Research Article Open Access

Intake of Mung Bean Protein Isolate Reduces Plasma Triglyceride Level in Rats

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Submission date: August 19, 2013; Acceptance date: September 29, 2013; Publication date: September 30, 2013

ABSTRACT

Background: Mung bean is well known as a starch source, but the physiological effects of mung bean protein have received little attention. In this study, we isolated mung bean protein from de-starched mung bean solutions, and investigated its influence on lipid metabolism.

Objective: The aim of this study is to clarify the influence of the lipid metabolism by consumption of mung bean protein isolate (MPI)

Methods: Diets containing either mung bean protein isolate (MPI) or casein were fed to normal rats for 28 days.

Results: Both groups ate the same amount of food, but the plasma triglyceride level, relative liver weight and liver lipid contents (cholesterol and triglyceride pool) in the MPI group were significantly lower than in the casein group. In the MPI group, the expression of sterol regulatory-element binding factor 1 (SREBF1) mRNA in the liver was significantly different when compared with the casein group. The significantly lower levels of insulin and free fatty acids in the MPI-fed rats may be due to the regulation of genes related to lipid metabolism in the

liver.

Conclusions: These results suggest that MPI may improve the plasma lipid profile by normalizing insulin sensitivity.

Keywords: mung bean, *Vigna radiata* L., 8S globulin, triglyceride, β-conglycinin, 7S globulin, insulin sensitivity, SREBF1

BACKGROUND:

Mung bean [*Vigna radiata* (L.) Wilczek] has been eaten since ancient times. In Asia, people consume it as a whole snack, as bean sprouts, or as noodles made from bean starch (harusame). The mung bean consists of approximately 60% carbohydrate by weight (starch), 25% protein, 1.5% lipids, and other minor components.

INTRODUCTION:

Previous studies of mung bean examined the physiological response to consumption of native starch or the flavonoids of the legume [1-5]. Kabir et al. reported that low glycemic index starch prepared from mung beans lowered lipogenesis when compared with high glycemic index starch (waxy cornstarch). This was attributed to the suppression of fatty acid synthase (FAS) expression in the liver of mice on a mung bean starch diet [1]. Cao and colleagues studied the relationship between the mung bean seed coat and protection against heat stress. Both vitexin and isovitexin, which are components of mung bean seed coat extract, remarkably reduced oxidative stress and increased glutathione levels [4].

Few studies have examined the relationship between the prevention of metabolic syndrome and the consumption of whole beans, sprouts, or mung bean extract [6-9]. Ethanol extracts from mung bean sprouts or seed coats showed a remarkable improvement of glucose tolerance in type 2 diabetic mice [9]. These findings suggest that consumption of starch or seed coats derived from mung beans may contribute to the prevention of metabolic disorders.

Recently, mung bean protein was extracted and isolated by a manufacturing process, but its physiological effects were not examined. Therefore, we set out in this study to determine whether mung bean protein isolate (MPI) could improve physiological parameters, such as elevated plasma lipid profiles, that are associated with metabolic disease.

MATERIALS AND METHODS:

Animals and Diets. The treatment of all the animals in this study followed the guidelines established by the Japanese Society for Nutrition and Food Science (Law No. 105 and Notification No. 6 of the Japanese government). The control group received vitamin-free casein (containing 88.1% crude protein, Oriental Yeast Co., Tokyo, Japan) and the experimental group received MPI (Glucodia^{TM}, 85.5% crude protein, Fuji Oil Co., Osaka, Japan). The amino acid composition (g/100 g protein) of MPI is as follows: Arg: 7.44, Lys: 7.22, His: 3.09, Phe: 6.74, Tyr: 3.28, Leu: 8.38, Ile: 4.26, Met: 1.38, Val: 5.19, Ala: 4.21, Gly: 3.53, Pro: 4.62, Glu+Gln: 18.59, Ser: 5.59, Thr: 3.24, Asp+Asn: 12.22, Trp: 0.99, Cys: 0.45. Experimental diets, which contained 20% of casein protein or MPI as a crude protein, were based on the AIN-93G formula as shown in Table 1 [10].

Table 1. Experimental Diets.

