Okara, a Soybean by-Product, Prevents High fat Diet-induced Obesity and Improves Serum Lipid Profiles in C57BL/6J Mice

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Received October 24, 2015
Revised January 13, 2016
Accepted January 16, 2016
Published online April 30, 2016

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pISSN 1226-7708
eISSN 2092-6456

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Abstract This study aimed to validate the anti-obesity effect of okara, a soyfood byproduct, in diet induced obese mice. Thirty two C57BL/6J male 4 week old mice were divided into four dietary groups ($n=8$) fed either normal diet (ND), high fat diet (HD), HD with 10% okara (OL), or HD with 20% okara (OH) for 12 weeks. Body weight gain and epididymal fat weight of OL and OH group were significantly lower than HD group. Similarly, the serum and hepatic lipid profiles in OH were significantly lower than HD group. The fecal triacylglycerol and TC levels in OL and OH increased compared to HD. Also, the expression of PPAR- α was higher in OH than HD group; PPAR- γ and FAS levels were lower in OH compared to HD. In this study, okara consumption appears to protect mice against diet induced obesity (DIO) and metabolic dysregulation related to obesity.

Keywords: soybean residue, anti-obesity, lipid profiles, dietary fiber, healthy promoting food

Introduction

Recently, changes in dietary habits and physical activity patterns have contributed to the increase in obesity, which is a risk factor for metabolic diseases, such as type 2 diabetes, insulin resistance, cardiovascular disease, stroke, and cancer (1,2). Consumption of diets high in fat is considered as a major etiological factor leading to obesity; whereas consumption of vegetables, fruits, and whole grains are known to lower the risk for obesity and metabolic disease (3). Dietary fiber intake from whole grains, vegetables, and fruits modulates parameters associated with the control of metabolic syndrome, like food intake, by increasing satiety and consequently improving glucose homeostasis, insulin sensitivity, glycemia, insulinemia, and blood lipids (4). A recent meta-analyses of prospective cohort studies report remarkably reduced diabetes risk in subjects consuming diets high in insoluble cereal dietary fiber and whole grains (5–7). Moreover, moderate weight loss due to low energy density and increased satiety has been reported to be comparable with both soluble and insoluble fiber (6,8,9).

Okara is the insoluble portion of the soybean which remains after the pureed soybeans are filtered during the production of bean curd, soymilk, and their derivatives (10). The production of every 1000 L of soy beverage can result in 250 kg of okara. Estimates based on soy milk consumption indicate that about 14 million tons of okara are annually generated worldwide (11). Dried okara contains about 50% fiber, 25% protein, 10% lipid, and other nutrients including isoflavones, lignans, phytosterols, etc. Moreover, these compounds have various

physiological and therapeutic functions, such as antioxidant activity, prevention of cardiovascular diseases, etc. (10), therefore, okara contains many valuable nutrients that might be useful for producing healthful food products. However, most of the okara produced is dumped as waste, but a small amount of it is used as animal feed, on account of its going stale rapidly, high cost of drying, and low digestibility, etc. (11,12).

Several studies on okara have been conducted to assess its functional ingredient composition and health-promoting attributes (10,13). *In vitro* experiments have indicated that okara has antioxidant activities (14). Another study evaluated the blood sugar, serum total cholesterol, and triglycerides of diabetic mice fed okara for 5 weeks, and this study showed that okara could markedly decrease plasma glucose and lipid concentrations, and protect liver and kidney of diabetic mice (10). Furthermore, Matsumoto *et al.* (15) reported that okara protects against obesity in ICR mice. Villanueva *et al.* (16) also conducted a study on the effect of soybean by-product on the lipid profiles in plasma, liver and feces of Syrian hamsters. However, those studies do not sufficiently explain the relationship between soybean by-product consumption and obesity. Therefore, the present study was designed to elucidate the effect of okara for managing diet induced obesity (DIO) in a rodent model. In order to assess the efficacy of okara as a functional food which prevents DIO we measured changes in body weight, food intake, serum and hepatic lipid profiles along with the changes in the expression of genes related to hepatic lipid metabolism.

