

# Cheonggukjang, a soybean paste fermented with *B. licheniformis*-67 prevents weight gain and improves glycemic control in high fat diet induced obese mice

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In this study, we investigated the anti-obesity effects of soybean paste—*Cheonggukjang*, fermented with poly gamma glutamic acid producing *Bacillus licheniformis*-67 in diet induced obese C57BL/6J mice. Forty male C57BL/6J mice aged 4 weeks were divided into four dietary groups; normal diet control, high fat diet control, high fat diet containing 30% of unfermented soybean and high fat diet containing 30% *Cheonggukjang* fermented with *Bacillus licheniformis*-67. After 13 weeks of dietary intervention the mice were sacrificed; serum and tissue samples were examined. Serum and hepatic lipid profile, blood glucose, insulin, leptin level were lower (<0.05) along with the body weight and epididymal fat pad weight in the 30% *Cheonggukjang* supplemented group compared with the high fat diet control group. The expression level of lipid anabolic gene was significantly decreased; whereas the expression level of lipid catabolic genes were significantly increased in the 30% *Cheonggukjang* supplemented group compared to the high fat diet control group. Collectively, these results suggested that intake of *Cheonggukjang* fermented with *Bacillus licheniformis*-67 significantly prevents obesity related parameters.

**Key Words:** *Cheonggukjang*, *B. licheniformis*, antiobesity, type 2 diabetes, high fat diet

Consumption of high energy diets followed by a sedentary lifestyle plays major role in obesity development characterized by increased fat deposition and elevated blood lipid profile. Obesity is accompanied with many diseases such as type-2 diabetes, hypertension and coronary heart disease.<sup>(1,2)</sup> Frequent intake of high calorie diet accelerates the impairment of insulin secretion and exacerbation of insulin resistance, with worsening type 2 diabetic symptoms.<sup>(3,4)</sup> If obesity associated disorders are left untreated, it will lead to a life threatening situation.

Currently there is a wide range of approaches available for obesity management, like phytotherapy, food therapy, bariatric surgery, pharmacotherapy etc. Natural products for obesity management are generally considered safe. Fermented food recently received greater attention in obesity prevention. One of the well known legumes used as fermented food is soybean. It is a well known source of flavonoids with greater health benefits. *Cheonggukjang* (CK) is a traditional fermented soybean food of Korea. It has been shown to have numerous health benefit, a study by Kwon *et al.*<sup>(5)</sup> indicated that 60% methanol fraction of traditionally fermented CK improves the insulin-stimulated glucose uptake in 3T3-L1 adipocytes via acting as an agonist of peroxisome proliferator-activated receptor (PPAR)- $\gamma$ ; similarly, it was observed that treatment of Min6 cells with 80% and 100% methanol frac-

tions of traditionally fermented CK increases glucose-stimulated insulin secretion. In addition, many animal studies indicated the anti-diabetic properties of CK which is correlated with the increased small peptides and isoflavones aglycones produced during the fermentation process.<sup>(6-10)</sup> Traditionally, CK is manufactured by fermentation of soybean for 72 h by naturally occurring bacteria in rice straw instead of using any specific species or strains of microorganisms. Therefore, the fermentative microbes in CK vary depending on regions and even with the same region from year to year.<sup>(11)</sup> The functionality of fermented foods, including CK depends on the milieu of the fermentative microbes.<sup>(12,13)</sup> Yang *et al.*<sup>(11)</sup> suggested that, within Korea the CK manufactured in some regions has lower anti-diabetic effect while from another region has higher. The difference in the functionality of CK is mainly due to the ambient microbe available in the environment of that particular area.<sup>(14)</sup> Therefore, it is necessary to manufacture CK using a specific microorganism that can retain its anti-diabetic properties without affecting the nature of CK. Traditionally fermented CK with the highest anti-diabetic effects are known to contain *Bacillus subtilis* (*B. subtilis*), *B. amyloliquefaciens* and *B. licheniformis*.<sup>(14)</sup> we hypothesized that one of these microorganism must be having an important role in the anti-diabetic property of CK. Since, *B. licheniformis* have been known as a good producer of poly- $\gamma$ -glutamic acid (PGA) which have shown anti-obesity effect in animal models.<sup>(15-17)</sup> Therefore, we chose *B. licheniformis*-67 as a starter in the preparation of CKB. The CKB thus produced was rich in PGA which was used in this study for the investigation of its functionality against diet induced obesity condition.

## Materials and Methods

**Preparation of CK.** CKB was supplied by the Korean Food Research Institute (Seongnam-si, Korea). In brief, soybeans were washed and soaked in the water for 18 h at 10°C. The soaked soybeans were then cooked for 30 min at 121°C. After cooling, the soybeans were inoculated with 5% *B. licheniformis*-67 and incubated at 40°C for 72 h. Samples were lyophilized at -70°C and mixed with the AIN-93 modified rodent diet. The amount of flavonoids in SS and CKB are shown in Table 1.