	Group	
g/100g diet	Casein	MPI
Ingredients		
Casein [#1]	22.7	-
Mungbean protein isolate [#2]	-	23.4
Corn starch	37.3	36.7
Dextrized corn starch	13.2	13.2
Sucrose	10.0	10.0
Soybean oil [#3]	7.0	7.0
Cellulose powder	5.0	5.0
Mineral mixture [#4]	3.5	3.5
Vitamin mixture [#5]	1.0	1.0
Choline bitartrate	0.25	0.25
Total	100	100

[#1]; Crude protein content is 88.1% as is.

[#2]; Crude protein content is 85.5% as is.

[#3]; Soybean oil contains 0.02w/w% *tert*-butylhydroquinone.

[#4]; AIN-93G-MX composition.

[#5]; AIN-93 VX composition.

Ten specific 6-week-old pathogen-free male Wistar rats, were purchased from Japan Crea (Tokyo, Japan). All the rats were housed individually in stainless steel cages under controlled conditions (temperature, 23 ± 1°C; humidity, 55 ± 5%; light, 0800–2000 h). After they were acclimated to commercial food (CRF-1, Oriental Yeast Co.) for 5 d, the rats were divided into two groups with similar average body weights and fasting blood glucose levels. Fasting blood samples were collected from the rats' tails. Blood glucose concentrations were measured using the Free Style Glucose Sensor (Nipro, Osaka, Japan).

Methods. Experimental diets and water were given *ad libitum* for 4 weeks. Food intake and body weight were recorded every day. At the end of the test period, after 6 h of food deprivation (0800–1400), arterial blood was withdrawn from the abdominal aorta into a heparinized syringe under isoflurane anesthesia. After the rats were sacrificed, several tissue samples, such as the liver and visceral fat pad (including the perirenal, epididymal, and mesenteric fat pads), were harvested, weighed, and stored in a deep freezer at -80°C.

Blood analysis. Plasma was separated by centrifugation (1,900 × *g*, 15 minutes, 4°C) and stored at 4°C until analysis. Insulin concentrations were measured using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (the Ultra-high Sensitivity Rat Insulin ELISA kit, Morinaga, Yokohama, Japan). Adiponectin concentrations were measured using an ELISA kit (the mouse/rat adiponectin ELISA kit, Otsuka Pharmaceutical Co., Tokyo, Japan). Other plasma characteristics were measured using a Fuji DRI-CHEM 7000V auto-analyzer (Fuji Film Co., Tokyo, Japan).

Liver lipid analysis. Lipids were extracted using a chloroform/methanol mixture (2:1, v/v) according to the method described by Folch et al. [11]. Liver triglyceride was separated from phospholipid with silicic acid and measured using the acetyl-acetone method described by Fletcher [12]. Liver total cholesterol was measured by using a cholesterol-digitonin reaction according to the method of Sperry and Webb [13]. Liver phospholipid was determined by measurement of inorganic phosphate as described by Feldman et al. [14].

Gene expression analysis. Liver total RNA samples were isolated using ISOGEN (NipponGene, Tokyo, Japan). Total RNA was reverse-transcribed into cDNA using the PrimeScript^{TM} RT reagent kit (Takara Bio, Tokyo, Japan). Real-time semi-quantitative reverse

transcription-polymerase chain reaction (RT-PCR) was performed using 25 ng of cDNA and 50 pM of Taqman probe primers (Taqman probe primers, Applied Biosystems, Foster City, CA) in an ABI PRISM 7300 sequence detection system (Applied Biosystems). These samples were denatured at 95°C for 10 min, followed by 40 PCR cycles. Each cycle consisted of treatment at 95°C for 30 s, 60°C for 30 s, and 72°C for 20 s. The following rat gene-specific Taqman primers were used: β-actin (Actb, Rn00667869) as housekeeping gene, liver X receptor (LXR, nuclear receptor subfamily 1- group H- member 3, Rn00581185), and sterol regulatory element binding transcription factor 1 (Srebf1, Rn01495769). After confirming that the amplification efficiency of all primer sets was similar, expression levels were calculated using the ΔΔCt semi-quantitation method according to the manufacturer's procedure, and relative comparison between the groups was then performed.

Statistis. Data (n = 5) are shown as the mean ± standard error of the mean (SEM). Statistical analysis was performed using the SPSS software (12.0J, SPSS, IBM Corporation, Armonk, NY). The results were analyzed using the Student's *t*-test ($p < 0.05$) to compare groups.