Materials and Methods

Animals and experimental diets Four-week-old male C57BL/6J mice were purchased from Center Animal Experiment (Seoul, Korea). Animals were maintained on a pellet diet (Research Diets, New Brunswick, NJ, USA) for 1 week, and then randomly divided into four groups ($n=8$) such that the average weight in each groups was comparable. The mice were housed in a temperature controlled environment with a 12 h light/dark cycle and were fed their respective experimental diets and water: normal diet (ND), high fat diet (HD), high fat diet with okara (soybean by-products) 10% (OL), and high fat diet with okara 20% (OH) ad libitum for 12 weeks (15). Dried okara was purchased from Seounok Food Company (Jeonju, Korea). Its nutritional data was analyzed by Korean Dasan Institute of Life and Science (Daejeon, Korea; analysis number 140227-6). Modified #D12450B and #D12492 diets were used as ND and HD, respectively. The compositions of the experimental diets are shown in Table 1. The feed intake and body weight were measured every other day and weekly, respectively. The experimental protocol was approved by the Animal Care and Use Committee of Chonbuk National University (CBU 2014-00025).

Collection of serum and tissue samples The mice were deprived of feed for 12 h and anesthetized with diethyl ether. After sacrificing, their blood samples were collected, and serum was separated from the blood by centrifugation at 1,100 $\times$ g for 15 min at 4°C (Micro 17R; Vision Scientific Co., Seoul, Korea), and stored at –80°C until analyzed. Liver tissues and epididymal fat were dissected for analysis, weighed quickly, and then stored at –80°C until analyzed. Fecal samples were collected on the last day of the experiment and freeze-dried. Feces were ground to produce a homogeneous powder and stored at –20 °C until analysis.

Biochemical analyses Hepatic and serum triacylglycerol (TAG) and total cholesterol (TC) were analyzed by an enzymatic method using a commercial kit (Asan Pharmaceutical Co., Seoul, Korea). The serum concentration of HDL-cholesterol was measured using a commercial kit (Asan Pharmaceutical Co.). LDL cholesterol (low density lipoprotein-cholesterol) level in the serum was calculated by the method of Friedewald *et al.* (17). The serum insulin concentration was measured by enzymatic method using a commercial mouse insulin ELISA kit (Shibayagi, Japan). Leptin concentration in the serum was measured by an enzymatic method using a commercial kit, Quantikine Immunoassay kit (R&D system, USA).

The fecal TAG and TC were extracted via a chloroform/methanol solution (2:1, v/v) from the feces using the Bligh and Dyer method (18). Briefly, chloroform/methanol solution was added to the homogenized feces, vortexed and centrifuged; the lower phase was collected and evaporated at room temperature under a fume hood (DaihanLabtech Co., Ltd., Namyangju, Korea). The remaining semi dried pellet was dissolved in 1% Triton X 100 (Yakuri Pure Chemicals

Table 1. Ingredient composition of diets

Ingredient (g)	Normal diet ¹⁾	High fat diet ²⁾		
		HD	OL	OH
Protein (%)	19.2	20	18	13
Carbohydrate (%)	67.3	20	20	26
Fat (%)	4.3	60	62	61
Okara	-	-	10	20
Casein, lactic	18.96	25.84	22.74	26.70
L-Cystine	0.28	0.39	0.34	0.23
Corn starch	29.86	-	-	-
Maltodextrin	3.32	16.15	14.21	9.53
Sucrose	33.17	8.89	7.82	5.25
Cellulose	4.74	6.46	5.69	3.8
Soybean oil	2.37	3.23	2.84	4.91
Lard	1.90	31.66	30.47	25.68
Mineral mix	0.95	1.29	1.14	0.76
Dicalcium phosphate	1.23	1.68	1.48	0.99
Calcium carbonate	0.52	0.71	0.63	0.42
Potassium citrate	1.56	2.13	1.88	1.26
Vitamin mix	0.95	1.29	1.14	0.76
Choline bitartrate	0.19	0.26	0.23	0.15
FD&C Blue Dye #1	-	0.01	0.01	-
Total(g)	100	100	100	100
Kcal	385	524	506	487
Kcal/g	3.8	5.2	5.1	4.9

¹⁾ND; #D12450B Modified diet with 4% fat (10% fat calories) content.

