Effects of Black Soy Peptide Supplementation on Glucose Control in Subjects with Prediabetes and Newly Diagnosed Type 2 Diabetes mellitus

Eun Na Rae Han

The Graduate School

Yonsei University

Department of Science for Aging

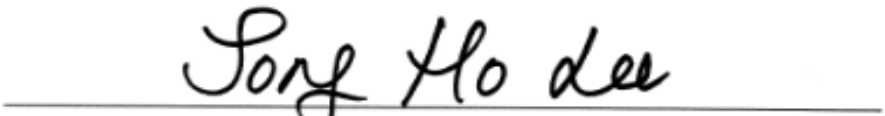
Effects of Black Soy Peptide Supplementation on Glucose Control in Subjects with Prediabetes and Newly Diagnosed Type 2 Diabetes mellitus

A Master's Thesis
Submitted to the Department of Science for Aging
and the Graduate School of Yonsei University
In partial fulfillment of the requirements
for the degree of Master in Science for Aging

Eun Na Rae Han

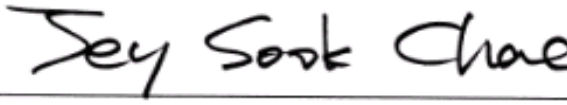
June 2010

This certifies that the master's thesis of
***Eun Na Rae Han* is approved.**



Thesis Supervisor: Jong Ho Lee

Thesis Committee Member : Yang Soo Jang



Thesis Committee Member : Jey Sook Chae

The Graduate School

Yonsei University

June 2010

CONTENTS

List of tables

Abstract

Effects of Black Soy Peptide Supplementation on Glucose Control in Subjects with Prediabetes and Newly Diagnosed Type 2 Diabetes mellitus

Eun Na Rae Han

Department of Science for Aging

The Graduate School

Yonsei University

The present study aimed to determine the effect of black soy peptide supplementation on glucose control in subjects with prediabetes (impaired fasting glucose; IFG or impaired glucose tolerance; IGT) and newly diagnosed type 2 diabetes mellitus (DM). In this double-blind, placebo-controlled study, subjects with prediabetes and type 2 DM were randomly assigned to the

placebo control group or the black soy peptide intervention group. We determined fasting serum concentrations of glucose, hemoglobin A1c (HbA1c), insulin, and free fatty acids (FFAs), performed a 2-h postload glucose test (2-h PG), and compared serum lipid profiles before and after the 12-week supplementation. Particularly, subjects with fasting glucose≥ 110 mg/dL who consumed black soy peptides demonstrated a tendency to the decrease of fasting glucose levels (p=0.098 by two tail-test / p=0.049 by one tail-test) and had a significant reduction in 2-h PG level (p=0.012 by two tail-test / p=0.006 by one tail-test), compared with baseline levels. The changes in 2-h PG levels were also statistically significant in the intervention group (-41.25 ± 13.67 mg/dL) compared with the placebo group (12.42 ± 9.80 mg/dL; p=0.015 by two tail-test / p=0.008 by one tail-test). In contrast, HbA1c levels were not significantly improved by the dietary intervention.

In conclusion, black soy peptide supplementation may be beneficial to control fasting blood glucose levels and 2-h postload glucose levels, particularly in subjects with fasting glucose ≥ 110 mg/dL.

KEY WORDS: Double-blind/placebo-controlled study, glucose control, impaired fasting glucose, impaired glucose tolerance, soy peptide.

1. Introduction

The incidence of type 2 diabetes mellitus (type 2 DM) has been lower in Asian populations compared with those in western countries. One of difference between Asian people and Western is that Asian diet is rich in soybean foods compared to Western diet [1]. Asian diet may help prevent and slow the progression of type 2 DM.

But, recently, high fat diet has increased, together with changes from the traditional Korean lifestyle to a Western lifestyle. Also, cardiovascular diseases such as atherosclerosis, coronary heart diseases (CHD) are not only increased, but type 2 diabetes is increased as well because of obesity, lack of exercise, social stress, overeating. And prevalence of type 2 diabetes has been increasing rapidly worldwide [2].

According to the 2007 National Health and Nutrition Examination Survey for Koreans, the prevalence of diabetes and impaired fasting glucose (IFG) were 9.7% and 16.1% in Korean adults (≥ 30 years old), respectively [3]. Diabetes is a common endocrine disorder that has become a worldwide health problem. It is characterized by chronic hyperglycemia with disturbances of macronutrient metabolism resulting from defects in insulin secretion, insulin action, or both. Chronic hyperglycemia causes diabetes-related

complications such as heart disease, retinopathy, kidney disease, and neuropathy [4].

Diabetes is diagnosed when the fasting plasma glucose concentration is consistently ≥ 126 mg/dL or when the plasma glucose concentration is consistently ≥ 200 mg/dL 2 hour after a 75-g glucose load (2-h postload glucose test; 2-h PG).

Both Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are not diabetes mellitus, but they are considered as pre-diabetes. So, they mean a high potential of progress to diabetes [5]. Impaired glucose regulation is associated with a high rate of cardiovascular disease (CVD) and mortality [6, 7].

It has been reported that foods or diets that produce a low glycemic response are associated with less insulin resistance and lower risk of type 2 diabetes [8] and CVD [9]. Among these foods, black soybean is a traditional component of Asian medicine used to treat conditions including diabetes, hypertension, and poor blood circulation [10]. Diets rich in soy protein and soy peptides have been reported to be beneficial in weight loss, improved insulin resistance and endothelial cell function, and reduced blood pressure and blood lipids [11-14]. Soy foods have a low glycemic index, suggesting that these soy-rich diets can improve blood glucose and insulin levels [15].

However, few studies have evaluated the effect of black soy peptide supplement on glucose control in humans. Therefore, we determined the effects of black soy peptide supplement on glucose control in Koreans with prediabetes and newly diagnosed type 2 DM.