RESULTS:

Daily food intake was the same in the casein-fed rats and the MPI-fed rats, as shown in Table 2. Final body weights were significantly different in the MPI group when compared to those in the casein group

Plasma triglyceride was significantly lower in the MPI-fed group, but other lipid profiles, such as cholesterol, were similar in both groups. Protein metabolic markers such as total protein, albumin, and blood urea nitrogen were significantly different between the two groups, but other liver metabolic markers (AST, ALT) were not changed. The plasma adiponectin level was significantly higher in the MPI group than in the casein group. Relative liver weight (g/100g of body weight) was significantly lower in the MPI-fed rats compared with the casein-fed rats. But, visceral fat pad weights in the MPI-fed group were higher than that in the casein-fed group. Liver triglyceride and cholesterol contents in the MPI group were significantly lower than the casein group

Table-2. Growth and physiological data in the casein group and the MPI group.

		Group					
		Casein			MPI		
n		5			5		
Body weight and food intake							
Initial body weight	(g)	141.3	±	1.6	141.1	±	1.9
Food intake	(g/d)	19.0	±	0.4	16.8	±	1.2
Final body weight	(g)	321.4	±	8.7	271.1	±	15.8 *
Characteristics of plasma							
Triglyceride	(mg/dL)	175.1	±	14.9	93.4	±	7.2 *
Total cholesterol	(mg/dL)	71.6	±	5.3	70.2	±	2.94
HDL-cholesterol [#1]	(mg/dL)	45.6	±	5.0	48.8	±	1.80
Glucose	(mg/dL)	87.6	±	5.4	86.8	±	3.1
Total protein	(g/dL)	5.22	±	0.02	4.74	±	0.06 *
Albumin	(g/dL)	3.40	±	0.00	3.14	±	0.07 *
BUN [#2]	(mg/dL)	14.8	±	0.4	19.6	±	1.7 *
AST [#3]	(U/dL)	53.6	±	1.9	61.2	±	2.8
ALT [#4]	(U/dL)	14.8	±	1.6	20.6	±	2.1
Adiponectin	(μg/mL)	2.80	±	0.12	10.83	±	0.71 *
Tissue weights [#5]							
Liver	(g/100gBW)	3.76	±	0.05	3.41	±	0.08 *
Perirenal fat pad	(g/100gBW)	2.03	±	0.20	2.69	±	0.19 *
Epididymal fat pad	(g/100gBW)	1.32	±	0.08	1.39	±	0.11
Mesenteric fat pad	(g/100gBW)	0.90	±	0.06	1.20	±	0.08 *
Liver lipid profiles							
Triglyceride	(mg/gliver)	14.82	±	0.84	7.34	±	1.10 *
Cholesterol	(mg/gliver)	1.77	±	0.05	1.57	±	0.06 *
Phospholipid	(mg/gliver)	37.7	±	0.37	35.5	±	0.88

Values are means ± SE (n=5). [#1]; High-density lipoprotein, [#2]; Blood urea nitrogen, [#3]; Aspartate aminotransferase, [#4]; Alanine aminotransferase, [#5;] values represent g tissue/100g body weight. *; statistical analysis was performed using Student's *t*-test ($p<0.05$).

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Liver triglyceride levels are controlled by several factors, such as fasting, insulin levels, FFA levels, and LXR as shown in Figure 1A. These factors controlled lipid metabolism-related gene expressions via SREBF1. The Srebf1 mRNA level in the MPI group was significantly lower than in the casein-fed group (Figure 1B). LXR mRNA was the same in both groups (Figure 1C). Although the plasma glucose level was the same in both groups, plasma insulin was significantly

lower in the MPI-fed rats as compared with that in the casein-fed rats (Figure 1D). Plasma FFA levels were also significantly lower in the MPI-fed rats (Figure 1E).

