Diabetes & Metabolic Syndrome: Clinical Research & Reviews xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

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

Original Article

Effect of Eicosapentaenoic acid (EPA) supplementation on cardiovascular markers in patients with type 2 diabetes mellitus: A randomized, double-blind, placebo-controlled trial

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
Eicosapentaenoic acid
Diabetes mellitus
Randomized clinical trial
Cardiovascular markers

ABSTRACT

Aims: Cardiovascular complications are one of main cause of increased mortality and morbidity among Diabetes Mellitus (DM) patients. Altered metabolism of sulphur amino acids in diabetes reflected as increases in concentration of methionine and cysteine/cystine in the blood which known as a markers of Cardiovascular Diseases (CVD). The aim of present study was to determine the effect of Eicosapentaenoic acid (EPA) supplementation on sulfhydryl amino acids and Atherogenic Index of Plasma (AIP) in patients with type 2 DM (T2DM).
Method: A randomized, double-blind, placebo-controlled clinical trial was performed in 36 control and patients with DM. The subjects were randomly assigned to obtain 2 g/d EPA (n = 18) or placebo (n = 18) for 8 weeks. Fasting serum level of Cystein and Methionine were measured using HPLC method and atherogenic index of plasma (AIP) as a proxy measure of atherosclerosis was computed.
Results: Eight weeks supplementation with EPA led to significant reductions in Met ($p < 0.002$) and Cys ($p < 0.001$) compared with the placebo ($p < 0.06$). In addition, compared to placebo a significant reduction in AIP were seen after taking EPA ($p < 0.04$).
Conclusion: EPA supplementation in patients with T2DM for eight weeks had beneficial effects on Met, Cys and AIP, which may attribute to the prevention of vascular complications in the T2DM patients.

1. Introduction

Diabetes mellitus (DM) is a chronic endocrine disorder with hyperglycemia in which insulin becomes disabled to carry out main role because of impaired insulin secretion, insulin resistance or both [1]. The prevalence of diabetes is rising all over the world and it is anticipated that the prevalence of type 2 DM) (T2DM) increase to more than 366 million people worldwide in 2030 [2]. T2DM is more prevalent than T1DM, and is responsible for 90% of diabetes cases [3]. Chronic hyperglycemia is contributed to many chronic microvascular and macrovascular complications such ascardiovascular diseases, neuropathy andnephropathy [4,5].

Recently, amino acids have been suggested as new biomarkers indicating metabolicmarks of insulin action [6]. Furthermore, a positive relationship between branched-chain and aromatic amino acids and risk for future insulin resistance and cardiometabolic disorders has been implicated [7–9].

Literature review revealed that inappropriate insulin action is associated with alteration in sulphur amino acid metabolism reflected as subtle increases in concentrationsof methionine and cysteine/cystine in the blood [10]. DM thorough coronary artery disease, dyslipidemia and hypertension may lead to cardiovascular complication [11]. Diabetes dyslipidemia is described as a mainroot for the development of atherosclerosis and cardiovascular complication [12]. Particularly, atherogenic dyslipidemia defines as high triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) levels directlyrelated with adverse clinical outcomes [13]. TheTG/HDL-C ratio consider as a marker of insulin resistance [14]. Furthermore, a greater TG/HDL-C ratio was implicatedwith cardiovascular disease [15] and type 2 diabetes patients [16]. The AtherogenicIndex of Plasma (AIP),calculated as log(TG/HDL-C), is useful formula in the estimation of atherosclerosis and coronary-heart disease than otherlipid profile measurements [17]. HDL are normally associated with reduced CVD risk because of their

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https://doi.org/10.1016/j.dsx.2018.03.003
1871-4021/

antioxidant and reverse cholesterol transport capabilities and their ability to stimulate glucose uptake and fatty acid oxidation contrasting insulin resistance [18]. However function of HDL may be flawed in T2DM due to increased lipid peroxidation [19].

We hereby present data of a double-blind, placebo-controlled, randomized; studyin T2DM patients receiving daily 2 g purified EPA or placebo for 8 weeks. Fasting levels of circulating Methionine and Cystien and AIPwere measured.

2. Subjects, materials and methods

2.1. Participants

All participants aged 30–65 years with type 2 diabetes diagnosis based on available guidelines (American Diabetes Association [20]) were recruited during February 2014 to June 2015 from Iran Diabetes Association, Tehran University of Medical Sciences (TUMS), Tehran, Iran.The cases were enrolled in the study if they met these inclusion criteria: [1] Affliction history of at least 1 year before the study [2] BMI <35 [3]; taking the antidiabetic's drug (s) dose at least for 3 months.All the subjects excluded if they [1] had history of Hypertension, Renal, Arrhythmia, Gastrointestinal, Hepatic, Endocrinological or hematological disease, [2] need to take insulin, do not use (noncompliance) supplements (<10%). The study was based on the Declaration of Helsinki. All the subjects filled up written informed consent after verification the study protocol by the TUMS ethical committee.The trial was registered in the Clinical Trials (www.clinicaltrials.gov) for registration of clinical trials (NCT03258840).

