Original Article

Effects of Soy Protein Isolate Supplementation on Biomarkers of Cardiovascular Disease in Type 2 Diabetic Patients

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ABSTRACT

Objective: To evaluate the effect of soy protein isolate (SPI) supplementation on some circulating biomarkers of cardiovascular disease (CVD): total homocysteine (tHcy), folate and vitamin B_{12} in type 2 diabetic patients. **Method:** Thirty-six type 2 diabetic patients were recruited. They received nutrition counseling and were randomized to SPI group (30 g/day of SPI containing 32 mg of isoflavones for 6 weeks) and control group (no SPI supplement). Anthropometry, blood pressure and biomarkers of CVD were examined at baseline and at the end of 6th week of the study. **Results:** There were no significant differences in anthropometric parameters, blood pressure and biomarkers of CVD between the 2 groups. At week 6, levels of either tHcy, folate, vitamin B12, or GFR between the two groups did not differ. However, in SPI group, tHcy levels were significantly decreased (P = 0.005) and folate levels were significantly increased from baseline (P = 0.002). **Conclusion:** This study indicated that supplementation of SPI with isoflavones potentially improves some biomarkers of CVD and may be beneficial on cardiovascular events in type 2 diabetic patients.

Keyword: soy protein isolate, biomarkers, cardiovascular disease, type 2 diabetic mellitus

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Introduction

Cardiovascular disease (CVD) is a major cause of morbidity and mortality among patients with diabetes, accounting for more than 80% of death in this population.[1,2] Numerous independent biological factors potentially predicting progression to CVD in this population may include glycemic control, lipid profile, total homocysteine (tHcy), folate and vitamin B_{12}. Circulating tHcy levels were significantly elevated in type 2 diabetic patients with atherosclerosis vascular disease when compared with those without vascular disease. In addition, low folate and vitamin B_{12} levels and reduced renal functions were responsible for the majority of elevation of tHcy levels.[3]

tHcy, an intermediate sulfur-containing amino acid, is formed during the conversion of methionine through re-methylation pathway which requires folic acid and vitamin B_{12} in this reaction.[4] Elevated circulating plasma tHcy levels in diabetic patients may contribute to the development of chronic complication and a higher rate CVD mortality.[5] It has been estimated that lowering plasma tHcy by 5 µmol/l may reduce the risk of cardiovascular death by approximately 10%.[6] In type 2 diabetic patients, plasma tHcy levels were significantly increased when serum folate and vitamin B_{12} levels decreased. Therefore, supplementation of folic acid and vitamin B_{12} in diabetic patients could potentially prevent future atherogenic processes and diabetes complications due to hyperhomocysteinemia.[7]

Soybean is an excellent source of protein, folate, and minerals. Soy product intake was inversely associated with plasma tHcy and positively correlated with serum folate.[8] Recent studies indicate that genisteine aglycone (54 mg/day) supplemented in postmenopausal women significantly decreased plasma tHcy compared to placebo after 3 years of treatment.[9] Turhan et al showed that supplementation of isoflavones 80 mg/day in postmenopausal women resulted in a significant decrease in plasma tHcy.[10] Several studies have shown favorable effects of soy protein, isoflavones, or both in improved glycemic control and serum lipid profile.[11,12]

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There is limited information on the benefits of soy protein isolate (SPI) contained isoflavones in reducing other biomarkers of CVD (tHcy, folate and vitamin B_{12}), especially in diabetes mellitus**.** The aim of this study was to evaluate the effect of SPI supplementation on some circulating biomarkers of CVD in type 2 diabetic patients including tHcy, folate and vitamin B_{12}, as well as GFR.

Subjects and Methods

Subjects

Thirty-six type 2 diabetic patients aged 35 years and over with fasting blood sugar (FBS) between 5.56 - 13.89 mmol/l, and using only sulfonylureas and/or biguanide were recruited to participate in this study. All subjects had blood pressure (BP) less than 160/100 mmHg, total cholesterol (TC) and triglyceride (TG) levels less than 6.21 mmol/l and 2.26 mmol/l, respectively and body mass index (BMI) between 18.5 - 29.9 kg/m^2. They also needed to meet the following criteria: not taking any antibiotics within the last 7 days before the study, not taking any nutritional supplements or herbal products, soy or soy product regularly, not being a vegetarian, not smoking and drinking alcohol regularly. They were also free from chronic diseases or conditions including liver or renal diseases, history of soy or soy products allergy, malnutrition, and surgery within 1 month before and during the study. The subjects were randomly divided into 2 groups (SPI and control groups) with 18 subjects each. Proportions of male and female subjects in the two groups were somewhat comparable, 8 males and 10 females in control group and 7 males and 11 females in SPI group. The experimental protocol was approved by the Ethics Committee of Chulalongkorn University, and written informed consent was obtained from each subject.