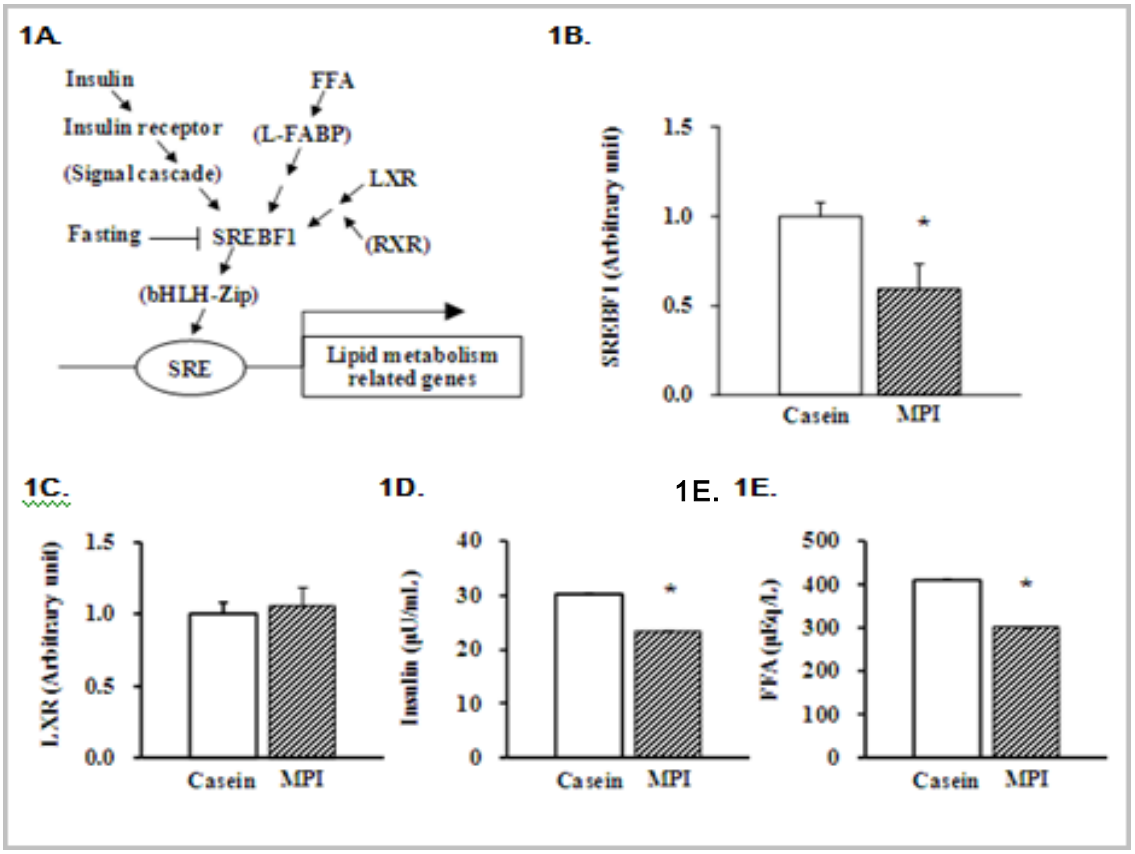

Figure 1. Various factors such as fasting, insulin level, free fatty acids (FFA) and liver X receptors (LXR) regulate the expression of genes related to lipid metabolism (Fig. 1A). These factors influence the activation of sterol regulatory-element binding factor 1 (SREBF1). If SREBF1 is activated, a bHLH-Zip (Basic helix-loop-helix leucine zipper) is cleaved from SREBP1. The bHLH-Zip binds to promoter regions (sterol regulatory element; SRE) and activates the expression of lipid metabolism-related genes. Fig. 1B and 1C showed the gene expressions of Srebf1 and LXR, respectively. Fig. 1D and 1E showed the plasma insulin level and plasma FFA level, respectively. Open columns and striped columns represent the casein-fed rats and MPI-fed rats, respectively. * showed a significant difference ($p<0.05$) using Student's *t*-test.

DISCUSSION:

Obesity is a serious problem because it is linked to diabetes, cardiovascular disease, and metabolic syndrome [15-17]. The World Health Organization has announced that many people are at critical risk of metabolic disorders such as obesity, and has recommended that proper

exercise habits and nutritionally balanced diets be incorporated into people's daily lives [18].

