

The Effects of Consumption of Bread Fortified With Soy Bean Flour on Metabolic Profile in Type 2 Diabetic Women: A Cross-over Randomized Controlled Clinical Trial

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ABSTRACT

Background: Diabetes mellitus is one of the most common chronic diseases in the world and has become a major threat for global health. Recent studies reported that the soy has beneficial effects in diabetic mellitus patients. The aim of this study was to assess the effects of soybean flour fortified bread consumption on metabolic profile in type 2 diabetic women.

Methods: This randomized, cross-over, controlled clinical trial was carried out in 30 type 2 diabetic women. At first, a 2-week run-in period was applied. Then, participants were randomly assigned to either intervention or control groups. Participants in the intervention group were asked to replace 120 g of soybean flour fortified bread with the same amount of their usual bread intake or other cereal products for 6 weeks. After a 4 weeks washout period, participants were crossed over for another 6 weeks.

Results: Mean (\pm standard deviation) age and body mass index of subjects was 45.7 ± 3.8 years and 29.5 ± 3.9 kg/m², respectively. The results of our study showed no significant effects of soybean flour fortified bread on metabolic profile. We found a reduction in serum triglycerides (change difference: -3.7 , $P = 0.82$), serum low-density lipoprotein-cholesterol (change difference: -11.2 , $P = 0.50$), insulin (change difference: -3.6 , $P = 0.7$), and homeostatic model assessment of insulin resistance (change differences: -0.57 , $P = 0.45$) after 6 weeks but these changes were not statistically significant. No significant effects of soybean flour fortified bread on serum concentrations of fasting blood sugar, glycated hemoglobin, high-density lipoproteins and total cholesterol levels were found.

Conclusions: Six weeks consumption of soybean flour fortified bread among diabetic patients had no significant effects on metabolic profile.

Keywords: Metabolic parameters, type 2 diabetes, soy bean

INTRODUCTION

Diabetes mellitus is one of the most common chronic diseases in the world^[1] and has become a major threat for global health.^[2] According to World Health Organization (WHO) report, the number of type 2 diabetic patients in the world will reach 300 million by 2025.^[1,3] WHO estimations show that more than 2 million diabetic patients were living in Iran in 2000 and have been estimated to be increased to more than 6.4 million in 2030.^[4] It has been shown that the prevalence of diabetes in Iran is 5–16.3%.^[4]

Diabetes complications include microvascular complications (e.g., nephropathy, retinopathy and neuropathy) and macrovascular complications (e.g., cardiovascular disease and stroke).^[4,5]

Several dietary factors have been reported to affect serum levels of glycemic indices and lipid profile. Currently, the effects of soybean and its components have been studied on many chronic diseases. Soybean is a member of legume family, which is a rich source of protein, fiber, vitamins, minerals, polyunsaturated fatty acids (PUFAs), isoflavones and phytoestrogens that might affect glycemic control and lipid profile.^[6]

Many animal and human studies reported that the soy has beneficial effects in diabetic mellitus patients. Several studies reported that soy consumption may be linked to a lower incidence of certain chronic diseases.^[7] Soy contains high amounts of isoflavones that improve vascular function.^[8] High consumption of legumes was shown to have a protective effect on the development of glucose intolerance and diabetes.^[6]

It seems that fortification of bread with soy flour can increase the quality of protein and improve its effects on human health. In addition, bread is a staple food in Iranian diet. About beneficial effects of soy on chronic disease, including type 2 diabetes, we aimed to fortify bread with soybean flour and evaluate its effects on metabolic profile in type 2 diabetic patients.

METHODS

Participants

This randomized, cross-over, controlled clinical trial was conducted in 30 premenopausal type 2

diabetic women aged 30–50 years. Exclusion criteria were use of oral hypoglycemic agents, insulin injection, lipid-lowering medications, use of hormone replacement therapy, hypo- and hyper-thyroidism and allergy to soybean. Participants who were pregnant or breastfeeding were also excluded. All participants were recruited from Endocrine and Metabolism Research Center of the Isfahan University of Medical Sciences. Participants were not undergoing dietary changes in the last 3 months. Sample size for this study was calculated based on suggested equation for cross-over trials:^[9] $N = ([z_{1-\alpha/2} + z_{1-\beta}]^2 \cdot s^2) / 2D^2$, with considering type 1 error of 5% and type 2 error of 20% (power = 80%). Low-density lipoprotein (LDL)-C was considered as key variable.^[10] According to this equation, we reached the sample size of 19 participants for the whole intervention. Since there are high dropouts in cross-over trials, we recruited 30 women in this study. All participants completed the study. Figure 1 outlines participant's flow throughout the study.

