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SHORT COMMUNICATION

Therapeutic lifestyle change diet enriched in legumes reduces oxidative stress in overweight type 2 diabetic patients: a crossover randomised clinical trial

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The objective of this study was to investigate whether a diet rich in legumes are associated with oxidative stress among type 2 diabetic patients. In a randomized, controlled, crossover clinical trial, 31 type 2 diabetic patients were randomly assigned to receive 2 diets, each for a period of 8 weeks: (1) The legume-free therapeutic lifestyle change (TLC) diet and (2) the legume-based TLC diet. Both diets were similar except that 2 servings of red meat were replaced by different types of legumes 3 days per week in the legume-based TLC diet. Oxidative stress biomarkers were measured at baseline and after 8 weeks. Compared to the legume-free TLC diet, the legume-based TLC diet significantly decreased malondialdehyde (−0.22 versus − 0.68 μmol/l; $P = 0.002$), oxidized-LDL (−0.9 versus − 2.3 mU/l; $P = 0.05$) and increased nitric oxide (0.40 versus 0.96 mM/l; $P = 0.03$) and catalase activity (1.2 versus 2.1 Iu/ml; $P = 0.05$).

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INTRODUCTION

Ameliorating oxidative stress through diets rich in antioxidants has been suggested as a new therapy for diabetes patients for reducing diabetic complications.[1] Efforts are being made to identify food products containing natural antioxidants, among which legumes are considered good sources of antioxidants for health promotion. The high content of total phenolic components, especially procyanidin of legumes, in particular, has a high antioxidant activity and scavenges free radicals;[2] moreover, other components of legumes, such as low glycemic index, protein, fiber, and vitamins, and minerals, such as magnesium, have also been reported to reduce oxidative stress.[3,4] Previous clinical trials investigating the effect of consumption of non-soy legumes on biomarkers of oxidative stress, have shown protective effects against lipid peroxidation biomarkers, and enhanced antioxidant defense in the liver and erythrocytes by increasing both enzymatic and non-enzymatic antioxidant systems.[5–7] Hence considering the limited data available on the effects of non-soy legumes on biomarkers of oxidative stress and the antioxidant defense system in diabetic patients, this study was conducted to investigate the effects of non-soy legume inclusion in the therapeutic lifestyle change (TLC) diet on biomarkers of oxidative stress among overweight type 2 diabetic patients.

MATERIALS AND METHODS

Participants

Details of this study have been reported previously.[8] Briefly, 40 diabetic patients, aged 50–75 years, with BMI ranging between 25 and 30 kg/m^2 were recruited from the clinic of Taleghani hospital, Tehran, Iran, during 2012. Using a crossover design, participants were randomly assigned to receive the 2 following diets (each for a period of 8 weeks): (1) Legume-free TLC diet; and (2) non-soy legume-based TLC diet. The legume-free TLC diet included a macronutrient composition of 50–60% carbohydrates, 15% protein, and 25–35% of energy from fat.[9] The non-soy legume-based TLC diet was the same as the legume-free TLC diet, except that 2 servings of red meat were replaced by different types of cooked legumes. Following the interventional period, there was a washout period for 4 weeks, after which the groups followed the alternate treatment also for 8 weeks. Random allocation to intervention groups was done by an assistant, using random sequencing generated in SPSS and all group allocation was blinded for the investigators. Of 40 participants, two dropped out due to changes in medications (one patient while receiving the first intervention diet and the other while receiving the second intervention diet). At the end of the trial, seven patients had poor compliance with the treatment protocol.[8] All participants provided written informed consent. This study was approved by the research council and the ethics committee of the Research Institute for Endocrine Sciences (RIES), Shahid Beheshti University of Medical Sciences (registered in http://www.irct.ir; ID number IRCT201202251640N7).