Mung bean is a traditional food in Asia, especially in China. In general, the high starch content of the mung bean is utilized in the form of noodles, but the protein itself is not used for food. The aim of this study was to examine the physiological changes that occur upon consumption of MPI, and to compare these changes with those induced by the animal protein, casein. There are several differences in amino acid composition between MPI and casein. Specifically, Arg, Gly, Asp/Asn and Pro in MPI are 2.3-, 2.2-, 1.9- and 0.5-fold the level found in casein. Our research suggests that the different amino acid composition of these two proteins in part explains their ability to significantly influence blood lipid profiles.

MPI consumption significantly reduced plasma triglyceride level. In contrast, neither fermented nor non-fermented, water-soluble mung bean extract could produce this effect in alloxan-induced diabetic mice [19]. Yeap and colleges suggest that the ability of MPI to reduce triglycerides and improve glucose tolerance is due to its γ-aminobutyric acid (GABA) and free amino acid content. Similarly, ethanol-extract of various mung bean fractions (sprout and seed coat) may help reduce the plasma triglyceride level [9]. Although these data show that various fractions of mung bean can affect the plasma lipid profile, our research strongly indicates that mung bean protein itself may be a major trigger for this phenomenon.

Fatty liver may cause serious metabolic disorders and lead to death by cirrhosis [20]. With this in mind, it appears that MPI may exert beneficial effects on liver metabolism and function. This is since MPI-fed mice had significantly lower liver weight, which correlated with a small but significant reduction in liver cholesterol content. Furthermore, liver triglyceride level in the control group was twice as high as in the MPI-fed group. Plasma adiponectin was about 3-fold higher in the MPI group when compared with casein-fed mice. This may contribute to a decrease in liver fat content, since the physiological functions of adiponectin are stimulation of fatty acid oxidation and improvement of insulin sensitivity in the liver [21]. Thus, MPI may accelerate lipid degradation via an adiponectin signal cascade in the liver. Together, these data lead us to infer that MPI may become a "functional food" supplement that has hepatoprotective properties.

Many reports have demonstrated the triglyceride-lowering effects of consuming coffee extracts, tea polyphenols, and soy proteins [22-24]. β-conglycinin, which is a component of soy protein, lowers triglyceride levels and reduces obesity [25]. Mechanistically, this is thought to be due to the suppression of SREBF1, which regulates the expression of genes that perform fatty acid synthesis in the liver [24]. In addition, the β-conglycinin-fed group was also found to lower

the plasma insulin level. A similar effect was observed in the MPI-fed rats in this study. These findings suggest that MPI has triglyceride-lowering effects similar to β-conglycinin.

The similarities between MPI and β-conglycinin with regard to their physiological effects may in part be due to their similar amino acid compositions and sequences [26]. For example, a previous study showed that mung bean protein, which mainly consists of 8S globulins, has 68% sequence identity and structural similarity with soy 7S globulins (also called β-conglycinin). The correlation analysis of amino acid compositions between the MPI and commercial β-conglycinin protein is r = 0.981 (our unpublished data). Further investigations are needed to clarify whether consumption of soy 7S globulins and mung bean 8S globulins induce common pathways in order to bring about physiological changes.

CONCLUSIONS:

To our knowledge, this is the first report to determine that mung bean protein itself shows hypolipidemic effects using normal rats fed under an otherwise general diet. Induction of adiponectin and reduction of triglyceride synthesis via insulin signaling may play a key role in these effects. In order to evaluate the physiological effects of MPI prior to human studies, it will be necessary to determine the efficacy parameters in rat models (under high fat diet or high glucose diet conditions). In addition, a comparison of MPI and soy β-conglycinin may reveal the physiological consequences of triglyceride suppression.

Competing Interests:

The authors have no financial interests or conflicts of interest.

Authors' Contributions:

* and †; These authors contributed equally to this work.

Abbreviations:

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; bHLH-Zip, Basic helix-loop-helix leucine zipper; BUN, Blood urea nitrogen; ELISA, Enzyme-linked immunosorbent assay; FAS, Fatty acid synthase; FFA, Free fatty acid; GABA, γ- aminobutyric acid; HDL, high-density lipoprotein; L-FABP, Liver-Fatty acid binding protein; LXR, Liver X receptor; MPI, Mung bean protein isolate; RXR, Retinoid X receptor; SRE, Sterol regulatory-element; SREBF1, Sterol regulatory-element binding factor 1

Acknowledgements and Funding:

We thank Michiru Sakaue and Saori Nakao for animal maintenance. This study did not receive any grants.

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