Ethical considerations

The study was approved by Ethical Committee of Isfahan University of Medical Sciences, Isfahan, Iran. Written informed consent was obtained from all patients before entering the study. The clinical trial registration code (IRCT2013061613684N1) was obtained from Iran center for registration of clinical trials (www.irct.ir).

Study procedures

To obtain detailed information about the lifestyle characteristics and the compliance of participants to soybean flour fortified bread, at first, a 2-week run-in period was applied. Participants were asked to continue their habitual diet and physical activity and wanted them to consume 1 serving/day of soybean flour fortified bread during this period to be prepared for the intervention and have a better compliance during the study. All participants completed 2 days dietary records (nonconsecutive days) and a 2 days physical activity record in the run-in period. After a 2 week run-in period, all measurements were done. Then, participants were random, using randomized block design method, assigned to either intervention (soybean flour fortified bread) or control groups (habitual diet) for 6 weeks. After the first phase of intervention, a 4-week wash-out period was applied. Participants

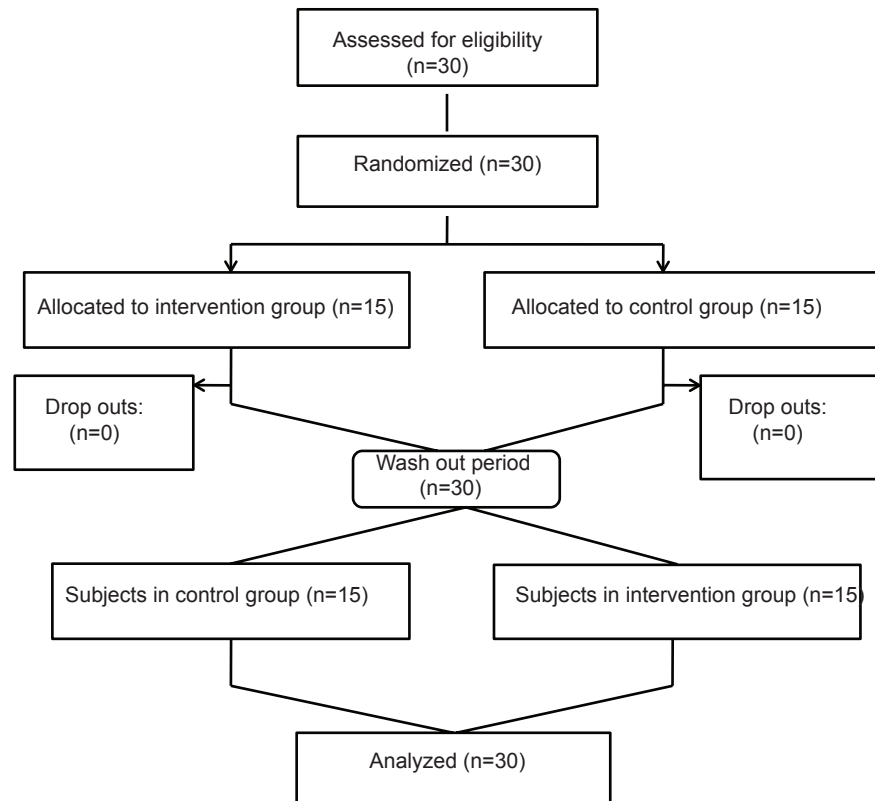


Figure 1: Participant flow chart

were crossed over for another 6 weeks. During wash-out period, subjects were asked to avoid any soy based foods and soybean flour fortified bread intake that they were received during the intervention. All measurements were taken at baseline and 6, 10 and 16 weeks. All participants completed a 3 days (2 week days and one weekend day) dietary and physical activity records once every 2 weeks during the study. Study diagram is shown in Figure 2.

- Group 1: Intervention control; treatment from soy bean flour fortified bread diet to habitual diet
- Group 2: Control intervention; treatment from habitual diet to soybean flour fortified bread diet.