Study Protocol

In this quasi-experimental study, subjects participated in a 10-week experiment with two consecutive periods: a 4-week pre-experimental period followed by a 6-week experimental period (SPI supplementation). In pre-experimental period, subjects received nutrition counseling to control blood glucose levels and personal information was obtained. Each subject was asked to maintain the amount of energy intake and level of physical activity, and to avoid taking additional soybean and soy products throughout the study.

After the pre-experimental period, fasting blood was obtained from each subject for determining baseline levels of CVD biomarkers including tHcy, folate and vitamin B_{12}. Glomerular filtration rate (GFR) was determined using Cockroft-Gault formula: GFR (ml/min) = [(140-age) × weight (kg)]/[(72 × serum creatinine (mg/dl))] for men, and multiplied by 0.85 for women. Habitual diet was assessed by a 3-day food record prior to their experimental period and during SPI supplementation period. The subjects in SPI group were instructed to consume 1 package of 30-g SPI/day (containing 32 mg isoflavones) for 6 weeks, by mixing with water, soup or other beverage. At the end of the study, venous blood was drawn after a 12-hour fasting to determine biomarkers of CVD, and BP and anthropometric measurements were also taken. Plasma tHcy was determined by a fluorescence polarization immunoassay using the IMx analyzer. Plasma levels of folate and vitamin B_{12} were determined by microbiological assay and Elecsys® immunoassay analyzers, respectively. The subject's compliance was assessed by interviewing and counting the remaining packs of SPI. In addition, any adverse effects that occurred during SPI supplementation were also asked over the telephone.

Statistical Analysis

Continuous data were expressed as mean ± standard deviation (SD). For comparison of the proportion of demographic data, chi-square test was used to test for significant difference between groups. Normal distribution of the data was checked by Shapiro-Wilk's statistics, skewness, and kurtosis. When the distribution of variables was normal, paired *t*-test and independent *t*-test were used to compare within group and between groups differences, respectively. Associations between continuous variables were described using Pearson's correlation coefficients. Significance was accepted with $P < 0.05$.

Results

During the 6-week SPI supplementation, no adverse events or side effects of SPI were reported. At baseline, age,

BMI, GFR and CVD biomarker levels were comparable between SPI and control groups (Table 1).

Table 1 Baseline clinical characteristics of subjects.

Characteristics	Mean ± SD		*p*-value†
	Control (n = 18)	Soy protein isolate (n = 18)	
Age (year)	61.56 ± 10.57	62.00 ± 7.87	0.887
Body mass index (kg/m^2)	24.80 ± 2.50	25.55 ± 1.58	0.350
Biomarkers of cardiovascular disease			
Total homocysteine (μmol/l)	12.72 ± 3.50	12.92 ± 4.06	0.200
Folate (ng/ml)	7.53 ± 4.10	6.91 ± 3.99	0.647
Vitamin B_{12} (pg/ml)	433.78 ± 104.19	445.82 ± 112.55	0.741
Glomerular filtration rate (ml/min)	72.16 ± 16.35	71.74 ± 19.74	0.953

† Independent *t*-test

In SPI group, at week 6, plasma tHcy levels were significantly decreased (P = 0.005) while folate levels were significantly increased from baseline (P = 0.002). The effect on vitamin B_{12} was not observed (Table 2). At week 6, levels of either tHcy, folate, vitamin B_{12}, or GFR between the two groups did not differ. Regarding correlation between tHcy and other CVD biomarkers, age and GFR, it was found at baseline, circulating tHcy was significantly inversely correlated with folate (r = -0.471, P = 0.004), GFR (r = -0.753, P < 0.001), vitamin B_{12} (r = -0.390, P = 0.019) and significantly positively correlated with age (r = 0.592, P < 0.001) (Table 3).