Oxidative stress biomarkers

Fasting venous blood samples were taken after 12–14 h overnight fast, at baseline and again after 8 weeks. Serum malondialdehyde (MDA) was measured spectrophotometrically by its reactivity with thiobarbituric acid using the TBARS Assay kit (Cayman Chemical Inc., Ann Arbor, MI, USA, CAT No. 10009055). The monoclonal

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antibody was used to quantify the concentration of serum oxidized-LDL (ox-LDL) using the ELISA kit (Mercodia Company, Uppsala, Sweden, CAT No. 10-1143-01). Serum total antioxidant capacity (TAC) was measured spectrophotometrically using the antioxidant assay kit (Cayman Chemical Inc. Ann Arbor, MI, USA, CAT No. 709001). Serum nitric oxide (NO) concentration was determined by first reducing the nitrate to nitrite using nitrate reductase (Sigma). Catalase activity was measured using commercial kits (Cayman Chemical Inc., Ann Arbor, MI, USA, CAT No. 707002).

Table 1. Geometric means of biomarkers of oxidative stress at baseline and after 8 weeks of intervention in type 2 diabetic patients

	Legume-free TLC diet	*Non-soy legume-based TLC diet*	P[a]
n	40	40	
MDA (μmol/l)			
Baseline	2.7 ± 0.04	2.5 ± 0.1	0.35
End of trial	2.5 ± 0.1[a]	1.8 ± 0.1[b]	3.2×10^{-5}
Change	− 0.22 ± 0.1	− 0.68 ± 0.1	0.002
Ox-LDL (mU/l)			
Baseline	12.6 ± 0.4	12.8 ± 0.5	0.67
End of trial	11.6 ± 0.3[a]	10.5 ± 0.2[b]	0.008
Change	− 0.9 ± 0.4	− 2.3 ± 0.5	0.05
TAC (μmol Trolox Equiv/l)			
Baseline	0.29 ± 0.01	0.28 ± 0.01	0.39
End of trial	0.28 ± 0.01	0.25 ± 0.01	0.07
Change	− 0.02 ± 0.01	− 0.04 ± 0.01	0.28
NO (mM/l)			
Baseline	2.8 ± 0.1	2.9 ± 0.10	0.74
End of trial	3.2 ± 0.2[a]	3.8 ± 0.2[b]	0.02
Change	0.40 ± 0.2	0.96 ± 0.2	0.03
Catalase activity (Iu/ml)			
Baseline	5.6 ± 0.6	5.6 ± 0.4	0.84
End of trial	6.8 ± 0.5[b]	7.8 ± 0.5[b]	0.04
Change	1.2 ± 0.5	2.1 ± 0.6	0.05

Abbreviations: MDA, malondialdehyde; NO, nitric oxide; Ox-LDL, oxidized-LDL; TAC, Total antioxidant capacity. Data are geometric means ± SE. Non-soy legume-based TLC diet, same as the legume-free TLC diet, except that 2 servings of red meat are replaced by different cooked legumes, such as lentils, chickpeas, peas and beans, 3 days per week. [a]*P*-value was obtained from paired *t*-test, using logarithmically transformed values of variables. [b]Significantly different from baseline values (obtain from paired *t*-test using logarithmically transformed values of variables) $P < 0.001$.

Statistical analysis

Normal distribution of all oxidative stress variables was checked with the Kolmogorov–Smirnov test, and because distributions of biomarkers were not normal, the logarithmically transformed values of these variables were used for all analysis. Paired *t*-test was used to compare baseline and end-of-intervention values in each group. In addition, Paired *t*-test was used to compare means of oxidative stress at the end of the intervention diets between groups. Geometric means for oxidative stress variables were calculated and reported. According to the intention-to-treat approach, first, imputation of missing data ($n = 2$) was done and after that, analyses were done using data for all participants ($n = 40$), whose results were similar to those of participants who had good compliance ($n = 31$).

Carry-over effect was assessed, using independent sample *t*-test; we compared mean values at end-of two intervention diets between patients, assigned to receive the legume-free TLC diet or the non-soy legume-based TLC diet at initiation of study.