**Animals and diets.** Male C57BL/6J mice, aged 4 weeks, were purchased from Charles River Laboratories (Tokyo, Japan).

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**Table 1.** Isoflavone content in soybean and *Chungkukjang* fermented with different *B. licheniformis*-67

Isoflavones ( $\mu\text{g/g}$ )	Soybean	CKB
Daidzin	99.25 $\pm$ 3.2	61.77 $\pm$ 5.07
Glycitin	45.31 $\pm$ 1.51	32.63 $\pm$ 0.48
Genistin	184.29 $\pm$ 5.0	104.90 $\pm$ 11.34
Malonyldaidzin	1,009.23 $\pm$ 5.6	211.04 $\pm$ 10.33
Malonylglycitin	157.20 $\pm$ 2.8	61.15 $\pm$ 3.90
Malonylgenistin	111.80 $\pm$ 3.4	93.47 $\pm$ 20.66
Acetyldaidzin	85.71 $\pm$ 4.7	42.10 $\pm$ 3.74
Acetylgenistin	101.25 $\pm$ 1.74	17.23 $\pm$ 2.06
Total glycosides	1,794.04 $\pm$ 21.2	624.28 $\pm$ 55.85
Daidzein	15.12 $\pm$ 2.4	138.27 $\pm$ 9.15
Glycitein	ND	74.29 $\pm$ 5.36
Genistein	24.71 $\pm$ 2.9	84.95 $\pm$ 4.99
Total aglycones	39.83 $\pm$ 2.6	297.51 $\pm$ 19.46
Total isoflavones	1,833.87 $\pm$ 45.1	921.80 $\pm$ 74.43

All values are mean  $\pm$  SD ( $n = 3$ ).

The animals were maintained on a pellet diet (Research Diets, New Brunswick, NJ) for 1 week, and then randomly divided into four groups ( $n = 10$ ): normal diet control (ND), high fat diet control (HD), high fat diet added with 30% unfermented soybeans (SS), high fat diet added with 30% CK fermented with *B. licheniformis*-67 (CKB). The compositions of the experimental diets (Research Diets, New Brunswick, NJ) are shown in Table 2. Animals were housed in a temperature-controlled environment with a 12 h light/dark cycle and were given free access to food and water during the entire study period. The feed intake and body weight were measured daily and weekly basis, respectively, using a digital weighing scale (Mettler Toledo PL6001-S). The experimental protocol was approved by the Animal and Use Committee of Chonbuk National University.

**Collection of serum and tissue samples.** After 12 h of overnight fasting, each mouse was deeply anesthetized with diethyl ether inhalation and blood was collected by orbital vein puncture. Serum was isolated from clotted blood by centrifugation at  $1,100 \times g$  for 15 min at  $4^\circ\text{C}$  (Micro 17R, Hanil Science In Co., Ltd, Gangneung, Korea). Tissues were carefully excised, rinsed and weighed. Both tissues and serum samples were stored at  $-80^\circ\text{C}$  until analyzed.

**Analyses of lipid profiles.** The hepatic lipids were extracted using chloroform/methanol solution (2:1, vol/vol) from the liver tissue using the Bligh and Dyer method.<sup>(18)</sup> Briefly, chloroform/methanol solution was added in the homogenized liver tissues, vortexed and centrifuged; the lower phase was collected and evaporated at room temperature under fume hood (Daihan Labtech Co., Ltd, Namyangju, Korea). The remaining semi dried pellet was dissolved in 10 ml/L Triton X-100 (Yakuri Pure Chemicals Co., Ltd, Kyoto, Japan). Resulting solution was used to estimate hepatic lipids. Serum as well as hepatic triglyceride, total cholesterol and serum HDL-cholesterol were measured using commercially available kit (Asan Pharmaceutical Co., Seoul, Korea).

**Glycemic control effects and oral glucose tolerance test (OGTT).** On the 5th, 7th, 9th and 11th week of the experimental period, after 12 h of over night fasting blood glucose was measured in the morning using a glucometer (Roche Diagnostics GmbH, Germany). Oral glucose tolerance test was measured from blood samples collected from the tail vein at an interval of 0, 30, 60, 120 and 180 min after oral administration of glucose (1 g/kg BW).

**Hepatic mRNA analyses.** Total RNA was extracted from liver tissue using Trizol reagent (Invitrogen Life Technologies; Carlsbad, CA) and the concentration was measured spectrophotometrically. The extracted RNA was reverse transcribed into complementary DNA (cDNA) using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Then the RNA expression level was quantified by a quantitative real-time PCR using SYBR Green PCR Master Mix (Applied

**Table 2.** The composition of experimental diets

Ingredient (g)	ND <sup>1)</sup>	HD <sup>2)</sup>		
	ND	HD	SS <sup>3)</sup>	CKB <sup>4)</sup>
Casein	18.96	25.84	32.56	34.9
Cystine	0.28	0.39	0.27	0.27
Corn starch	29.86	—	11.47	8.63
Maltodextrin	3.32	16.15	11.05	11.05
Sucrose	33.17	8.89	6.08	6.08
Cellulose	4.74	6.46	4.42	4.42
Soybean oil	2.37	3.23	7.44	7.93
Lard	1.9	31.66	21.66	21.66
Mineral mix	0.95	1.29	0.88	0.88
Dicalcium phosphate	1.23	1.68	1.15	1.15
Calcium carbonate	0.52	0.71	0.49	0.49
Potassium citrate	1.56	2.13	1.46	1.46
Vitamin mix	0.95	1.29	0.88	0.88
Choline bitarrate	0.19	0.26	0.18	0.18
FD & C Yellow dye	—	—	—	—
FD & C Blue dye	—	0.01	—	—
Total	100	100	100	100
Kcal	385	524	514	511
Kcal/g	3.9	5.2	5.1	5.1

1) Normal diet; AIN-93 modified diet with 10% kcal from fat. 2) High fat diet; AIN-93 modified high fat diet with 60% kcal from fat. 3) SS; high fat diet plus 30% unfermented soybean. 4) CKB; high fat diet plus 30% CK fermented with *B. licheniformis*.