2. Background

2.1. Soy bean (Soy protein)

2.1.1. The characteristic of soy bean

Soybean is the genus *Glycine* and the cultivar *Glycine max*. Soybean is a singular food because of its rich nutrient content. Soybean contains vegetable protein, oligosaccharides, dietary fiber, phytochemicals, minerals [16-18]. Soybean is a protein source and the protein content of soybean is about 40% [16]. Soybean protein is low in sulphur amino acids, being methionine the most significant limiting amino acid, followed by cystine and threonin [19]. Most beans are very low in fat, but soybeans are an exception because their fat content is the highest among the beans, containing 47% of energy from fat [17]. And the predominant fatty acid is linoleic acid comprising approximately 53% of the total fatty acid content of soybean [19]. So soybean could be a good source to increase the linolenic acid intake.

Soybean represents an excellent source of high quality proteins, it has a great amount of dietary fiber and a low content in saturated fat [20]. So, soybean has shown to reduce risk of many diseases.

2.1.2. The effects of soy proteins on glucose metabolism

Recently, characterization and positive health effects of soybeans have been studied. Many studies in humans and experimental animals have examined whether the consumption of soy-containing diets have an effect on glucose and lipid metabolism and on hormones controlling their metabolism [21]. Most of the nutritional intervention studies performed in animals and humans have shown beneficial health effects on soybean protein [21-24]. In one study, soy protein induced a lower postprandial insulin-glucagon ratio in healthy and hypercholesterolemic subjects than did casein [25]. Soy proteins are rich in arginine and glycine, which are involved in insulin and glucagons secretion from the pancreas. Decreased plasma insulin by soy protein may be due to decreased release from the pancreas or increased hepatic removal. Thus, the decrease in cholesterol seen with soy protein may be due to the decreased insulin-glucagon ratio caused by arginine and glycine [26]. In healty pigs, soy-protein feeding compared with casein decrease postprandial serum concentration of insulin and glucose [27]. In a study in ovariectomized cynomolgus monkey, soy protein significantly improved insulin sensitivity and glucose effectiveness compared with casein [28].

Clinical studies also suggest that soy isoflavones may improve glucose

control. Nutritional studies performed in animals and intervention studies with humans suggest that ingestion of soy protein with isoflavones improves glucose control and reduces insulin resistance [21]. The study revealed the during fasting both glucose and insulin levels were significantly reduced by soy isoflavones (100mg/day) and conjugated estrogens (0.625mg/day) [29]. Among the ingredients in a soybean diet, isoflavones are related to improvements in type 2 diabetes and hyperlipidemia [21, 30, 31].

2.1.3. The effects of soy proteins on lipid metabolism

Most of the studies have been focused on soybean protein as a possible source of prevention against cardiovascular disease. This positive effect may be due to a decrease in serum cholesterol concentration [20]. Soy protein had a beneficial effect on serum lipid profile and renal function [32]. After soy consumption, total cholesterol decreased significantly, in agreement with another study in which subjects consumed a diet with 50% of soy protein [33].

Also, Clinical studies suggest that soy isoflavones may improve serum lipid profile. The isoflavones in soy protein can also play a key role modifying lipid profiles [34].

Despite these beneficiary effects of soy on serum lipid concentration, there is some evidence that reports no significant change in serum lipid level after soy protein intake.

2.2. Soy peptides

2.2.1. The characteristic of soy peptides

Soy peptides derived from isolated soybean protein are the major functional components. The soy grits undergo extensive hydrolysis during the fermentation process. As a result, many anti-nutritive factors of soybean are inactivated. Furthermore, high solubility and soy digestibility are achieved. So, soy peptides are very different form other soy products in their nutritive properties. The intermediate form is the product of protein and amino acids can be, and soybean peptides are generated by fermentation enzyme. Therefore, soy peptides are rapidly absorbed into the body, helping prevent and slow the progression of various chronic diseases.

2.2.2. The advantages of black soy peptides

Black soy bean has been used to as a component in oriental medicines to treat diabetes, hypertension, blood circulation, and so on [35]. Black soy bean represents higher the antioxidant effects than other yellow soybean because of anthocyanin pigment series of physiologically active substances in seed coat [36]. And anthocyanins (ANT) from black soybean seed coats may be used to prevent the development of diabetes, or furthermore modulate type 2 DM and its complications [36]. As the efficacy of flavonoids such as ANT is dose dependent, 50mg/kg ANT showed the protective effects against various oxidative stresses [37, 38] and on streptozotocin-induced diabetic rat [36].

Black soy bean also contains glycitein, which is isoflavones in black soy bean, not founded in yellow soy bean.

Nutritional invention studies have shown that a black soy peptide-based diet has antiobesity effect. Novel peptide mixtures derived from black soybean peptides (BSP) inhibit the development of diet-induced obesity in mice by activating leptin-like signaling and AMP-dependent protein kinase (AMPK) [39]. BSP also inhibits the elevation of plasma triglyceride concentrations in diabetic (*db/db*) mice through multiple mechanisms, including the inhibition of hepatic ER stress [40].

Therefore the intake of Black soy peptides produced from black soybean attributed to being a possible way to fight obesity and lowering cholesterol [39, 41].

2.3. The beneficial effects of soy peptides

According to the inside and outside of the country studies related to functional peptides, the studies associated to hypertension are the most active, followed by obesity (weight loss), but the studies connected with glucose control are not relatively enough yet.

It is reported that soy peptides as an excellent source of high quality vegetable protein have the bio-actives that are weight loss in person with obese, improvement of insulin resistance and endothelium function to a blood vessel, decline of blood pressure, and diminution of cholesterol and lipid [42]. But the benefits and mechanism of action of black soy peptides for preventing of treating diabetes are an area of much needed research that would include experimental animal models and clinical human studies.

3. SUBJECTS AND METHODS

3.1 Study population

Subjects with prediabetes (IFG or IGT) and newly diagnosed type 2 DM no requiring medication treatment according to doctor`s suggestion were recruited from the National Health Insurance Corporation Ilsan Hospital in Goyang and CHA General Hospital in Seoul, Korea. Exclusion criteria included 1) previous history of diabetes; 2) abnormal liver or renal function; 3) history of CVD or cancer; 4) pregnancy, breast feeding, or intending to become pregnant during time of study; and 5) thyroid or pituitary disease. IFG was defined as fasting glucose between 100 and 125 mg/dL and IGT was defined as 2-h postload glucose levels 140-199 mg/dL. Newly diagnosed type 2 diabetes was defined as fasting glucose ≥ 126 mg/dL or 2-h PG level ≥ 200mg/dL and who had never been diagnosed for DM. Written informed consent was obtained from all subjects, and the protocol was approved by the Ethics Committee of the Yonsei University and the Institutional Review Board of National Health Insurance Corporation Ilsan Hospital. Seventy-five subjects (18–69 years old) met the study criteria and those who consented to

participate in the trial were included in this study (n = 55). Study participants were randomly assigned to placebo group or intervention group. Subjects in the intervention group received three pouches containing black soy peptides (4.5-g supplement per day for 12 weeks). The former group received a placebo that had a similar appearance to the black soy tablet.