²⁾high fat diet, #D12492 Modified high fat diet with 35% fat (60% fat calories) content; OL, HD with 10% of soybean by-product (okara); OH, HD with 20% of soybean by-product (okara)

Co., Ltd., Kyoto, Japan). The resulting solution was used to estimate fecal TAG and TC. The contents of TAG and TC were determined with assay kits, as described above for liver samples.

Quantitative real-time PCR For quantitative real-time PCR analysis, total RNA was extracted using trizol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and the concentration and the quality were measured spectrophotometrically at 260 and 280 nm. For the real-time PCR, 1 µg of extracted RNA was reverse transcribed into first-stand cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Then amplification was performed in a cDNA mixture on the 7500 Real time PCR system (Applied Biosystems) using SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacture's protocol. Relative quantification of gene expression with real-time PCR data was calculated relative to β -actin.

Statistical analysis Data are presented as means $\pm$ SD. All data were analyzed by one-way analysis of variance (ANOVA) using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). The differences among groups were assessed by Duncan's multiple range test. *P* values <0.05 were considered significant.

Results and Discussion

Obesity plays a crucial role in the development of disorders such as diabetes mellitus, hyperlipidemia, and hypertension (15,19). The soybean by-product, okara, is rich in compounds that can modulate the DIO. In this study we have shown the beneficial effects of okara intake on the prevention of DIO and modulation of lipid metabolism in a mouse model. We demonstrated that diets containing okara- which is rich in dietary fiber, prevents weight gain in high fat diet-induced obese mice. Earlier preliminary research using Japanese dried okara (10, 20, and 40%) supplemented high fat diets, demonstrated that it can prevent DIO (15). In the current study we used a similar composition of Korean okara (10 and 20%) combined with a high fat diet to explore the molecular mechanism involved.

In this study, the feed intake was not significantly different among ND, HD, OL, and OH-fed mice, but the energy intake in ND-fed mice was significantly lower than the other three groups ($p<0.05$).

Initial body weights were not significantly different among four groups; however, OL and OH fed mice had significantly lower final body weights and weight gain compared to HD-fed mice in a dose-dependent manner ($p<0.0001$). Matsumoto *et al.* (15) reported significantly lower body weight of mice fed with 20 and 40% okara to the basal diet for 10 weeks significantly was lower than that of mice fed the basal diet, indicating a preventive effect in the obesity. Also, Antonio *et al.* (13) and Préstamo *et al.* (20) observed a decrease in body weight gain from the second week in rats fed okara, compared to the control animals. As mentioned above, both soluble and insoluble fiber lead to moderate weight loss due to low energy density and increased satiety (6,8,9). Moreover, FER (food efficiency ratio) was also significantly lower in OL and OH-fed mice than HD-fed mice ($p<0.0001$) (Table 2). FER was obtained by calculating the ratio between body weight gain (g) during the experimental period and total feed consumed (g) during the entire experimental period. In this study, weight gain of OL and OH groups was significantly lower compared to HD group, although the feed intake in four groups was not significant, thus, indicating a lower FER in OH and OL group compared to the HD group.

In addition, liver and epididymal fat weights in OL- and OH-fed

mice were significantly lower compared to HD-fed mice ($p<0.0001$). Likewise, the epididymal fat weight to body weight ratio in OH-fed mice was remarkably lower than HD-fed mice ($p<0.0001$) (Fig. 1). Fat accumulation has been correlated with the body weight and body weight gain (21,22), thus, it can be assumed that okara suppressed body weight gain by suppressing increases in adipose tissue mass.