**Table 3.** Primer sequences for real time PCR

Primer	Sequences
CPT-1	F: 5'-AAAGATCAATCGGACCTAGACA-3' R: 5'-CAGCGAGTAGCGCATAGTCA-3'
ACO	F: 5'-CCAACATGAGGACTATAACTTCCT-3' R: 5'-TACATACGTGCCGTGAGGCTT-3'
LXR $\alpha$	F: 5'-GCGTCCATTCAGAGCAAGTGT-3' R: 5'-TCACTCGTGGACATCCAGAT-3'
PPAR $\alpha$	F: 5'-GGATGTCACACAATGCAATTCGCT-3' R: 5'-TCACAGAACGGCTTCCTCAGGT-3'
$\beta$ -actin	F: 5'-ATGGATGACGATATGGCT-3' R: 5'-ATGAGGTAGTCTGTCAGGT-3'

List of primers used for analyzing the respective lipid metabolic genes. CPT1, carnitine palmitoyltransferase I; ACO, acyl CoA oxidase; LXR  $\alpha$ , liver X receptor  $\alpha$ ; PPAR $\alpha$ , peroxisome proliferator-activated receptor alpha;  $\beta$ -actin, beta actin.

Biosystems, Woolston, Warrington, UK) and the 7500 Real Time PCR system (Applied Biosystems) according to the manufacturer's protocol. Gene specific primers used in this study are given in Table 3. Relative quantification of gene expression with real-time PCR data was calculated relative to  $\beta$ -actin.

**Analysis of insulin and leptin.** Serum insulin and leptin levels were measured using commercially available kits (Shibayagi, Shibukawa, Japan) and Quantikine immuno assay kit (R&D system, Minneapolis, MN) respectively.

**Statistical analyses.** The data were analyzed by one-way ANOVA using SPSS ver. 12.0 (SPSS Inc., Chicago, IL). Values are expressed as mean  $\pm$  SD. The differences among groups were assessed using Duncan's multiple range tests. Statistical significance was considered at  $p < 0.05$ .

## Results

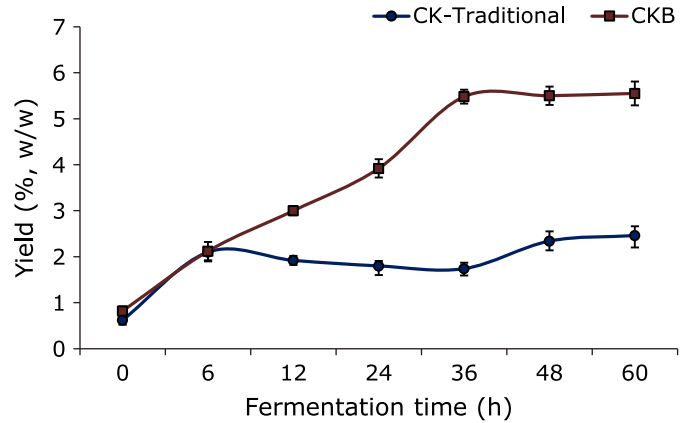
**$\gamma$ -PGA (Polyglutamic acid) yield in CK fermented traditionally or by *B. licheniformis*-67.**  $\gamma$ -PGA yield (Fig. 1) from CK fermented by traditional method or by using *B. licheniformis*-67 was measured and compared. It was observed that the production of  $\gamma$ -PGA increased from 12 h onwards in the *B. licheniformis*-67 fermented CKB compared to the traditionally fermented CK.

**Weight gain and feed efficiency ratio.** The effect of CKB on body weight is shown in Table 4. Initially the body weight of animals in all the groups were in a similar range. After 13 weeks of the experimental period, the final body weight of animals in the HD group was significantly higher than the rest of the group. However, CK supplementation group showed significantly lower final body weight compared to the HD group. There was no significant difference observed in feed intake between the groups. But, the feed efficiency ratio was significantly lower in the CK and unfermented soybean supplemented group compared to the high fat diet control group (Table 4).