3.2 Materials

The black soy peptide supplement and placebo were both provided by Nongshim Co., Ltd (Dongjak-Gu, Seoul, Korea). Black soy peptides (BSP) were obtained using an enzyme hydrolysis method. And placebo included lactose and dextrin (95%, 5% respectively). The forms of both BSP supplement and placebo were tablet.

3.3 Methods and Measurements

3.3.1 Study procedures

We designed a randomized, double-blinding, placebo-controlled human experiment tests. Before starting test supplements treatment through randomized assignment, Fasting plasma glucose test and glycosylated hemoglobin (HbA1c) test were carried out. During the run-in period of one week, we educated to usual dietary intake and physical activities and to write food diary and activity diary while 3 days (2 days on weekday, 1 day on weekend). After the end of observation on a week, we selected a suitable subject and divided subjects into the test group or a group of placebo. The subjects assigned to two groups would be taken test supplements (or placebo) for 12weeks (3 in one day, one in tablet (a tablet 20 capsules)).

3.3.2 Anthropometric parameters, blood pressure measurements, blood collection protocol and laboratory tests

Body weight and height were measured in the morning with the subjects unclothed and without shoes. Body mass index (BMI) was calculated as body weight in kg divided by height in square meters (kg/m^2). Waist circumference was measured with paper tape horizontally at the umbilicus in the standing position after normal expiration. Body composition was determined by a foot-to-foot bioelectrical impedance analyzer while standing erect with bare feet on the analyzer footpads (Tanita, Japan) to obtain lean body mass (kg), fat mass (kg), and body fat (%). Blood pressure was measured using the left arm of the seated patient with an automatic blood pressure monitor (TM-2654, A&D, Tokyo, Japan) after a 10-min rest. The average of three measurements was recorded for each subject.

Venous blood specimens were collected in EDTA-treated and plain tubes after a 12-h fast. The tubes were immediately covered with aluminum foil and placed on ice until they arrived at the laboratory room (within 1-3hr) and centrifuged into plasma or serum then, stored at -70℃ until analysis.

3.3.3 Fasting plasma glucose, 2hr postload glucose test, Insulin, Hemoglobin A1c, Free fatty acid

After 12 hours, fasting blood glucose in venous blood was measured by a glucose oxidase using method a Beckman Glucose Analyzer (Beckman Instrument, Irvine, CA, USA). 2hr postload glucose test also was determined after 12 hours fasting. The 75g of glucose was dissolved in 250-350ml of water to drink 5-15minutes. Subjects drank a 75g glucose solution after an overnight fast. Venous blood specimens were collected before and 120 min after glucose ingestion. Glucose was determined by the glucose oxidase method using the Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA). Insulin in venous blood was measured by radio-immunoassays with commercial kits from Immuno Nucleo Corporation (Stillwater, MN, USA) after 12 hours of fasting. Glycosylated hemoglobin (HbA1c) was measured using immunotrubidimetric analyzer. And free fatty acids (FFAs) were analyzed with a Hitachi 7150 analyzer (Hitachi Ltd, Tokyo, Japan).

3.3.4 Serum lipid profile and apolipoprotein A-I and B

Fasting serum concentrations of total cholesterol (TC) and triglyceride (TG) were measured using commercially available kits with the Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). High-density lipoprotein (HDL) cholesterol was measured from the supernatant by enzymatic methods. Low-density lipoprotein (LDL) cholesterol was indirectly estimated in subjects with serum TG levels < 400 mg/dL (4.52 mol/L) using the Friedewald formula: LDL-cholesterol = total-cholesterol-{HDL-cholesterol + (triglycerides/5)}. In subjects with serum TG concentrations ≥ 400 mg/dL, LDL cholesterol was determined directly by an enzymatic method on a Hitachi 7150 Autoanalyzer. Serum apolipoprotein A-I (apo A-I) and apo B were determined by turbidimetry at 340 nm using a specific anti-serum (Roche, Switzerland).

3.3.5 Assessment of food intake and physical activity

Usual food intake was assessed by a 24-h recall method; a semi-quantitative food frequency questionnaire was used to confirm that the data collected by the 24-h recall method was representative of the usual dietary pattern [43]. Nutrient intake data were calculated as mean values from the database referenced above. Total calorie expenditure (TEE; kcal/d) was calculated from basal metabolic rate, 24-h physical activity [44], and the food specific dynamic action. The basal metabolic rate for each subject was calculated with the Harris-Benedict equation [45].

3.4 Statistical analysis

Statistical analyses were performed with SPSS ver. 17.0 for Windows (Statistical Package for the Social Science, SPSS Inc., Chicago, IL). Each variable was examined for normal distribution, and significantly skewed variables underwent log transformation. For descriptive purposes, mean values of untransformed and unadjusted variables are presented. Baseline characteristics and comparisons between the intervention and control groups were evaluated by Student's *t*-test for continuous variables. A paired *t*-test was used to evaluate the effects of soy peptide or placebo within each group before and after the intervention (in subjects number < 20 years old, a non-parametric test was used). Only results from the participants who completed the intervention program were analyzed (n = 42). Results are expressed as mean ± standard error of the mean (SEM). A p-value < 0.05 was considered statistically significant (we present that two-tailed p-value / one-tailed p-value).

4. Results

4.1 Baseline characteristics and dietary intake change of the study participants

Twenty-one of 28 participants (75%) assigned to the soy peptide supplement group and 21 of 27 (78%) of those assigned to the control group completed the intervention and underwent blood analysis at their 12-week visit.

