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Original Research Article

TCF7L2-rs7903146 polymorphism modulates the effect of artichoke leaf extract supplementation on insulin resistance in metabolic syndrome: a randomized, double-blind, placebo-controlled trial

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ABSTRACT

Background: Transcription factor 7-like 2 (TCF7L2)-rs7903146 polymorphism is associated with increased risk of type 2 diabetes. The response of insulin and insulin resistance to artichoke leaf extract (ALE) may be affected by TCF7L2-rs7903146 polymorphism.
Objective: This study examined the effects of ALE supplementation on metabolic parameters of the TCF7L2-rs7903146 polymorphism in patients with metabolic syndrome (MetS).
Design, setting, participants and interventions: This double-blind clinical trial was conducted on 80 patients with MetS in Sina Clinic, Khoy, Iran. The patients were randomized into ALE or placebo groups to receive either ALE (1800 mg/d as four tablets) or matching placebo for 12 weeks.
Main outcome measures: Anthropometric indices, blood pressure, glucose and lipid profile levels were measured before and after the study. Moreover, patients were genotyped for TCF7L2 polymorphism.
Results: ALE supplementation decreased insulin level and the homeostasis model assessment of insulin resistance (HOMA-IR) in patients with the TT genotype of TCF7L2-rs7903146 polymorphism ($P < 0.05$). There was no significant interaction between blood pressure, glucose and lipid profile response to ALE supplementation.
Conclusion: The responses of insulin and HOMA-IR to ALE supplementation have shown an interaction with single-nucleotide polymorphism rs7903146 in TCF7L2.
Trial registration: Iranian Registry of Clinical Trial IRCT201409033320N9.

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1. Introduction

Metabolic syndrome (MetS), the constellation of abdominal fat, dyslipidemia, hypertension and hyperglycemia, is a major public health problem throughout the world [1]. MetS increases the risk of several chronic diseases including cardiovascular diseases and type 2 diabetes [2].

Recent evidence has indicated that a polymorphism in transcription factor 7-like 2 (TCF7L2) could predispose individuals to MetS [3–5]. The variant rs7903146 of TCF7L2, which is located between exons 4 and 5 on chromosome 10, can influence pancreatic β and α cell metabolism [6,7], and proglucagon gene expression in intestinal endocrine cells [8]. TCF7L2-rs7903146 may mediate the response of an individual to treatment [9,10].

Artichoke (*Cynara cardunculus* var. *scolymus* L.) is a member of the Asteraceae family and is used as a medicinal herb [11]. Hydroalcoholic and aqueous extracts of artichoke leaf have long been used in the treatment of some diseases such as dyspepsia and hepatobiliary diseases. The main bioactive constituents of artichoke leaf extract (ALE) are phenolic acids (e.g., caffeic acid, mono- and dicaffeoylquinic acids), bitter sesquiterpene lactones

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(cynaropicrin) and flavonoids (luteolin) [12]. There is some evidence of antioxidant [13,14], antidyspeptic [15], hepatoprotective [13], antimicrobial [16], hypolipidemic [17–22] and hypoglycemic [21] properties of ALE. In human studies, ALE was well tolerated, without any serious adverse effects [20–22]. Moreover, ALE has not been shown to have any genotoxic or mutagenic effects [23].

Limited human studies have evaluated the effects of ALE on metabolic parameters and insulin resistance with conflicting results [20–22,24]. Therefore, this study examined the effects of ALE on metabolic parameters in patients with MetS, considering the modulation of the response by TCF7L2-rs7903146 polymorphism.

2. Subjects and methods

2.1. Study design and participants

This randomized, double-blind, placebo-controlled clinical trial was conducted on patients with MetS. The participants were recruited from outpatients referred to Sina Clinic in Khoy, Iran from November 2014 through May 2015. Eighty patients with MetS from 20 to 50 years of age; with no history of self-reported or clinically diagnosed biliary tract obstruction or gallstones, diabetes mellitus, cardiovascular diseases, hypo- or hyperthyroidism, cancer or liver dysfunction; not taking fat-lowering, antihypertensive or corticosteroids medications, or antioxidant supplements; not following weight loss program; not being hypersensitive to artichoke family plants and their products; not being pregnant, breast-feeding, or menopausal; and not smoking were recruited to our study. MetS was defined according to the Joint Interim Statement of 2009 Criteria as having three out of 5 of the following criteria: high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL for men and < 50 mg/dL for women, and fasting blood sugar (FBS) $\geq$ 100 mg/dL, triglyceride (TG) $\geq$ 150 mg/dL, systolic blood pressure (SBP) $\geq$ 130 mmHg or diastolic blood pressure (DBP) $\geq$ 85 mmHg and waist circumference (WC) $\geq$ 95 cm in both sexes, based on a report of the Iranian National Committee of Obesity [25,26].