**Table 4.** Effect of CK on body weight, food intake and feed efficiency ratios in mice

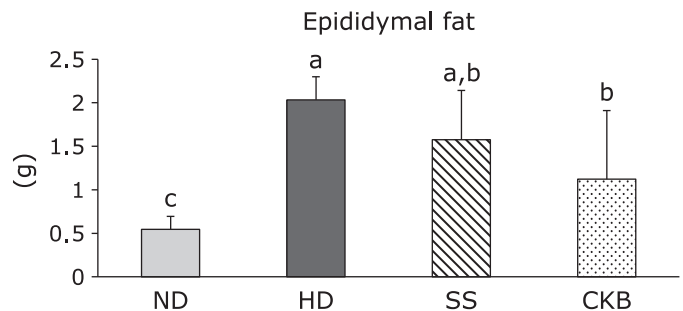
Groups	ND	HD	SS	CKB
Initial body weight (g)	18.86 $\pm$ 0.97	19.11 $\pm$ 0.88	18.85 $\pm$ 0.78	18.41 $\pm$ 0.84
Final body weight (g)	27.19 $\pm$ 1.26 <sup>c</sup>	37.58 $\pm$ 5.07 <sup>a</sup>	33.88 $\pm$ 4.08 <sup>b</sup>	32.27 $\pm$ 3.12 <sup>b</sup>
Body weight gain (g)	6.87 $\pm$ 1.95 <sup>c</sup>	14.71 $\pm$ 5.08 <sup>a</sup>	11.63 $\pm$ 4.19 <sup>b</sup>	11.01 $\pm$ 2.95 <sup>b</sup>
Feed intake (g/day)	2.56 $\pm$ 0.20	2.47 $\pm$ 0.21	2.38 $\pm$ 0.26	2.47 $\pm$ 0.27
Feed efficiency ratio	0.03 $\pm$ 0.01 <sup>c</sup>	0.07 $\pm$ 0.03 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>b</sup>	0.05 $\pm$ 0.01 <sup>b</sup>

All values are mean  $\pm$  SD. Values with different letters are significantly different by ANOVA with Duncan's multiple range test at  $p < 0.05$ . ND, AIN-93 modified diet with 10% kcal from fat; HD, AIN-93 modified high fat diet with 60% kcal from fat; SS, high fat diet plus 30% unfermented soybean; CKB, high fat diet plus 30% CK fermented with *B. licheniformis*-67. Feed efficiency ratio = weight gain per day/diet intake per day.

**Fig. 1.** Yield of PGA in *Cheonggukjang* (CK-Traditional) fermented by either traditional method or using *B. licheniformis*-67 (CKB).

**Epididymal fat pad weight.** As shown in Fig. 2, the epididymal fat pad weight was significantly higher in all the high fat diet fed groups compared to the normal diet fed ND group. However, the CK supplemented CKB group showed significantly lower epididymal fat pad weight compared to the high fat diet control HD and unfermented soybean supplemented SS group.

**Serum and liver lipid levels.** The effects of CKB supplementation on serum as well as hepatic lipid profiles are shown in Table 5. Serum triglyceride (TG) level was not significant among the entire group. Whereas serum total cholesterol (TC) was significantly low in the CK supplemented CKB group compared to the high fat diet control HD group. The serum HDL-cholesterol

**Fig. 2.** Effect of CK fermented with *B. licheniformis*-67 on epididymal fat in mice. All values are mean  $\pm$  SD. Values with different letters are significantly different by ANOVA with Duncan's multiple range test at  $p < 0.05$ . ND, AIN-93 modified diet with 10% kcal from fat; HD, AIN-93 modified high fat diet with 60% kcal from fat; SS, high fat diet plus 30% unfermented soybean; CKB, high fat diet plus 30% CK fermented with *B. licheniformis*-67.

**Table 5.** The effect of CK on lipid profiles in mice

Groups	ND	HD	SS	CKB
Serum (mg/dl)				
TG	122.61 ± 21.25	124.15 ± 14.94	112.78 ± 12.08	122.58 ± 21.50
TC	112.15 ± 9.80 <sup>c</sup>	146.08 ± 22.60 <sup>a</sup>	132.29 ± 19.85 <sup>a,b</sup>	128.61 ± 13.22 <sup>b</sup>
HDL-c	89.40 ± 16.26 <sup>b</sup>	108.85 ± 16.13 <sup>a</sup>	85.41 ± 22.65 <sup>b</sup>	90.05 ± 15.70 <sup>b</sup>
HDL-c/TC (%)	80.77 ± 12.30	76.23 ± 14.69	64.93 ± 16.80	72.18 ± 5.94
Liver (mg/g)				
TG	9.28 ± 1.71 <sup>b</sup>	11.91 ± 1.59 <sup>a</sup>	10.17 ± 2.17 <sup>a,b</sup>	10.75 ± 1.67 <sup>a,b</sup>
TC	3.78 ± 0.70 <sup>a,b</sup>	4.39 ± 0.83 <sup>a</sup>	3.22 ± 0.96 <sup>b</sup>	3.30 ± 0.99 <sup>b</sup>

All values are mean ± SD. Values with different letters are significantly different by ANOVA with Duncan's multiple range test at  $p < 0.05$ . ND, AIN-93 modified diet with 10% kcal from fat; HD, AIN-93 modified high fat diet with 60% kcal from fat; SS, high fat diet plus 30% unfermented soybean; CKB, high fat diet plus 30% CK fermented with *B. licheniformis*-67.

concentration was significantly higher in the HD group than the rest of the group. Hepatic TG in both the experimental groups (SS and CKB) were at an intermediate range between the ND and HD groups and were not significantly different from either. TC concentration in the liver was significantly lower in both of the experimental groups (SS and CKB) compared with high fat diet control HD group.