General baseline characteristics were similar between the two groups (Table 1). No significant differences in age, initial weight, BMI, weight-hip ratio (WHR), lean body mass (LBM), systolic blood pressure (SBP), or diastolic blood pressure (DBP) were observed between the two groups before or after the intervention. Table 2 presents dietary nutrient intake at baseline and after the 12-week intervention. Total calorie intake (TCI), TEE, and dietary macronutrient intake did not differ significantly between groups ($p > 0.05$).

Table 1. Characteristics of study participants.

	Black soy peptides (n=21)		Placebo (n=21)	
	Baseline	**Week 12**	**Baseline**	**Week 12**
Age (years)	56.8 ± 1.53		57.6 ± 2.01	
Height (cm)	161.2 ± 1.58		162.5 ± 2.09	
Weight (kg)	62.6 ± 1.47	62.4 ± 1.56	65.8 ± 1.95	65.8 ± 2.04
BMI (kg/m^2)	24.1 ± 0.50	24.0 ± 0.53	24.8 ± 0.38	24.8 ± 0.42
WHR	0.91 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.91 ± 0.01
LBM$^{\int}$(kg)	44.9 ± 1.68	45.3 ± 1.82	46.5 ± 2.02	45.8 ± 1.98
Fat (%)	28.5 ± 1.79	27.7 ± 1.73	29.6 ± 1.80	30.8 ± 1.45
SBP (mmHg)	125.1 ± 3.22	128.2 ± 2.61	126.7 ± 2.93	124.2 ± 2.91
DBP (mmHg)	73.6 ± 2.36	75.1 ± 1.93	74.5 ± 2.12	74.3 ± 2.07

Mean±SEM. $^{\int}$Analyzed after log transformation. $^{\S}p<0.1$ compared with baseline.
BMI: body mass index, WHR: weight-hip ratio, LBM: lean body mass, SBP: systolic blood pressure, DBP: diastolic blood pressure.

Table 2. Daily dietary nutrient intake at baseline and after the 12-week intervention.

	Black soy peptides (n=21)		Placebo (n=21)	
	Baseline	**Week 12**	**Baseline**	**Week 12**
TEE (kcal/d)	2020 ± 59.6	2028 ± 60.3	2043 ± 65.7	2046 ± 66.4
Estimated daily nutrient intake				
TEI (kcal/d)	2094 ± 56.7	2099 ± 57.5	2168 ± 67.1	2175 ± 68.6
CHO (%)	61.6 ± 0.18	61.6 ± 0.19	61.6 ± 0.15	61.7 ± 0.17
PRO (%)	16.8 ± 0.24	16.9 ± 0.21	16.7 ± 0.16	16.7 ± 0.18
Fat (%)	21.6 ± 0.21	21.4 ± 0.16	21.8 ± 0.20	21.8 ± 0.21
Chol (mg/d)	204.6 ± 26.9	180.6 ± 14.3	270.0 ± 41.4	237.4 ± 16.1

Mean±SEM. TEE: total energy expenditure, TEI: total energy intake, CHO: carbohydrate, PRO: protein, Chol: cholesterol.

4.2 Levels of glucose and related biomarkers

Table 3 presents levels of glucose and related markers after fasting and after the 2-h PG. The intervention group demonstrated somewhat lower fasting glucose levels (baseline: 121.62 ± 2.96 mg/dL, after soy peptide supplementation: 117.95 ± 4.06 mg/dL, p = 0.166 / p = 0.083) and 2-h PG levels (baseline: 219.71 ± 18.66 mg/dL, after soy peptides: 200.48 ± 15.03 mg/dL, p = 0.194 / p = 0.097) after the 12-week intervention, although this difference was not significant. In addition, FFAs were elevated after the 12–week dietary intervention (p = 0.126 / p = 0.063). We also observed the changes of the above variables in subjects with fasting glucose ≥ 110 mg/dL. At baseline, black soy peptide supplementation was associated with a tendency to the decrease of fasting glucose levels (-4.88 ± 2.79 mg/dL, p = 0.098 / p = 0.049) and a significant reduction in 2-h PG levels (baseline: 243.13 ± 20.44 mg/dL, after soy peptides: 201.88 ± 17.45 mg/dL, p = 0.012 / p = 0.006). Changes in 2-h PG levels were also statistically significant in the intervention group (-41.25 ± 13.67mg/dL) compared with the placebo group (12.42 ± 9.80 mg/dL; p = 0.015 / p = 0.008) in subjects with fasting glucose ≥ 110 mg/dL at baseline. In contrast, no significant differences in HbA1c, insulin, and FFA levels were observed.

Table 3. Glucose-related biomarkers at baseline and after the 12-week intervention.

	Black soy peptides (n=21)		Placebo (n=21)	
	Baseline	**Week 12**	**Baseline**	**Week 12**
Fasting levels				
Glu (mg/dL)	121.6 ± 2.96	118.0 ± 4.06	115.4 ± 3.03	114.4 ± 3.61
HbA1c (%)[∫]	6.70 ± 0.14	6.65 ± 0.14	6.42 ± 0.13	6.45 ± 0.14
Ins (μIU/dL)[∫]	11.9 ± 1.63	17.2 ± 5.89	10.6 ± 0.61	11.2 ± 0.91
FFA (μEq/L)	429.8 ± 41.6	503.7 ± 45.3	515.3 ± 40.0	516.2 ± 42.4
2-h postload glucose levels				
Glu (mg/dL)	219.7 ± 18.7	200.5 ± 15.0	182.6 ± 13.3	185.4 ± 14.7

Mean±SEM. [∫]Analyzed after log transformation. [§]$p<0.1$ compared with baseline.
Glu: glucose, HbA1c: hemoglobin A1c, Ins: insulin, FFA: free fatty acid.

Table 4. Glucose-related biomarkers in subjects with fasting glucose ≥ 110 mg/dL.