The lipid profiles in serum, liver and feces are shown in Table 3. The serum TAG levels in the supplemented groups (OL and OH) were remarkably and dose-dependently lower compared to HD-fed mice with ($p<0.01$). Moreover, OL- and OH-fed mice had lower TC levels as well as LDL cholesterol levels in the serum than HD-fed mice ($p<0.001$). However, HDL cholesterol level was not significantly different among four groups. Our data is similar to that of Kim *et al.* (23), showing lipid lowering effect of fermented soybean fibers; however, the okara used in our study was not fermented indicating that the dietary fiber and phytochemical in okara are beneficial. The groups fed with okara had remarkably lower hepatic TAG levels than the HD-fed mice ($p<0.05$). Meanwhile, the TC levels in OL- and OH-fed mice were not significantly different compared to HD-fed mice. Likewise, Choi and Jo (24) reported that isoflavones in soybean had beneficial effects on serum cholesterol and non-HDL cholesterol in growing female rats; however, it didn't affect body weight gain as well as hepatic cholesterol.

The TAG and TC in fecal excretions of OL- and OH-fed mice were significantly higher compared to HD-fed mice. The fecal TAG levels in the okara supplemented groups increased in a dose-dependent manner ($p<0.0001$). Diets enriched in dietary fibers (oat bran, barley, wheat germ, etc) increase fecal fat excretion in humans and experimental animals (25). Besides, Pande and Srinivasan (4) reported that cluster beans, as a source of soluble dietary fiber, has the potential to improve hypercholesterolemia through increasing fat excretion and regulating serum lipids. It suggests that okara may exert positive effects on serum lipid metabolism by increasing the fat excretion and decreasing serum lipids that may cause coronary heart disease (CHD).

As circulating serum leptin is positively correlated to visceral adiposity, it is an ideal indicator for assessing obesity not only humans, but also experimental animals (22,26,27). Furthermore, it

Table 2. Body weight and body composition

Groups	ND ¹⁾	HD	OL	OH
Feed intake (g/day)	2.12±0.04	1.90±0.13	2.04±0.06	2.04±0.03
Energy intake(kcal/day)	7.78±0.16 ^b	9.55±0.69 ^{a2)}	10.02±0.30 ^a	9.66±0.12 ^a
Initial body weight (g)	20.36±1.17	20.10±0.98	20.80±0.86	21.00±1.18
Final body weight (g)	25.59±2.15 ^c	37.46±4.27 ^a	32.69±4.84 ^b	31.09±1.20 ^b
Weight gain (g/12wks)	5.23±2.18 ^d	17.21±3.70 ^a	13.47±2.17 ^b	10.12±1.24 ^c
FER ³⁾	0.03±0.01 ^d	0.10±0.02 ^a	0.08±0.01 ^b	0.06±0.01 ^c

¹⁾ND, #D12450B Modified diet with 4% fat (10% fat calories) content; HD, #D12492 Modified high fat diet with 35% fat (60% fat calories) content; OL, HD with 10% of okara; OH, HD with 20% of okara.

²⁾All values are mean±SD ($n=8$). Values with different superscripts letters are significantly different among ND, HD, OL, and OH by ANOVA with Duncan's multiple range test at $p<0.05$.

³⁾FER (Food efficiency ratio) = body weight gain during the experimental period (g) / total feed intake during the experimental period (g)

Fig. 1. Effects of okara supplementation on liver weight, epididymal fat weights, and the ratio fat to body weight of mice fed the different diets. All values are given as means $\pm$ SD ($n=8$). Statistical significance of means was determined using one-way ANOVA followed by Duncan's multiple-range post hoc test. Values not sharing a common superscript letter are significantly different at $p<0.05$. ND, #D12450B Modified diet with 4% fat (10% fat calories) content; HD, #D12492 Modified high fat diet with 35% fat (60% fat calories); OL, HD with 10% of okara; OH, HD with 20% of okara