**Glycemic control effects.** As shown in Fig. 3a, after the 5th week of experiment, the whole blood glucose level was significantly increased in the high fat diet fed groups compared to the normal diet fed group. Seventh week onwards a decline in fasting blood glucose was observed in all the groups when compared to the earlier observation. However a significant reduction in fasting blood glucose was observed in the CKB group compared to HD and SS group.

**OGTT.** We examined the effect of CKB on glucose clearance through OGTT (Fig. 3b); blood glucose levels were measured at 0, 30, 60, 120 and 180 min after oral administration of 1 g/kg BW of glucose. The blood glucose was significantly decreased in the CKB group compared to the HD and SS group at 120 and 180 min after the glucose administration, indicating an improved state of glucose clearance in CKB group compared to the SS and HD group.

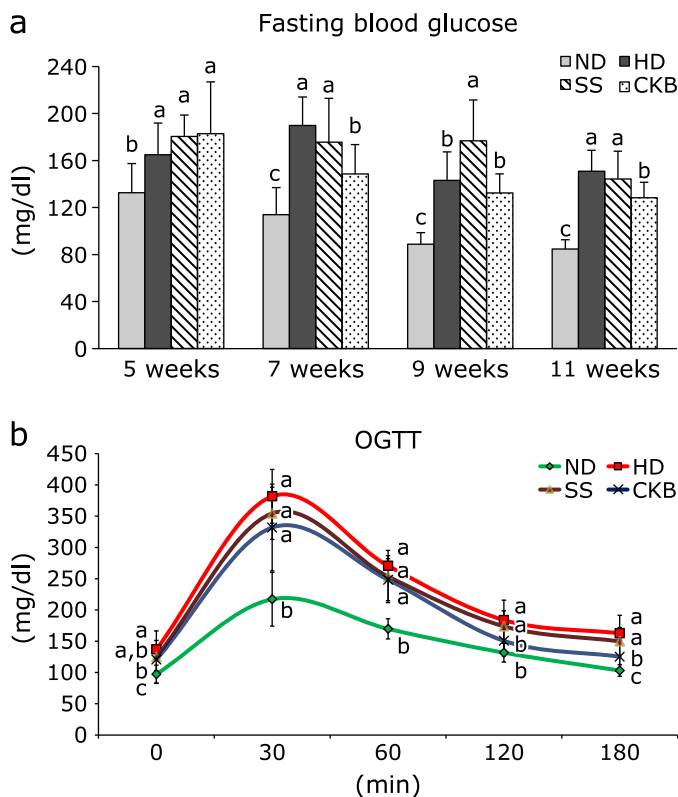
**Hepatic mRNA levels.** The changes in hepatic lipid metabolic genes were analyzed (Fig. 4). The Carnitine palmitoyltransferase-1 (CPT-1) and acyl-CoA oxidase (ACO) mRNA levels associated with lipid oxidation and mitochondrial function were significantly higher in the CKB group than the HD groups; and were similar to the ND group. The regulator of cholesterol biosynthesis, liver x receptor alpha (LXR $\alpha$ ) mRNA level was significantly lower in the CKB group than both HD and ND groups. No significant difference was observed in the LXR $\alpha$  mRNA level between the CKB and SS group, however the level was lower in the CKB compared to SS.

The peroxisome proliferator activated receptor alpha (PPAR $\alpha$ ) mRNA level was highest in the SS group compared to the rest of the group, however the CKB group showed similarity with the ND group.

**Analyses of serum insulin and leptin.** The serum insulin and leptin level are shown in Fig. 5a and b respectively. Serum insulin level was significantly lower in the SS and CKB groups compared with HD group, whereas the serum leptin levels were significantly lower in the CKB group than SS and HD groups.