	Black soy peptides (n=16)		Placebo (n=12)	
	Baseline	**Week 12**	**Baseline**	**Week 12**
Fasting levels				
Glu (mg/dL)	126.6 ± 2.92	121.7 ± 4.68	124.7 ± 3.15	124.5 ± 3.85
HbA1c (%)∫	6.83 ± 0.17	6.78 ± 0.16	6.77 ± 0.11	6.78 ± 0.14
Ins (μIU/dL)∫	12.3 ± 1.57	19.3 ± 7.68	9.73 ± 0.82	10.9 ± 0.92
FFA (μEq/L)	465.4 ± 42.3	530.8 ± 51.9	515.5 ± 52.7	549.4 ± 44.5
2-hr postload glucose levels				
Glu (mg/dL)	243.1 ± 20.4	201.9 ± 17.5*	204.5 ± 13.2	216.9 ± 17.2

Mean±SEM. ∫Analyzed after log transformation. *p<0.05,**p<0.01 compared with baseline. Glu: glucose, HbA1c: hemoglobin A1c, Ins: insulin, FFA: free fatty acid.

4.3 Lipid profiles

Subjects receiving black soy peptides showed a tendency to reduced Apo B levels ($p = 0.054$ / $p = 0.027$) and a tendency to increased LDL cholesterol ($p = 0.055$ / $p = 0.028$). Total cholesterol was also higher in the intervention group, but this was not statistically significant ($p = 0.160$ / $p = 0.080$). No significant differences were observed for the other variables. Among subjects with fasting glucose $\geq$ 110 mg/dL, black soy peptide supplementation was associated with a tendency to reduction in Apo B levels ($p = 0.055$ / $p = 0.028$; data were not shown).

Table 5. Serum lipid profiles at baseline and after the 12-week intervention.

	Black soy peptides (n=21)		Placebo (n=21)	
	Baseline	**Week 12**	**Baseline**	**Week 12**
TG (mg/dL)[∫]	128.1 ± 17.8	126.6 ± 19.0	128.1 ± 10.3	129.1 ± 14.7
TC (mg/dL)	186.5 ± 6.93	193.3 ± 6.38	190.8 ± 6.51	191.5 ± 7.22
HDL-c (mg/dL)	46.5 ± 2.81	44.9 ± 2.11	45.6 ± 1.92	45.8 ± 1.91
LDL-c (mg/dL)	114.4 ± 5.66	123.1 ± 5.21[§]	119.6 ± 6.86	119.9 ± 6.81
Apo A-I (mg/dL)	145.7 ± 5.72	139.7 ± 4.61	152.5 ± 4.31	147.3 ± 4.63
Apo B (mg/dL)	86.1 ± 4.03	80.9 ± 4.62[§]	89.7 ± 4.16	87.1 ± 5.96

Mean±SEM. [∫]Analyzed after log transformation. [§]$p<0.1$, *$p<0.05$ compared with baseline.
TG: triglyceride, TC: total cholesterol, HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol, Apo A-I: apolipoprotein A-I, Apo B: Apolipoprotein B.

5. Discussion

The purpose of the present study was to determine the effects of black soy peptide supplementation on glucose control in Koreans with prediabetes and newly diagnosed type 2 DM. We found that black soy peptide supplementation may reduce fasting glucose levels and 2-h PG levels, particularly among individuals with fasting glucose $\geq$ 110 mg/dL. Diabetes is a serious worldwide health problem [46]. The number of people with type 2 diabetes is expected to increase rapidly within the next 25 years, with an estimated 42% increase in developed countries [47]. Hyperglycemia results from a complex interplay between insulin sensitivity and secretion, with a failure of pancreatic beta cells to compensate sufficiently for the increased insulin requirement induced by insulin resistance [48]. Chronic hyperglycemia causes diabetes-associated complications [4], thus, strict control of blood glucose may be essential in IFG or IGT.

Soy protein and peptides as major dietary vegetable proteins have been reported to produce various physiological effects, including weight loss in obese subject and reductions of insulin resistance, cholesterol, and blood pressure [11-14].

Many intervention studies reported the effect of soy or soy drived product

on glucose metabolism in humans. However, the effects are still controversial [49, 50]. A study by Tsai et al. observed that in obese subjects with type 2 diabetes, soy polysaccharide (10g) to standard test meal significantly reduced the increase in postprandial serum glucose and triacylglycerol concentrations. This effect appears to have been due to smaller increases in glucagon and pancreatic polypeptide and larger increases in somatostatin concentrations. There was no significant effect on serum insulin concentrations [48]. Taniguchi et al. (2008) showed that consuming naturally viscous vegetables (50 g natto, 60 g Japanese yams, and 40 g okra) with white rice reduced acute glycemia and insulinemia [51]. Fujita et al. (2001) demonstrated that soybean-derived Touchi extract, an α-glucosidase inhibitor, appears to exert extensive and useful effects in subjects with borderline and mild diabetes [52]. Lui et al, reported that soybean supplementation (15g soy protein + 100mg isoflavone) during 6 month, did not show the favorable effect of glymeic control and insulin sensitivity, but showed the favorable effect of 2 h postload glucose [53].

The meta-analysis published by Anderson et al [22] in 1995 reported the effect of soy supplement (47g/day) on the blood lipid level; significant reductions in total cholesterol (23.2 mg/dL), LDL cholesterol (21.7 mg/dL), and triglycerides (13.3 mg/dL), but a non-significant increase in HDL cholesterol (1.2 mg/dl). In another study, metaregression analyses showed a

dose-response relation of soy protein and isoflavone supplementation with the changes in serum lipids. Which presented that soy protein supplementation reduced serum lipids in adults regardless of hypercholesterolemia [23]. Anderson et al. [33] studied the effect of soy protein and (1g protein/kg body wt for 8week) in type 2 diabetic subjects with obesity and hypertension and observed a reduction of total cholesterol and triacylglycerol concentrations and the improvement of hyperlipidemia. Hermansen et al. [13] also reported that type 2 diabetic subjects, treated with the soy-based dietary supplement (50g isolated soy protein, 165mg isoflavone, 20g soy cotyledon fiber) for 6 weeks had significant reduction in LDL cholesterol (10%), the LDL/HDL ratio (12%) and Apo B100 levels (30%). Such changes in lipid levels have been shown to associated with less CAD [54–56]. The reduction of ApoB levels may be associated with the PPARr expression, activated by soy supplemention [57]. In our study, we observed a significant reduction in Apo B levels(6 %) after black soy peptide supplement.