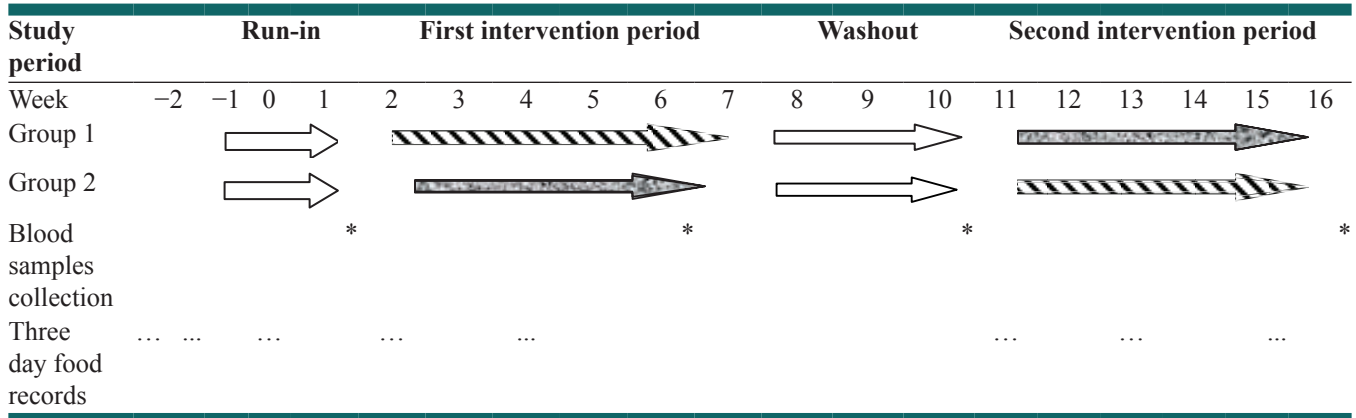
Interventions

Soybean flour fortified bread was prepared by replacing 30% of the wheat flour by soybean flour. Participants in the intervention group were asked to substitute 120 g of soybean flour fortified bread with the same amount of their habitual bread intake. Fresh bread was given to participants every week. Bread packages were frozen before use by participants. Individuals in the control group were asked not to consume soy-based foods during this

phase of the study. The dietitian monitored bread intake weekly, if bread intake was outside the recommended amount, Participants were trained on how to use bread properly. Nutrient composition of soybean flour fortified bread is shown in Table 1.

Assessment of biomarkers

After 12 h fasting, blood samples were collected between 7:30 and 9:00 a.m at Endocrine and Metabolism Research Center Laboratory. Blood samples were centrifuged after 30 min of blood collection. Fasting blood sugar (FBS) was measured by enzymatic colorimetric method using glucose oxidase. Total cholesterol and triglyceride kits (Pars Azmoon Inc., Iran) were used for measuring these items. Glycated hemoglobin (HbA1C) was measured in the whole blood by cation exchange chromatography method with Pars Azmoon kit. Serum insulin levels were measured by ELISA (Demeditec, Germany). Insulin resistance index homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin ($\mu\text{IU}/\text{Ml}$) \times fasting glucose (mmol/L)/22.5.^[11] Serum total cholesterol and triglyceride (TG) levels were assayed using

Figure 2: Study diagram**Table 1:** Nutrient composition of soy bean flour fortified bread

Nutrient	Amount/100 g
Fat	7.2
Carbohydrate	44.31
Protein	14.1
Moisture	28.24
Ash	2.5

enzymatic colorimetric tests with cholesterol esterase and cholesterol oxidase and glycerol phosphate oxidase, respectively. High-density lipoproteins (HDL)-C was assayed after precipitation of the apolipoprotein B containing lipoproteins with phosphotungstic acid. LDL-C was calculated based on Fried Ewald formula.^[12]

Anthropometric assessment

Measurement of height was done using a tape measure in standing the position without shoes while shoulders were relaxed. Weight was measured to the nearest 100 g using a digital scale in light clothing (Seca, Hamburg, Germany). Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters. Waist circumference was measured at the smallest circumference. To avoid subjective error, all measurements were done by the same person. Blood pressure was measured in a seated position by the use of a mercurial sphygmomanometer.

Statistical methods

Data analysis was performed using SPSS version 18 software (SPSS Inc., Chicago, IL, USA). All subjects were included in the final

analysis. Paired *t*-test was used to examine the main effects by comparing the mean differences of variables in two groups. Carry-over effect and period effect were checked using *t*-test. Results are described as mean \pm standard deviation ($\bar{x} \pm SD$). $P < 0.05$ were considered as statistically significant. Dietary records were assessed using nutritionist IV software.

RESULTS

Baseline characteristics of study participants are presented in Table 2. Mean ($\pm SD$) age of study participants was 45.7 ± 3.8 years, and mean BMI was 29.5 ± 3.9 kg/m². Dietary intakes of participants at study baseline are shown in Table 3. Mean energy intake of participants was 1449 kcal: 54% as carbohydrate, 17% as protein and 29% as fat. Participants received 7.2% of their energy from saturated fatty acids, 11.2% from PUFAs, and 7% from monounsaturated fatty acids. Mean daily intake of dietary fiber, calcium and folate was 9 g, 469 mg, and 308 μ g at study baseline, respectively. No significant difference was found in physical activity between two groups during the study (intervention: 36.7 ± 3.2 vs. 37.2 ± 3.4 MET-h/d for control, $P = 0.4$).