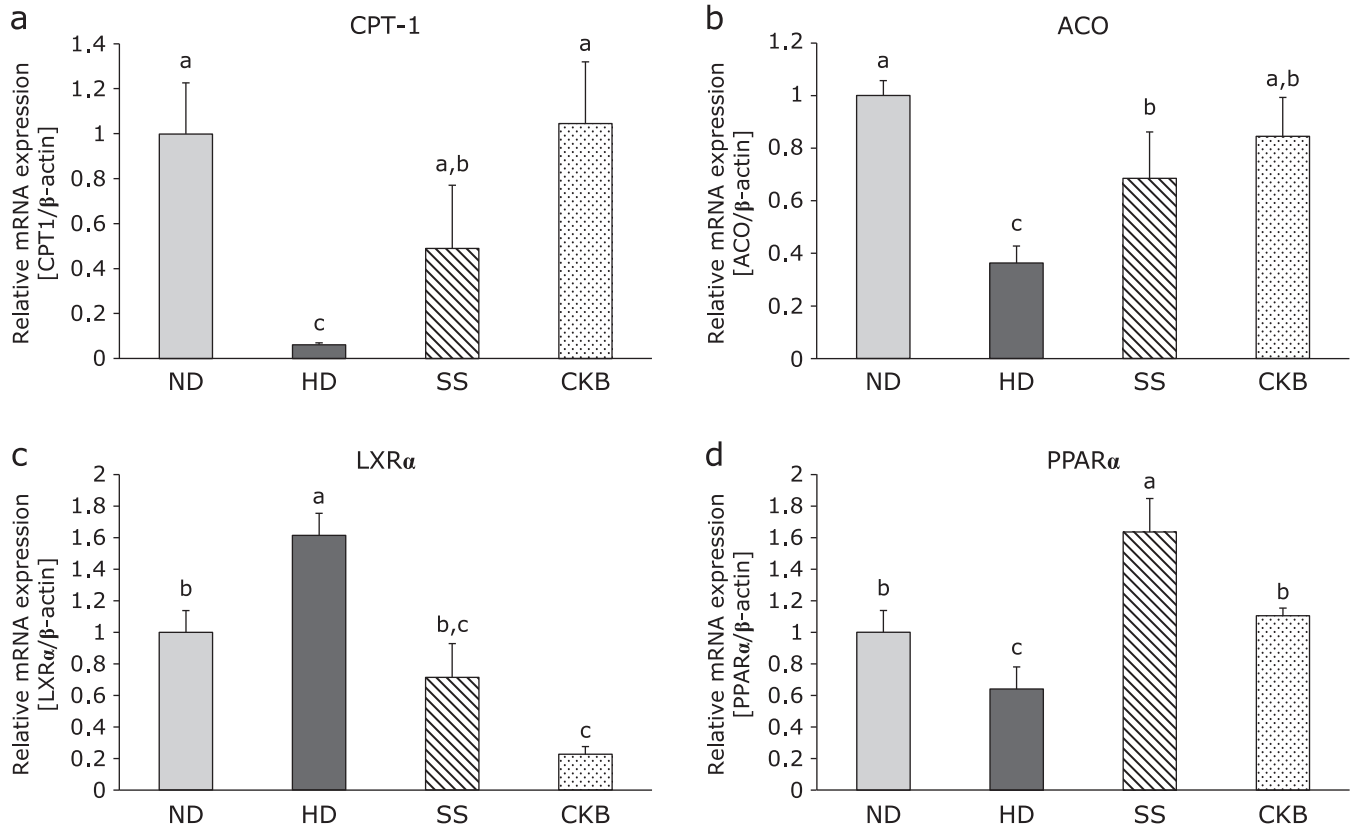
## Discussion

The C57BL/6J mouse strain is sensitive to high fat diet induced weight gain.<sup>(19,20)</sup> The high fat diet intake lead to increase in body weight, adipose tissue weight and promotes hyperlipidemia and hyperglycemia in rodents.<sup>(21,22)</sup> In this current study, body weight gain and feed efficiency ratio were significantly higher in the

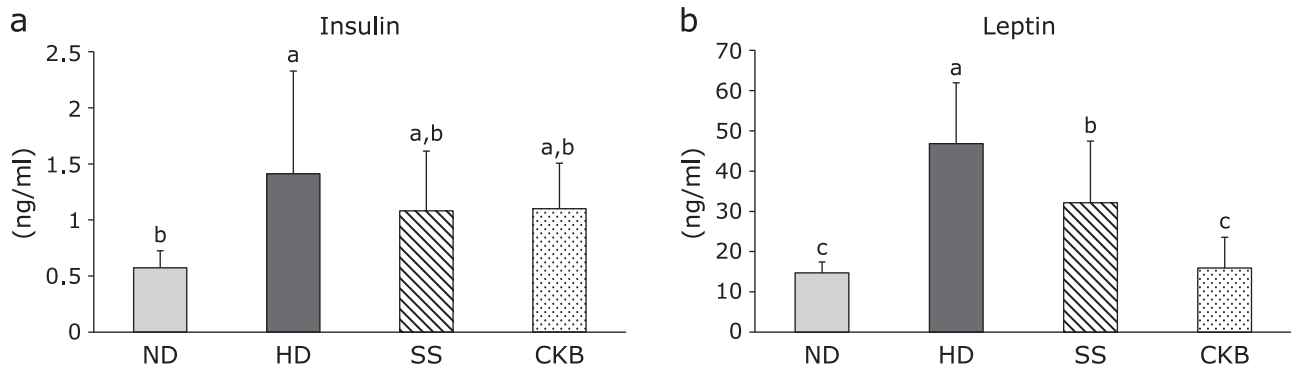


**Fig. 3.** (a) The effect of CK fermented with *B. licheniformis*-67 on fasting whole blood glucose concentration. (b) The effect of CK fermented with *B. licheniformis*-67 on oral glucose tolerance test. All values are mean ± SD. Values with different letters are significantly different by ANOVA with Duncan's multiple range test at  $p < 0.05$ . ND, AIN-93 modified diet with 10% kcal from fat; HD, AIN-93 modified high fat diet with 60% kcal from fat; SS, high fat diet plus 30% unfermented soybean; CKB, high fat diet plus 30% CK with *B. licheniformis*-67.