Anthocyanins in the black soybean seed coat play a role in regulating glucose transporter 4 and preventing insulin resistance as well as pancreatic apoptosis, supporting the use of black soybean as a potential anti-diabetic treatment [36].

A few studies have also reported that black soy peptides possess anti-

obesity and hypolipidemic actions [35, 39, 41]. Kim et al. (2007) showed that peptides derived from black soybean hydrolysate could inhibit adipogenesis in an in vitro model [35], suggesting a potential anti-obesity effect through control of adiposity. Similarly, Jang et al. (2008) reported that novel peptide mixtures derived from black soybean inhibited diet-induced obesity in mice by activating leptin-like signaling and AMP-dependent protein kinase (AMPK) [39]. In addition, recent studies have suggested that black soy peptide may activate the insulin-signaling pathway and improve the endoplasmic reticulum stress, making it a potential therapeutic peptide for type 2 diabetes [40].

In our study, we additionally subdivided study subjects according to the glucose levels <110 mg/dL or above and performed the paired t-test and independent t-test. We found the significant differences in subjects with the glucose ≥ 110 mg/dL before and after the intervention. [glucose : 0 wk ; 126.6 ±2.92 mg/dL, 12 wks ; 121.7±4.68 mg/dL, p=0.098 by two tail-test / p=0.049 by one tail-test / 2hr PG: 0 wk ; 243.1±20.44 mg/dL, 12 wks ; 201.9±17.45 mg/dL, p=0.012 by two tail-test / p=0.006 by one tail-test]

The present randomized, controlled trial conducted in Korean adults demonstrated that black soy peptide supplement may reduce fasting glucose levels and 2-h postload glucose levels, particularly in subjects with fasting glucose ≥ 110 mg/dL. However, HbA1c levels were not significantly

changed by the dietary intervention in the present study. Larger clinical studies with subjects who have high fasting glucose levels are needed to confirm the beneficial effects of black soy peptide on glucose and related biomarkers.

References

[1] Setchell KDR, Cassidy A: Dietary isoflavones: Biological effects and relevance to human health. J Nutr 1999; 129:758-767.

[2] Raquel Villegas, Yu-Tang Gao, Gong Yang, Hong-Lan Li, Tom A Elasy, Wei Zheng, and Xiao Ou Shu: Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai women's health study, Am H Clin Nutr. 2008; 87:162-7.

[3] Ministry of Health and Welfare: The fourth Korea National Health and Nutrition Examination Survey (KNHANES Ⅳ), Korea, 2007, pp256-257.

[4] Yki-Jarvinen H: Glucose toxicity. Endocr Rev 1992; 13:415-431.

[5] Makoto T, Kimko I, Hideyuki E, Takeo K, Hideo M, Akira S: Impaired Glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose, Disease Care, Vol.22, No.6, June 1999.

[6] Claudi T, Midthjell K, Holmen J, Fougner K, Kruger O, Wiseth R: Cardiovascular disease and risk factors in persons with type 2 diabetes diagnosed in a large population screening: the Nord-Trondelag Diabetes Study, Norway. J Intern Med 2000; 248(6):492-500.

[7] Midthjell K, Kruger O, Holmen J, Tverdal A, Claudi T, Bjorndal A, Magnus P: Rapid changes in the prevalence obesity and known diabetes in an adult Norwegian population: the Nord-Trongdelag Health Surveys: 1984-1986 and 1995-1997. Diabetes care 1999; 22:1813-1820.

[8] Schulze MB, Liu S, Rimm EB, Manson JE, Willett WC, Hu FB: Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. Am J Clin Nutr 2004; 80:348-356.

[9] Beulens JW, de Bruijne LM, Stolk RP, Peeter PH, Bots ML, Grobbee DE, van der Schouw YT: High dietary glycemic load and glycemic index increase risk of cardiovascular disease among middle-aged women: a population-based follow-up study. J Am Coll Cardiol 2007; 50:14-21.

[10] Cho Y, Park S, Shin HJ, Jang KH, Kang SA, Choue RY: Comparative estrogenic effect of Yak-kong and Soy bean on the proliferation of human osteoblastic cell line, MG63. Kor J Nutr 2001; 34:905-911.

[11] Erdman jr JW: Control of serum lipids with soy protein. New Engl J Med 1995; 333:313-315.

[12] Friedman M, Brandon DL: Nutritional and health benefits of soy proteins. J Agric Food Chem 2001; 49:1069-1086.

[13] Hermansen K, Sondergaard M, Hoie L, Carstensen M, Brock B: Beneficial effects of soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. Diabetes Care 2001; 24:228-233.

[14] Merritt JC: Matabolic Syndrome: soybean foods and serum lipids. J Natl Med Assoc 2004; 96:1032-1041.

[15] Blair RM, Henley EC, Tabor A: Soy foods have low glycemic and insulin response indices in normal weight subjects. Nutr J 2006; 5(35):1-10.

[16] Anderson JW, Smith BM, Washnock CS. Cardiovascular and renal benefits of dry bean and soybean intake. Am J Clin Nutr 1999; 70: 464-474.

[17] Messina MJ. Legumes and soybean: overview of their nutritional profiles and health effects. Am J Clin Nutr 1999; 70: 439-450.

[18] Liu KS. Chemistry and nutritional value of soybean components. In Soybeans: Chemistry, Technology and Utilization. Aspen Publ. Inc.: Gaithersberg, Maryland, USA, 1999; pp.25-113.

[19] Messina MJ. Soybeanfoods: their role in disease prevention and

treatment. In Soybeans: Chemistry, Technology and Utilization. Chapman and Hall: New York, USA, 1997; pp. 442-447.

[20] I. Mateos-Aparicio, A. Redondo Cuenca, M. J. Villanueva-Suarez and M. A. Zapata-Revilla: Soybean, a promising health source. Nutr Hosp. 2008; 23:305-312.

[21] Bhathena SJ, Velasquez MT. Beneficial role of dietary phytoestrogens in obesity and diabetes: American Journal of Clinical Nutrition. 2002; 76:1191-1201.

[22] Anderson JW, Johnstone BM, Cook-Newell ME: Meta-analysis of the effects of soy protein intake on serum lipids. New England Journal of Medicine, 1995; 333:276-282.