The study was approved by the Ethics Committee of Tabriz University of Medical Sciences (reference number: 93120). All patients signed the informed consent. The trial was registered at the Iranian Registry of Clinical Trials (http://www.irct.ir/, IRCT201409033320N9).

Sample size was calculated based on information obtained from the study of Rondanelli et al. [21] on the change of low-density lipoprotein cholesterol (LDL-C) level in response to ALE. Using the Pocock Formula, with a confidence level of 95% and a power of 90%, the sample size was determined to be at least 33 patients in each group. Anticipating a dropout rate of 30%, 40 patients were assigned for each intervention group.

2.2. Randomization and intervention

The permuted block randomization procedure by Random Allocation Software, version 1.0 was used for randomization, with a block size of four and with stratification for age and sex. Patients were allocated into two groups: (1) ALE group, receiving 1800 mg/d of ALE as four tablets; (2) placebo group, receiving 1800 mg/d of placebo as four tablets. Participants were directed to take supplements as one tablet before breakfast, one before dinner and two before lunch for 12 weeks.

2.3. Supplements preparation

The leaves of fresh globe artichoke were obtained from Iran's Medicinal Plants Cultivation Company in Qazvin, Iran. After drying the leaves, 600 mL of an ethanol–water solution (7:3) was added to 100 g of leaf powder and incubated at 40 °C for 12 h. The hydroalcoholic extract was concentrated under vacuum and tablets were made. ALE and placebo tablets were prepared by Dineh Pharm. Co (Qazvin, Iran) with matching color and size in similar opaque plastic bottles. Each tablet of ALE contained 450 mg of the hydroalcoholic extract of artichoke leaf, which was characterized by at least 4%–5% chlorogenic acid. The spectrophotometer was used for standardization, based on the protocol of *Iranian Herbal Pharmacopoeia* [27]. Briefly, 20 random samples of ALE tablets were chopped and equal amounts of one tablet were dissolved in 250 mL of ethanol–water (50:50). The solution was heated over a boiling water bath for 30 min, centrifuged at 4000 r/min for 10 min and then filtered with a 0.2 μm syringe filter. The filtered solution (1 mL) was mixed with 2 mL of 0.5 mol/L hydrogen chloride, 2 mL of Arnow's nitrite-molybdate reagent, 2 mL of 1 mol/L sodium hydroxide and 3 mL of water. The absorbance of samples and standards was determined at (505 ± 2) nm.

2.4. Anthropometry, blood pressure, dietary intake and physical activity assessments

Height, weight and WC were measured at baseline and the end of the study. Blood pressure was measured in a seated position using an automated digital sphygmomanometer (Microlife A100-30, Berneck, Switzerland). Measurements were taken twice, with a 5-minute rest in between. The mean of the two measurements was used to represent the value.

Dietary intakes were assessed using a 3-day 24-hour food recall (one weekend and two working days) and were analyzed with the *Nutritionist IV* software modified for Iranian foods (First Databank Inc., Hearst Corp., San Bruno, CA, USA). The short form of the International Physical Activity Questionnaire (IPAQ-SF) was used to detect physical activity levels [28]. According to the scoring method of IPAQ-SF, physical activity level was defined as "high," "moderate" and "low" levels [29].

2.5. Laboratory analysis

Fasting blood samples were obtained after 12-hour fast before and after the intervention. Serum total cholesterol (TC), HDL-C, TG and FBS were measured enzymatically by an automatic biochemical Hitachi 717 analyzer (Hitachi, Boehringer Mannheim, Japan) with Pars-Azemoon kits (Tehran, Iran) on the same day of blood sampling. Friedewald's formula was used for LDL-C level determination as follows: LDL-C = TC – (HDL-C + TG/5) [30]. Insulin level was assayed using a human enzyme-linked immunosorbent assay kit (Monobind Inc., Lake Forest, CA, USA). Insulin resistance index was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) [31].

2.6. Genotyping

Blood samples were stored at −80 °C in tubes containing anticoagulant ethylenediaminetetraacetic acid. Genomic deoxyribonucleic acid was extracted using the conventional phenol chloroform extraction method. The polymerase chain reaction-based method of screening with mismatch primer modification for the TCF7L2-rs7903146 was carried out as previously described in detail [32]. The final product was digested with RsaI enzyme (Thermo Fisher Scientific, Vilnius, Lithuania), for 16 h at 36 °C. The digested product was separated by electrophoresis on 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and stained with ethidium bromide. The resulting fragments 79 and 26 bp were referred to as TT homozygotes, 105, 79 and 26 bp as TC heterozygotes and 105 bp as CC homozygotes.

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