The effects of soybean flour fortified bread intake on metabolic profiles are indicated in Table 4. The results of our study showed no significant effects of soybean flour fortified bread on metabolic profile. We found a reduction in serum TGs (change difference: -3.7 , $P = 0.82$), serum LDL-C (change difference: -11.2 , $P = 0.50$), insulin (change difference: -3.6 , $P = 0.7$), and HOMA-IR (change differences: -0.57 , $P = 0.45$)

Table 2: Baseline characteristics of study participants (*n*=30)

Variable	Mean	SD
Age (year)	45.7	3.8
Weight (kg)	73.8	10.7
Height (cm)	158	5.3
BMI (kg/m ²)	29.5	3.9
Waist circumference (cm)	87.4	6.7
Hip circumference (cm)	99.04	4.6

SD=Standard deviation, BMI=Body mass index

Table 3: Dietary intakes of study participants during run-in period (*n*=30)

Variable	Mean	SD
Macronutrients		
Energy (kcal)	1449	766
Carbohydrate (g/day)	196	134
Protein (g/day)	63	49.3
Fat (g/day)	48	17.8
Micronutrients		
Saturated fats (g/day)	11.7	5.01
PUFA (g/day)	18.3	9.2
MUFA (g/day)	11.5	4.8
Calcium (mg/day)	469	287
Magnesium (mg/day)	195.8	271.7
Folate (µg/day)	308.3	686.6
Vitamin C (mg/day)	50.9	37.5
Dietary fiber (g/day)	9	3

SD=Standard deviation, PUFA=Polyunsaturated fatty acid, MUFA=Monounsaturated fatty acid

after 6 weeks, but these changes were not statistically significant. No significant effects of soybean flour fortified bread on serum concentrations of FBS, HbA1C, HDL and total cholesterol levels were found. No significant effect of soybean flour fortified bread intake on weight, waist circumference and BMI was seen compared with the control group.

DISCUSSION

The results of our cross-over randomized clinical trial study showed that daily consumption of 120 g soybean flour fortified bread for 6 weeks had no significant effect on lipid profile and glycemic indices.

Abnormal metabolic profile might lead to increased risk of chronic diseases. Several dietary factors can influence serum levels of lipids and glycemic parameters. Soy products have been

reported to positively affect glycemic and lipid profiles. In this study, we calculated HOMA-IR as a measure of insulin resistance. However, it was not significantly affected. Our findings are consistent with the earlier study. Where, Gobert *et al.*^[3] showed soy protein isolate consumption did not significantly affect HbA1C, HOMA-IR, fasting blood glucose and insulin levels in type 2 diabetes. In line with our study, Dent *et al.* showed that 40 g/d soy protein consumption in premenopausal women had no effect on lipid profile.^[13] In contrast to our findings, a meta-analysis among diabetic patients indicated that soy protein could decrease serum concentrations of total cholesterol, LDL-C and TGs.^[14] In one study that conducted in type 2 diabetes patients with obesity, Anderson *et al.* observed a reduction in serum cholesterol and triacylglycerol levels.^[15] In an earlier study among postmenopausal women, Chiechi *et al.* indicated that a soy-rich diet had beneficial effects on lipid profile.^[16] Mechanisms of lipid-lowering effect of soy are not clear. However, there are some mechanisms suggested for hypocholesterolemic and hypoglycemic effect of soy, such as: Soy fiber content,^[17,18] trace component such as plant setrols, phytates, folates, and oxalates. As well as amino acid profile of soy is one of the suggested mechanisms. The higher arginine to lysine and methionine ratio in soy might be responsible for its hypocholesterolemic effects. Animal studies have shown that the amino acids lysine and methionine were hypercholesterolemic, while arginine was hypocholesterolemic.^[19] Also soy bean β-conglycinin (7S globulin), a protein exists in soy, up-regulate LDL receptors and decreased serum levels of cholesterol.^[20] The beneficial effects of soy may be due to soy proteins. Soy protein contains a high amount of arginine and glycine. These amino acids are associated with decreased serum cholesterol levels arginine and glycine also involved in insulin and glucagon secretion from the pancreas. The insulin to glucagon ratio is a major determinant of the metabolic directions in lipid and glucose metabolism and thereby a major contributor to metabolic homeostasis.^[21] Decrease in plasma insulin that caused by soy protein may be due to decreased in insulin release from the pancreas or increased hepatic removal. Furthermore, cholesterol-lowering effect of soy protein may be due to the decreased insulin to glucagon ratio caused by arginine and glycine.^[21,22] Findings of Moriyama *et al.* study