Table 3. Serum, liver, and fecal lipid profiles

	ND ¹⁾	HD	OL	OH
Serum (mg/dl)				
Triacylglycerol	101.79 $\pm$ 21.48 ^b	143.81 $\pm$ 39.88 ^{a2)}	112.30 $\pm$ 23.64 ^b	85.88 $\pm$ 32.01 ^b
Total cholesterol	179.77 $\pm$ 23.21 ^b	354.14 $\pm$ 52.04 ^a	222.73 $\pm$ 34.25 ^b	214.97 $\pm$ 58.12 ^b
LDL cholesterol	69.45 $\pm$ 22.28 ^c	199.53 $\pm$ 57.88 ^a	178.25 $\pm$ 54.90 ^{ab}	120.45 $\pm$ 60.65 ^{bc}
HDL cholesterol	93.39 $\pm$ 16.29	115.77 $\pm$ 21.16	107.61 $\pm$ 16.24	97.41 $\pm$ 10.38
Liver (mg/g)				
Triacylglycerol	15.05 $\pm$ 3.33 ^b	19.38 $\pm$ 5.18 ^a	16.25 $\pm$ 1.39 ^{ab}	13.17 $\pm$ 2.52 ^b
Total cholesterol	2.48 $\pm$ 0.63	3.05 $\pm$ 0.41	2.51 $\pm$ 0.57	2.18 $\pm$ 0.70
Feces (mg/g)				
Triacylglycerol	0.21 $\pm$ 0.06 ^c	0.32 $\pm$ 0.13 ^c	0.57 $\pm$ 0.13 ^b	0.78 $\pm$ 0.15 ^a
Total cholesterol	0.76 $\pm$ 0.09 ^c	1.33 $\pm$ 0.42 ^b	1.67 $\pm$ 0.24 ^a	1.84 $\pm$ 0.20 ^a

¹⁾ND, #D12450B Modified diet with 4% fat (10% fat calories) content; HD, #D12492 Modified high fat diet with 35% fat (60% fat calories) content; OL, HD with 10% of okara; OH, HD with 20% of okara

²⁾All values are mean $\pm$ SD ($n=8$). Values with different superscripts are significantly different among ND, HD, OL, and OH by ANOVA with Duncan's multiple range test at $p<0.05$.

was reported that the epididymal adipose tissue secretes the highest quantity of leptin compared to white adipose tissue from other regions (22,28). In our study, serum leptin levels in OL- and OH-fed mice were significantly lower compared to HD-fed mice in a dose-dependent manner ($p<0.0001$) (Fig. 2). Also, serum insulin levels in OL- and OH-fed mice were significantly lower than HD-fed mice ($p<0.0001$) (Fig. 2). Our data showed that the okara supplementation lowers the fat accumulation and thus results in lower circulating leptin. This study suggests that the consumption of okara, a rich source of dietary fiber, has a beneficial effect against hyperlipidemia. We measured the expression levels of hepatic genes involved in lipid metabolism, to elucidate the mechanism of the normalization of hyperlipidemia by okara. The relative mRNA expression levels of

genes involved in hepatic lipid metabolism are shown in Fig. 3. PPAR- α plays crucial regulatory role in beta-oxidation (22,29), and governs the expression of CPT-1 and ACOX which are involved in mitochondrial and peroxisomal fatty acid oxidation respectively (22,28); whereas, PPAR- γ and FAS are crucial for hepatic lipogenesis. FAS is known to regulate fatty acid synthesis and lipogenesis and acts as a common molecular target for central appetitive and peripheral metabolic regulation (15,30,31). In our study, the hepatic PPAR- α mRNA expressions in OL- and OH-fed mice were significantly higher compared to ND- and HD-fed mice ($p<0.01$). CPT-1 mRNA was also increased in OH- fed mice compared with HD-fed mice, but the level of CPT-1 mRNA in OL-fed mice was similar to HD-fed mice ($p<0.05$). Moreover, the expression levels of ACOX mRNA in OH-fed mice were

Fig. 2. Effects of okara supplementation on serum levels of leptin and insulin in mice fed the different diets. All values are given as means $\pm$ SD ($n=8$). Statistical significance of means was determined using one-way ANOVA followed by Duncan's multiple-range post hoc test. Values not sharing a common superscript letter are significantly different at $p<0.05$. ND, #D12450B Modified diet with 4% fat (10% fat calories) content; HD, #D12492 Modified high fat diet with 35% fat (60% fat calories); OL, HD with 10% of okara; OH, HD with 20% of okara

