did not have vitamin E concentrations before starting ezetimibe. Moreover, ezetimibe was prescribed along with statins in fixed combinations. We do not know the possible effects of statins on vitamin E; nevertheless, a previous study addressing this issue reported no negative impact of statins on plasma vitamin E level[31]. Moreover, the present findings are restricted to patients with type 2 diabetes and therefore they cannot be generalized to other populations. The strengths of our study include the number of patients, the care to exclude subjects who took vitamin supplements during the six months before the study, and the completeness of the database.

CONCLUSIONS

In conclusion, we found that therapy with ezetimibe can reduce plasma levels of α-tocopherol by inhibiting the NPC1L1 protein. Future studies are needed to address the clinical implications of this finding.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Approval and Informed Consent

The study was approved by the Local Ethics Committee of the Hospital Trust of Verona, with protocol number No. 3853CESC on July 6, 2022. Informed consent was obtained from each participant. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki and its latest amendments.

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Authors' Contributions

AT contributed to the preparation of data, NV contributed to the preparation of data, NR contributed to the preparation of data, EP laboratory analyses of vitamins, MG laboratory analyses of vitamins, ED laboratory analyses of vitamins and critically reviewed the manuscript, GZ analysis of data and wrote the manuscript.

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Data Availability
Data may be requested from the corresponding author upon reasonable motivation.

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