Discussion

In the present study, after 6 weeks of SPI supplementation in type 2 diabetic patients, plasma tHcy levels were decreased, whereas plasma folate levels were increased from baseline. The intake of 30-g SPI containing 32 mg of isoflavones per day was well tolerated and did not cause significant side effects.

An increase in the plasma level of tHcy, an intermediate in the catabolism of methionine, has been identified as a risk factor for CVD. Elevated tHcy concentrations are associated with a higher risk for coronary artery disease (CAD) in subject with type 2 diabetes than in non-diabetic subjects.[13]

Table 2 Effects of soy protein isolate supplementation on biomarkers of cardiovascular disease.

Parameters		Mean ± SD		p-value †
		Control (n = 18)	Soy protein isolate (n = 18)	
Total homo-cysteine (μmol/l)	Baseline	12.72 ± 3.50	12.92 ± 4.06	
	Week 6	13.60 ± 3.67	11.63 ± 3.22*	0.090
	Mean change	0.88 ± 2.07	-1.29 ± 1.72	
Folate (ng/ml)	Baseline	7.53 ± 4.10	6.91 ± 3.99	
	Week 6	9.44 ± 6.27	10.33 ± 5.36‡	0.353
	Mean change	1.91 ± 5.26	3.42 ± 3.20	
Vitamin B_{12} (pg/ml)	Baseline	433.78 ± 104.19	445.82 ± 112.55	
	Week 6	429.71 ± 112.9	444.64 ± 85.11	0.657
	Mean change	-4.07 ± 95.17	-1.18 ± 85.10	
GFR (ml/min)	Baseline	72.16 ±16.35	71.96 ± 18.97	
	Week 6	71.77 ± 27.73	71.53 ± 18.80	0.975
	Mean change	-0.39 ± 18.57	-0.44 ± 5.92	

†Independent *t*-test comparing between groups at week 6

* P = 0.005 for significant difference from baseline (within group) (paired t-test)

‡ P = 0.005 for significant difference from baseline (within group) (paired t-test)

Table 3 Correlations of baseline total homocysteine with age, GFR and cardiovascular disease biomarkers (N = 36).

Biomarker	*Pearson's correlation coefficient (r)*	*P*-value
Folate (ng/ml)	-0.471	0.004
Vitamin B_{12} (pg/ml)	-0.390	0.019
Age (years)	0.592	< 0.001
GFR (ml/min)	-0.753	< 0.001

In this study, baseline tHcy was positively correlated with age. The increase in plasma tHcy level with increasing age may be due to decreased renal function, decreased B-vitamins absorption and other factors associated with aging process.[14]

The amount of tHcy in the blood is regulated by folate, vitamin B_{12} and vitamin B_6.[15] Lowering tHcy concentrations by 25% from current levels would reduce the risk of ischemic heart disease by 11%.[16] This study found that the supplementation of SPI for 6 weeks significantly increased plasma folate and decreased tHcy concentrations from baseline.

This study also found that plasma tHcy were significantly inversely correlated with folate and vitamin B_{12}. These findings are consistent with previous studies in that plasma tHcy levels were significantly reduced with soy protein or its

isoflavones.[8,9] Han et al reported that soy formula-fed infants had the highest serum folate than human milk-fed infants.[17] Furthermore, the decrease in plasma tHcy levels in this study may be due to the folate in SPI supplemented products. Supplementation of folate > 500 µg/d has shown to reduce elevated tHcy levels[18] and SPI supplemented in this study was found to contain folate 211 µg/100 g, which was higher than other sources of folate such as kidney beans, asparagus and green beans (144, 95 and 37 µg of folate/100 g respectively). Moreover, this study found that tHcy levels were associated directly with renal functions and were increased substantially with a decrease in GFR, the indicator of renal function. Mezzano et al showed that plasma tHcy was elevated in chronic renal failure patients.[20] Elevation of tHcy found in chronic renal failure was not because of impaired urinary excretion but the impaired metabolism of tHcy by the kidney instead.[21]

Conclusion

The present study has shown that soy protein isolate containing isoflavones may have the additional benefits, including decreased plasma tHcy and increased folate levels. It is thus reasonable to conclude that SPI containing isoflavones potentially improves some biomarkers of cardiovascular disease in type 2 diabetic patients. However, studies with larger subject size and longer period are needed before a recommendation on the use of soy protein isolate for preventing cardiovascular disease in individuals with high risk can be made.

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