[23] Reynolds K, Chin A, Lees KA, Nguyen A, Bujnowski D, He J: A meta-analysis of the effect of soy protein supplementation on serum lipids. American Journal of Cardiology, 2006; 98:633-640.

[24] Trujillo J. Ramirez V, Perez J, Torre-Villalvazo I, Torres N, Tovar AR, Munoz RM, Uribe N, Gamba G, Bobadilla NA: Renal protection by a soy diet in obese Zucker rats is associated with restoration of nitric oxide generation. American Journal of Physiology - Renal Physiology 2005; 288; F108-F116.

[25] Sanches A, Hubbard RW. Plasma amino acids and the insulin/glucagons ratio as an explanation for the dietary protein modulation of atherosclerosis. Med Hypoth 1991; 36: 27-32.

[26] Howe JC. Postprandial response of calcium metabolism in postmenopausal women to meals varying in protein level/source. Metabolism 1990; 39: 1246-52.

[27] Beynen AC, West CE, Spaaij CJ, et al. Cholesterol metabolism, digestion rates and postprandial changes in serum of swine fed purified diets containing either casein or soybean protein. J Nutr 1990; 120: 422-30.

[28] Wagner JD, Cefalu WT, Anthony MS, et al. Dietary soy protein and estrogen replacement therapy improve cardiovascular risk factors and

decrease aortic cholesteryl ester content in ovariectomized cynomolgous monkeys. Metabolism 1997; 46: 698-705.

[29] Cheng, S.Y., Shaw, N.S., Tsai, K.S., Chen, C.Y., 2004. The hypoglycemic effects of soy isoflavones on postmenopausal women. J. Women Health (Larchmt) 13, 1080-1086.

[30] Cederroth CR, Vinciguerra M, Gjinovci A, Kuhne F, Klein M, Cederroth M Caile D, Suter M, Neumann D, James RW, Doerge DR, Wallimann T, Meda P, Foti M, Rohner Jeanrenaud F, Vassalli JD, Nef S: Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism. Diatetes. 2008; 57:1171-1185.

[31] Crouse Ⅲ JR, Morgan T, Terry JG, Ellis H, Vitolins M, Burke GL: A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Archives of Intenal Medicine, 1999; 159:2070-2076.

[32] L Azadbakht, R ShakerHosseini, S Atabak, M Jamshidian, Y Mehrabi and A Esmaill-Zadeh. Beneficiary effect of dietary soy protein on lowering plasma levels of lipid and improving kidney function in type Ⅱ diabetes with nephropathy. European J. Clin Nutr, 2003, 57, 1292-1294.

[33] Anderson JW, Blake JE & Smith BM (1998); Effects of soy protein on renal function and proteinuria in patients with type 2 diabetes. Am. J. Clin. Nutr. 68(Suppl), 1347s-1353s.

[34] Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF & Erdman JW (1998) : Soy protein and isoflavones. Am. J. Clin. Nutr. 68, 1375s.

[35] Hyun Jeong Kim, In Young Bae, Chang-won Ahn, Suyong Lee and Hyeon Gyu Lee: Purification and identification of adipogenesis inhibitory peptide from black soybean protein hydrolysate. Peptides 28, 2007; 2098-2103.

[36] Irina tsoy Nizamutdinova, Yong Chun J, Jong Il C, Sung Chul S, Sung Joong L, Han Geuk S, Jae Heun L, Ki Churl C and Hye Hung K: The

anti-diabetic effect of anthocyanins in streptozotocin-induced diabetic rats through glucose transporter 4 regulation and prevention of insulin resistance and pancreatic apoptosis. Mol. Nutr. Food Res. 2009, 53, 1419-1429.

[37] Kim, H. J., Tsoy, I., Park, J. M., Chung, J. K. et al., Anthocyanins from soybean seed coat inhibit the expression of TNF-α-induced genes associated with iischemia/reperfusion in endothelial cell by NF-κB-dependent pathway and reduce rat myocardial damages incurred by ischemia and reperfusion *in vivo.* FEBS *Lett.* 2006, 580, 1391-1397.

[38] Tsoy, K., Park, H. B., Kim, Y. M., Chung, J. I. et al., Anthocyanins from black soybean seed coats inhibit UVB-induced inflammatory cylooxygenase-2 gene expression and PGE2 production through regulation of the nuclear factor-KappaB and phosphatidylinositol 3-kinase/Atk pathway. J. Agric. Food Chem. 2008, 56, 8969-8974.

[39] E-H Jang, J-S Moon, JH Ko, C-W Ahn, H-H Lee, J-K shin, C-S Park and J-H Kang: Novel black soy peptides with antiobesity effects: activation of leptin-like signaling and AMP-activated protein kinase. International Journal of Obesity, 2008; 32:1161-1170.

[40] E-H Jang, Jeong Hyeon Ko, Chang-Won Ahn, Ho-Hyun Lee, Jae-Kil Shin, Seong-Jun Chang, Chang-Shin Park, Ju-Hee Kang. In vivo and in vitro application of black soybean peptides in the amelioration of endoplasmic reticulum stress and improvement of insulin resistance. Life Sciences 2010; 86: 267-274

[41] Shin Joung Rho, Soojin Park, Chang-Won Ahn, Jae-Kil Shin and Hyeon Gyu Lee: Dietetic and hypocholesterolaemic action of black soy peptide in dietary obese rat. J sci food and Agric, 2007; 87:908-913.

[42] Hyong Joo Lee: Health functional peptides from soybean foods. Korea Soybean Digest, 1998; 15(1):16-22.

[43] J.S. Shim, K.W. Oh and I. Suh et al., A study on validity of a 299 semiquantitative food frequency questionnaire of Korean adults, Korean J Community Nutr7 (2002), pp. 484–494.

[44] Christian JL, Greger JH: Nutrition for Living. San Francisco, The Benjamin/Cummings Publ Comp Inc, 1991, p111.

[45] American Dietetic Association: Handbook of Clinical Dietetics, 2nd ed, New Haven, Yale University Press, 1992, pp5-39.