The sample size was determined to allow for 20% attrition and noncompliance and to provide 85% power and 95% confidence interval to detect a minimal difference of 0.5 μmol/l in MDA between two diet interventions.

RESULTS

After consumption of both the legume-free TLC- and the non-soy legume-based TLC diets, MDA and ox-LDL decreased significantly and NO and catalase activity increased significantly from baseline values. Compared to the legume-free TLC diet, the non-soy legume-based TLC diet significantly decreased MDA, ox-LDL and increased NO and catalase activity. Serum TAC decreased significantly after consumption of non-soy legume-based TLC diet, although no difference was observed between the two diets (Table 1). The carry-over effect has been shown in Table 2. There was no significant difference in mean values of oxidative stress in the two intervention diets between diabetic patients, assigned to receive either the legume-free TLC diet or the non-soy legume-based TLC diet at initiation of the study.

DISCUSSION

Results of the present study indicate that replacement of 2 servings of red meat by non-soy legumes in an isocaloric TLC diet, 3 days per week, improves biomarkers of oxidative stress among overweight diabetic patients, independent of weight change. Consistent with our findings, a previous study found that substitution of a serving of red meat with an alternative protein food source was associated with lower inflammatory markers, insulin concentrations, and endothelial dysfunction.[10]

Table 2. Baseline concentrations of stress oxidative biomarkers and carry-over effect between two intervention diets according to diet intervention at initiation of the study

	Values for diabetic patients that assigned to received legume-free TLC diet at initiation of the study		*Values for diabetic patients that assigned to received non-soy legume-based TLC diet at initiation of the study*		P-value[a]	
	Mean values for baseline	*Mean values for two interventions diet*	*Mean values for baseline*	*Mean values for two interventions diet*	*Baseline value between two groups*	*Carry-over effect*
MDA (μmol/l)	2.6 ± 0.1	2.1 ± 0.1	2.6 ± 0.1	2.2 ± 0.04	0.78	0.11
Ox-LDL (mU/l)	12.6 ± 0.6	11.3 ± 0.4	13.5 ± 0.4	10.8 ± 0.4	0.26	0.29
TAC (μmol Trolox Equiv/l)	0.29 ± 0.01	0.26 ± 0.01	0.28 ± 0.02	0.26 ± 0.01	0.24	0.92
NO (mM/l)	3.0 ± 0.2	3.8 ± 0.1	2.7 ± 0.1	3.4 ± 0.2	0.11	0.14
Catalase activity (Iu/ml)	6.3 ± 0.6	7.3 ± 0.4	5.5 ± 0.5	8.5 ± 0.6	0.51	0.15

Abbreviations: MDA, malondialdehyde; NO, nitric oxide; Ox-LDL, oxidized-LDL; TAC, Total antioxidant capacity. [a]Independent sample *t*-test.

Decrease in plasma MDA and ox-LDL concentrations in the legume-based TLC diet could be due to the antioxidative effects of total phenolic compounds, viz. procyanidin of legumes, that has high antioxidant activity and scavenges free radicals.[2] In addition, legumes are an excellent source of dietary fiber, each serving providing 2–4 g fiber, which enhances the capacity to detoxify free radicals and reduces lipid peroxidation.[3] High contents of magnesium and low glycemic index in legumes may also reduce oxidative stress.[3,4] Furthermore, substitution of animal protein with plant protein may be another component of the non-soy legume-based TLC diet that could reduce oxidative stress.[2] Another protective mechanism by which legumes improve oxidative stress may be mediated by reduction in dyslipidemia and hyperglycemia.[8] In conclusion, consumption of legumes improves biomarkers of oxidative stress among overweight diabetic patients.

Not blinding of patients and investigators because the study was a dietary intervention is another limitation of the present study, however blinding was maintained for investigators involved in the outcome assessment and data analysis until study completion.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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