Table 4: The effects of soy bean flour enriched bread intake on metabolic profiles in type 2 diabetic women^a using adjusted paired *t*-test (controlling for baseline)

Variables	First period		Wash-out	Second period		Main effect	
	Baseline	6 th week		10 th week	16 th week	Effect size ^d	<i>P</i>
FBS							
Group 1 ^b	140±42.6	142±61.4		142.2±34.1	156.1±58.8	4±3.3	0.24
Group 2 ^c	173.2±38.3	149.7±33		168.7±39.3	157.7±40.3		
TG							
Group 1	133.5±56	136±48.3		133.3±53.1	151.8±41.8	-3.7±16.3	0.82
Group 2	184.9±93.8	176.3±82.4		210±109.4	178.6±88.1		
Total cholesterol							
Group 1	174±36.4	161.4±34.1		170±39.7	179.6±53	7.4±11.8	0.53
Group 2	193.1±35.3	167.7±24.6		183.6±40.5	178.3±35.6		
HDL-C							
Group 1	43.3±6.2	43.5±8.2		42.5±6.8	37.4±6.4	1.9±1.8	0.32
Group 2	37.4±6.4	42.5±8.8		41±8.7	44.9±9.3		
LDL-C							
Group 1	104±29.8	89.9±30.1		101.2±30.3	101.9±43	-11.2±16.7	0.50
Group 2	118.8±36.3	90.1±24.7		100.8±31.8	98.2±33		
Insulin							
Group 1	11.8±6.9	12±7.2		12±6.6	12.9±6.8	-0.36±0.95	0.70
Group 2	7.7±3.8	8.7±3.4		11.2±5.2	10.8±3.5		
HbA1C							
Group 1	7.2±1.8	6.8±1.6		6.9±1.7	6.9±1.5	0.55±0.3	0.82
Group 2	7.8±1.6	7±1		7.6±1.42	7.6±1.5		
HOMA-IR							
Group 1	4.3±4.22	4.2±3.2		4.2±2.9	4.8±2.9	-0.05±0.54	0.9
Group 2	3.4±2.2	3.6±2.6		4.9±3.4	4.2±2		

^aData are means±SD, ^bGroup 1=Treatment from soy bean flour enriched bread diet to habitual diet, ^cGroup 2=Treatment from habitual diet to soy bean flour enriched bread diet, ^dMain effect=Mean difference between treatment and control groups (means±SE) and its *P* value. SD=Standard deviation, SE=Standard error, FBS=Fasting blood sugar, HOMA-IR=Homeostasis model assessment-insulin resistance, LDL-C=Low-density lipoprotein-cholesterol, HDL-C=High-density lipoprotein-cholesterol, TG=Triglyceride, HbA1C=Glycated hemoglobin

showed that the soy β-conglycinin diet reduced serum TG levels by acceleration of β-oxidation, inhibition of fatty acids synthase and increasing fecal excretion of TG and also decreased serum levels of insulin and glucose.^[23] A meta-analysis on 23 clinical trial indicated that improvements in HDL-C were only occurred in studies of more than 12 week duration,^[24] whereas our study duration was 6 week. In a randomized, double-blind, cross-over clinical trial,^[25] dietary supplementation with soy phytoestrogens for 12 weeks in 32 diabetic postmenopausal women, favorably affect serum insulin, HOMA-IR, HbA1C, LDL and total cholesterol, but no significant change were seen in HDL-C and TG levels. The controversial findings might be explained by different study designs,

characteristics of study participants or intervention duration.

According to our knowledge, the present study is the first study that examined the effects of soybean flour fortified bread intake on metabolic profiles among diabetic women. Strengths of our study are the cross-over design, a high percentage of participants who completed the study and enriching bread with soy to facilitate in soy consumption by participants. Despite the strength points, there are some limitations that deserve attention. It was not possible for us to use a double-blind design due to texture and taste of the bread. Also, daily distribution of bread package was impossible. The effects of soy on metabolic profile may be linked to the dosage of soy. We were not able to increase the soybean

flour dosage in bread more than 30% because of unfavorable effects on texture and taste of the bread.

CONCLUSIONS

Our findings suggest that daily consumption of 120 g soybean flour fortified bread for 6 weeks had no significant effects on serum levels of metabolic parameters in diabetic women. Further studies with longer duration might be needed.

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