2.2. Study design

This was a randomized, placebo-controlled, double-blind parallel-group clinical trial. At study baseline and after stratificationfor gender and age, Subjects were randomly allocated to receive 2 g/day of the softgels of EPA (n = 18) ([softgels containing Eicosapentaenoic acid ethyl ester (75%), Mino Pharmaceutical Co. Iran]) (supplied as 1-g softgels) or placebo (Edible paraffin by Mino Pharmaceutical Co., Iran) (n = 18) for 8 weeks.EPA and its placebo were in the same appearance such as colour, shape, size and packaging, which coded by the manufacturer to ensure blinding. Randomized allocation sequence and assigned participants to the groups was done by study technician allocate patients to EPA group and placebo group. Randomized allocation was not disclosed to the researchers and patients until the main analysis.

The participants were strictly advised to not change their usual diets and nutritional habits, dosage and type of medication during study to avoid their possible effect on the study findings. Compliance to the EPA supplementation was controlled through phone interviews, weekly and asking participants to return the medication containers, monthly.

2.3. Sample size calculation

Sample size calculated according to type one (α) and type two errors (β) as 0.05 and 0.20 (power = 80%), respectively and according to the previous study. Standard deviation (SD) and difference in mean or effect size (d) of serum Paraoxonase considered as 11.61 U/ml and 11.4 U/ml respectively as the key variables [21]. Although we needed 16 subjects in each group but because of possible dropouts 36 participants were participated in the study.

2.4. Anthropometry and physical activity assessment

Body weight was measured without shoes and in a minimum clothes condition by the use of a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was calculated to the nearest 0.1 cm by using aninelastic tape measure (Seca, Hamburg, Germany). BMI was calculated as weight in kg divided by height in meters squared. Waist circumference was assessed by tape measure (Seca; Germany) at the midpoint between the costal margin and the iliac crest.

2.5. Nutritional and medical history assessment

Allpatents filled up a general questionnaire regarding demographic variables (age, sex) and lifestyle habits (including the history of smoking, alcohol consumption)medical and drug history (heart rate, and measurements of systolic, diastolic blood pressure (SBPandDBP)), family history of diseases (diabetes, hyperlipidemia and hypertension, cardiovascular, etc) at the beginning and end of study.

Dietary intake was measured using a 24-h recall method for 3 days (including 2 working days and 1 weekend day) a week before and at the end of supplementation. The dietary recalls were analyzed using Nutritionist IV software (First Databank, San Bruno, CA, USA) adjusted for Iranian foods.

2.6. Biochemical assessment

Fasting blood samples (10 ml) (overnight fast for 10–12 h) were obtained from the antecubital vein at the beginning and the end of study (8th week) then centrifuged (3000 rpm for 10 min at 4 °C for serum acquisition) and stored at −80 °C until analysis.

2.7. Chemicals

Amino acid standard (Cys and Met), tri-*n*-buthylphosphine, dimethylformamide, sodium acetate, 7-Fluoro-2,1,3-Benzoxadiazole-4-Sulfonamide (ABD-F), were from Sigma (St. Louis, MO, USA). Acetonitrile, methanol and tetrahydrofuran was from Merck (Merck, Germany).

2.8. Sample preparation and HPLC analysis

The HPLC method includes reduction of the plasma samples with tri-*n*-butylphosphine, in dimethylformamide in order to reduce thiols and to decouple them from proteins. Then sample were mixed with trichloroacetic acid solution containing Na2EDTA under vigorous vortexing, followed by centrifugation. At last borate buffer, NaOH and ABD-F were added to the cleared supernatant.

The sample injected in reverse phase (RP) high-performance liquid chromatographer (Agilent, 1260). The the chromatographic column (Intersil ODS–3 V, 5 μm-4.6*250 mm, C/N 5020-01802) was equilibrated with acetonitrile in acetate and methanol buffer then amino acids concentration fluorimetrically measured (Excitation: 340, Emission: 450, 37C, pH = 7). Quantization was performed by a standard curve equation in a range of 0.05–10 μM. Tests with standard fresh solutions were frequently injected during analysis.

2.9. Atherogenic index of plasma equation

Based on previous studies atherogenic index of plasma were calculated according to following formula:

Atherogenic Index of Plasma: [Log(Triglycerides/HDL-Cholesterol)]

2.10. Statistical analysis

Normal distribution of all variables was tested by the Kolmogorov-Smirnov test. All variables were reported as mean ±standard deviation (SD).Within group comparisons were

Please cite this article in press as: M.H. Golzari, et al., Effect of Eicosapentaenoic acid (EPA) supplementation on cardiovascular markers in patients with type 2 diabetes mellitus: A randomized, double-blind, placebo-controlled trial, Diab Met Syndr: Clin Res Rev (2018), https://doi.org/10.1016/j.dsx.2018.03.003