high fat diet fed groups compared with the normal diet fed group. Interestingly, animals fed with high fat diet along with CKB showed a lower gain in body weight compared with the groups fed with either high fat diet alone or along with SS. Korean fermented soybean foods have been known to avert weight gain in animals fed with high fat diet.<sup>(23)</sup> In our previous study, we showed that, animals fed with 40% of traditional CK containing high fat diet had 24% lesser body weight gain compared to high fat control group; however, in this current study animals on 30% CKB fermented with *B. licheniformis*-67 showed 25% lesser weight gain.<sup>(24)</sup> Consumption of a diet high in fat promotes adiposity,



**Fig. 4.** Effect of CK on mRNA levels of lipid metabolism-related genes in the liver of C57BL/6J mice. All values are mean  $\pm$  SD. Values with different letters are significantly different by ANOVA with Duncan's multiple range test at  $p < 0.05$ . ND, AIN-93 modified diet with 10% kcal from fat; HD, AIN-93 modified high fat diet with 60% kcal from fat; SS, high fat diet plus 30% unfermented soybean; CKB, high fat diet plus 30% CK fermented with *B. licheniformis*-67. (a) CPT-1, carnitine palmitoyltransferase-1; (b) ACO, acyl-CoA oxidase; (c) LXR $\alpha$ , liver X receptor  $\alpha$ ; (d) PPAR $\alpha$ , peroxisome proliferator-activated receptor  $\alpha$ .



**Fig. 5.** Concentrations (a) insulin and (b) leptin in serum. All values are mean  $\pm$  SD. Values with different letters are significantly different by ANOVA with Duncan's multiple range test at  $p < 0.05$ . ND, AIN-93 modified diet with 10% kcal from fat; HD, AIN-93 modified high fat diet with 60% kcal from fat; SS, high fat diet plus 30% unfermented soybean; CKB, high fat diet plus 30% CK fermented with *B. licheniformis*-67.

resulting in the increase in epididymal fat pad weight which is positively correlated with body fat.<sup>(25,26)</sup> We observed that, supplementation of CKB was effective in averting the gain of epididymal adipose tissue mass compared to untreated (HD) group as well as unfermented soybean supplemented (SS) group. Earlier we reported that intake of high fat diet with 40% traditional CK lowered 26% weight gain of epididymal adipose tissue,<sup>(24)</sup> however in our current study, we observed that consumption of high fat diet with 30% CKB fermented with *B. licheniformis*-67 was more efficient

in averting the gain of epididymal adipose tissue which was around 45% lower than the high fat diet control. The CKB sample used in this study was richer in  $\gamma$ -PGA than the traditional CK, as a result of fermentation by *B. licheniformis*-67. A recent study suggested that  $\gamma$ -PGA both in the presence and absence of isoflavones decreases epididymal adipose tissue mass.<sup>(17)</sup> The adipose tissue gains its mass as a result of accumulation of ingested dietary fat as triglyceride. However, increased amount of fatty acid oxidation averts the storage of the fats in adipose tissue and other

peripheral organs.<sup>(27)</sup> Liver plays a vital role in the metabolism of the dietary fat. Therefore, we measured the level of expression of crucial genes involved in hepatic lipid metabolism. The PPAR $\alpha$  plays a central role in the oxidation of fatty acids. It is expressed primarily in tissues that have a high level of fatty acid catabolism such as liver.<sup>(28)</sup> Takahashi and Ide<sup>(29)</sup> showed that the PPAR $\alpha$  mRNA level increases with increased dietary soy protein intake, which clearly implies that soy protein increases the expression of PPAR $\alpha$  in a dose dependent manner. In our study also the CKB group showed a significant increase in the expression of PPAR $\alpha$  compared with the HD group. However the expression level was lower in the CKB group compared to the SS group, the differences in PPAR $\alpha$  mRNA levels between these two groups (CK and SS) may be due to the amount of isoflavones. Isoflavones are capable of regulating the expression of PPAR $\alpha$ .<sup>(30)</sup> In our study the isoflavones concentration in CKB was lower compared with the SS, that may be the reason for PPAR $\alpha$  expression in the SS group was higher than the CKB group.

The two vital subcellular organelles involved in fatty acid  $\beta$ -oxidation are mitochondria and peroxisome. The rate limiting enzyme for mitochondrial  $\beta$ -oxidation is CPT-1 while in peroxisome is ACO. We measured the mRNA expression level for these two enzyme. Liver and/or skeletal muscle CPT-1 mRNA expression increases as a result of supplementation soy protein as well as  $\gamma$ -PGA and helps in preventing weight gain.<sup>(17,31)</sup> Soh *et al.*<sup>(24)</sup> also showed that CPT-1 mRNA levels were increased in a high fat diet plus CK 40% supplementation group. Our study was consistent with these three studies; indicating that supplementation of either steamed soybean or fermented soybean increases the CPT-1 mRNA expression level in both SS and CKB groups compared to HD group. Apart from CPT-1 regulating the mitochondrial  $\beta$ -oxidation, the mRNA expression level of enzyme ACO for peroxisomal fatty acid oxidation was notably higher in both SS and CKB group. Earlier study by Takahashi and Ide<sup>(29)</sup> suggests that soy protein supplementation increases the ACO activity, which was similar to our result. The reason for higher expression level of ACO mRNA expression level in the CKB group than the SS group could be due to the changes in the flavonoid levels along with the higher protein level in CK. Some studies suggested that the expression of both CPT-1 and ACO genes are up-regulated by fatty acid and acyl derivatives through PPAR $\alpha$  dependent pathway but other findings showed that long-chain FA regulate CPT-1 through a PPAR $\alpha$  independent pathway.<sup>(32–37)</sup> The mRNA expression level of PPAR $\alpha$  was lower in CKB while the CPT-1 and ACO was higher as compared to the SS group, suggesting that the expression of CPT-1 and ACO might be regulated in PPAR $\alpha$  dependent as well as independent pathway. Though we did not measure the level of hepatic long-chain FA, however the results of CPT-1 and ACO mRNA level suggest that there might be an increase in the hepatic long-chain FA in CKB compared to SS group, further studies, however is needed to be carried out to verify the changes in hepatic long-chain FA as a result of CKB supplementation.