Fig. 3. Effects of okara supplementation on the expression of genes in the liver. All values are given as means $\pm$ SD ($n=8$). Statistical significance of means was determined using one-way ANOVA followed by Duncan's multiple-range post hoc test. Values not sharing a common superscript letter are significantly different at $p<0.05$. NS is not significant. ND, #D12450B Modified diet with 4% fat (10% fat calories) content; HD, #D12492 Modified high fat diet with 35% fat (60% fat calories); OL, HD with 10% of okara; OH, HD with 20% of okara

higher than HD-fed mice ($p<0.05$); and were similar to those of ND-fed mice. Meanwhile, the hepatic PPAR- γ mRNA level in OH-fed mice was remarkably lower than HD-fed mice. Similarly, the level of FAS mRNA in OH-fed mice decreased compared to HD-fed mice, however, that of OL-fed mice was not significantly different from the HD-fed mice ($p<0.01$). ACC, which is the rate-limiting enzyme in fatty acid

synthesis (32), was not greatly affected by the OL and OH diets and thus the level of ACC was not significantly different among the four groups. In congruence with our data, a recent report by Bae *et al.* (33) suggested that, soybean paste supplementation in animals does not produce any significant changes in the ACC mRNA levels as compared with the high fat diet. In this study, although the ACC

mRNA did not show any significant difference, the outcome of lipogenic pathway was notable with the lowest concentrations of hepatic triglycerides in the OH group; suggesting the existence of alternative pathways. Dietary phytoestrogen in soybean have been reported to activate the AMP activated protein kinase (AMPK) (34). Therefore, one of the possibilities could be the inactivation of ACC via phosphorylation by AMPK leading to decrease in the synthesis of TAG. These results demonstrated that a diet containing okara may improve hepatic lipid metabolism through increasing lipid catabolism and decreasing the anabolism.

Our study had certain limitations. The first, we didn't measure the anti-nutrients in okara, such as phytic acid and lectin, etc. Okara has a little amount of phytic acid (35). Several previous studies mentioned the anti-nutrients in soybean products are decreased by intentional processing (35,36). Also, many studies improved the safety of okara consumptions through using okara as a material in their study (10,13,15,18,20). The second, the malabsorption of fat soluble vitamins and minerals after okara intake during 12 weeks was not examined in our study. Mahle and Patton (37) mentioned that neither vitamin A and carotene excretion in feces was increased nor were plasma vitamin A and carotene levels affected after long-term intake of hydrophilic mucilloid in human study. However, the result of Kelsay *et al.* (38) is the contrast to previous study. Kasper (39) reported that a single test is not appropriated to determine whether long-term administration of high dietary fiber amount leads to lacking of essential serum nutrients or not. So a study on the effect of fat soluble vitamins excretion after okara intake is needed to be conducted in further.

In conclusion, our data supported the hypothesis that okara may prevent DIO and improve serum lipid profiles in high fat diet induced obese mice. Furthermore, it showed positive results through comparing between the control diets (ND, HD) and the supplemented diets (OL, OH). A diet containing okara had hypolipidemic efficacy in C57BL/6J mice; moreover, okara was effective for preventing weight gain, especially, visceral fat. Okara consumption increased the mRNA expressions of genes related to hepatic β -oxidation resulting in lower serum TAG, TC, and LDL cholesterol in mice. Our findings suggest that okara, soybean by-products may have potential as a phytochemical and dietary fiber to manage DIO as well as a method of reutilization of the soy waste.

Acknowledgments This work was supported by the "Industry-Academic Cooperation Technical Development (Project No. C0028597)" from Small and Medium Business Administration. The authors thank Dr. P.B. Tirupathi Pichiah for helpful discussions in animal experiments. We also thank Mina Kim, Cho Rong Bae, and Yeon Hee Park for technical support.

Disclosure The authors declare no conflict of interest.

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