[46] Glümer C, Jørgensen T, Borch-Johnsen K: Prevalences of diabetes and impaired glucose regulation in a Danish population: the Inter99 study. Diabetes Care 2003; 26(8):2335-2340.

[47] King H, Aubert RE, Herman WH: Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabeties Care 1998; 21: 1414-1431.

[48] Tsai AC, Vinik AI, Lasichak A, Lo GS. Effects of soy polysaccharide on postprandial glucose, insulin, glucagon, pancreatic polypeptide, somatostatin, and triglyceride in obese diabetic patients. Am J Clin Nutr 1987; 45: 596–601.

[49] Daniel R. Christie, MD; Jan Grant, RN; Betty E. Darnell, MS; Victoria R. Chapman, MPH; Amalia Gastaldelli, PhD; Cynthia K. Sites, MD, Metabolic effects of soy supplementation in postmenopausal Caucasian and African American women: a randomized, placebo-controlled trial. Am J Obstet Gynecol 2010; 202: xx.

[50] Colleen P. Gobert, Elizabeth A. Pipe, Sarah E. Capes, Gerarda A. Darlington, Johanna W. Lampe and Alison M. Duncan: Soya protein does not affect glycaemic control in adults with type 2 diabetes. British Journal of Nutrition 2010, 103, 412-421.

[51] Taniguchi A, Yamanaka-Okumura H, Nishida Y, Yamamoto H, Taketani Y, Takeda E.: Natto and viscous vegetables in a Japanese style meal suppress postprandial glucose and insulin responses. Asia Pac J Clin Nutr 2008; 17(4): 663-668.

[52] Fujita H, Yamagami T, Ohshima K: Long-term ingestion of a fermented soybean-derived Touchi-extract with alpha-glucosidase inhibitory activity is safe and effective in humans with borderline and

mild type-2 diabetes. J Nutr 2001; 131(8): 2105-8

[53] Zhao-min Liu, Yu-ming Chen, Suzanne C Ho, Yee Ping Ho, and Jean Woo: Effects of soy protein and isoflavones on glycemic control and insulin sensitivity: a 6-mo double-blind, randomized, placebo-controlled trial in postmenopausal Chinese women with prediabetes or untreated early diabetes. Am J Clin Nutr 2010; 91: 1394-401.

[54] Kannel WB, Castelli WP, Gordon T: Cholesterol in the prediction of atherosclerotic disease: new perspectives based on the Framingham Study. Ann Intern Med., 1979, 90: 85-91.

[55] Arntzenius AC, Kromhout D, Barth JD, Reiber JH, Bruschke AV, Buis B, van Gent CM, Kempen-Voogd N, Strikwerda S, vander Velde EA: Diet, lipoproteins, and the progression of coronary atherosclerosis: the Leiden intervention Trial. N Engl J Med., 1985, 312: 805-811.

[56] Lamarche B, Moorjani S, Lupien PJ, Catin B, Bernard PM, Dagenais GR, Despres JP: Apolipoprotein A-1 and B levels and the risk of ischemic heart disease during a five-year follow-up of men in the Quebec cardiovascular study. Circulation, 1996, 94: 273-278.

[57] Martin J. Ronis, Ying Chen, Jamie Badeaux, and Thomas M. Badger. Dietary soy protein isolate attenuates metabolic syndrome in rats via effects on PPAR, LXR, and SREBP signaling. J. Nutr. 2009, 139:1431-1438.

국문 요약

당뇨 전 단계 또는 초기 제 2형 당뇨군에서 검정콩 펩타이드 섭취 시 혈당에 미치는 영향

당뇨병은 흔한 내분비계 장애로서 전세계적으로 대두되는 만성질환으로 알려져 있다. 공복혈당장애와 내당능장애는 당뇨병으로 진행될 가능성이 높은 당뇨병 전기로서 이 시기에 혈당 조절을 통한 예방이 중요하다고 할 수 있다. 대두는 혈당지수가 낮은 식품으로서 대두에 풍부하게 함유된 식이섬유는 혈당과 인슐린 저항성을 개선시키는 것으로 알려져 있다.

본 연구에서는 한국인 18—69세에서 당뇨 전 단계이거나 혈당 강하제를 복용하지 않는 초기 제 2형 당뇨군 55명을 대상으로 무작위 배정, 이중맹검, 플라시보 대조군 인체 시험을 실시하여,

검은콩 펩타이드 섭취 시 (12주간 4.5g/day) 혈당 조절 및 지질대사에 미치는 영향을 확인하였다.

나이, 체중, 체질량지수, 허리-엉덩이 둘레, 제지방량, 수축기 혈압, 이완기 혈압과 같은 인체계측치에서 두 그룹간에는 유의적인 차이는 없었다. 0주와 12주 후에서 식이섭취 변화에서도 유의적인 차이가 없었다.

0주 공복혈당 수치가 110mg/dL 이상인 대상자에서 검은콩 펩타이드 섭취 시 공복혈당이 감소하는 경향이 있었으며(-4.88 ± 2.79 mg/dL, 양측검정; p = 0.098 / 단측검정; p = 0.049) 식후 2시간 혈당은 유의적으로 감소하였다(0주: 243.13 ± 20.44 mg/dL, 12주 후: 201.88 ± 17.45 mg/dL, p = 0.012 / p = 0.006). 군간 비교 시, 식후 2시간 혈당 변화 값은 검은콩 펩타이드 섭취군과 위약군 간에 유의적인 차이가 있었다(실험군: -41.25 ± 13.67mg/dL, 위약군: 12.42 ± 9.80 mg/dL; p = 0.015 / p = 0.008). 반면, 당화혈색소와 인슐린, 유리지방산은 유의적인 차이가 없었다.

지질 농도 관련해서는 검은콩 펩타이드 섭취 시 아포지단백질 B농도가 감소하는 경향(p = 0.054 / p = 0.027)을 보였다.

결론적으로 4.5g/day 검정콩 펩타이드 섭취는 공복혈당이

110mg/dL 이상인 대상자에서 식후 2시간 혈당 감소에 도움을 줄 수 있을 것으로 사료된다.

핵심 되는 말: 당뇨 전 단계, 초기 제 2형 당뇨, 검은콩 펩타이드, 혈당 조절, 아포지단백질 B