The lipid anabolic gene, LXR $\alpha$  is highly expressed in the tissues playing crucial role in lipid metabolism, such as liver, kidney, adipose tissue, skeletal muscle, small intestine and macrophages.<sup>(38)</sup> Earlier study reported that supplementation of soy protein in obese rodents lowers the expression of LXR $\alpha$ .<sup>(39,40)</sup> Similarly, in our study also supplementation of CKB and SS with higher protein content was effective in lowering the hepatic LXR $\alpha$  mRNA level which was not observed in the HD group. The higher lipid catabolism than anabolism as observed by the higher expression of PPAR $\alpha$ , CPT-1 and ACO; and lower expression of LXR $\alpha$ , reflected in several parameters including lower body weight gain and epididymal adipose tissue weight. Leptin is a hormone secreted by adipose tissue with epididymal adipose tissue as a major contributor.<sup>(41)</sup> Higher amount of adiposity has been positively related with high level of circulating leptin, a

condition known as hyperleptinemia. Surgical removal of epididymal adipose tissue was found to lower the circulatory serum leptin level,<sup>(42)</sup> suggesting that the decrease in epididymal adipose tissue mass has a direct impact on the circulatory leptin level. In our study decrease in the epididymal adipose tissue mass as a result of supplementation of CKB and SS resulted in decrease secretion of leptin and thereby averting the hyperleptinemia condition. In rodents prolong consumption of high fat diet induces hyperinsulinemia, impairs glucose tolerance and promotes adipose tissue inflammation.<sup>(43–45)</sup> In our study also, animals fed with high fat diet for 13 weeks showed higher levels of serum insulin compared to the animals on a normal diet. However, animals supplemented with CKB or SS showed a lower insulin level compared to the non supplemented HD group. Soybean isoflavonoids and protein consumption alleviate some of the pathology associated with type 2 diabetes, such as insulin resistance and impaired glycemic control.<sup>(46–48)</sup> Isoflavonoids improves anti-diabetic actions through estrogen receptors.<sup>(49)</sup> In a clinical trial it was shown that supplementation of soy phytoestrogen for 3 months significantly lowered insulin level.<sup>(50)</sup> In our study the lower insulin level could be because of the soy proteins along with the naturally occurring phytoestrogens that are structurally and functionally similar to estradiol.<sup>(51)</sup>

In insulin resistance state, an increased level of insulin along with high level of fasting blood glucose can be observed indicating a type-2 diabetic condition. In such state, the adipose tissue, liver and muscles fail to uptake glucose in response to increasing serum insulin. Oral glucose tolerance test measures the insulin resistance. The greater rate of blood glucose clearance elucidates the higher insulin sensitivity while the *vice versa* indicates insulin resistance. Soybean and its products are very well known for their anti-diabetic properties.<sup>(52)</sup> In our study a significant decrease in the blood glucose level was observed in the CKB group after 120 and 180 min of oral glucose administration (1 g/kg BW). The higher rate of glucose clearance in the CKB group in spite of the low level of serum insulin compared to the HD group suggests a higher insulin sensitivity in this group. In addition, it was also observed that overnight fasting blood glucose was lower in the CKB group from 7th week onward compared to both the HD group as well as SS group. Several studies on animals and few on humans evaluated the effects of fermented soybeans on glucose metabolism.<sup>(53,54)</sup> Glucose metabolism is a complex process which is governed by peptide and steroid hormones and is influenced by diet. A study in rodents supplemented with soy rich diet elucidated that soy phytoestrogen activates AMP-activated protein kinase (AMPK) and results in improved glucose as well as lipid metabolism.<sup>(55)</sup> AMPK is an energy sensor and pharmacological activation of AMPK promotes glucose uptake, fatty acid oxidation, mitochondrial biogenesis, and insulin sensitivity.<sup>(56)</sup> It has been reported that consumption of fermented soybeans shows higher bioavailability of isoflavones—a class of phytoestrogens, compared to non-fermented soybean.<sup>(57)</sup> Kwon *et al.*<sup>(10)</sup> showed that CK improved glucose homeostasis by enhancing hepatic insulin sensitivity and insulinotropic actions in 90% pancreatectomized rats. One of the key component poly-gamma glutamic acid, in the CKB used in our study has been reported to increase the expression level of AMPK mRNA level.<sup>(17)</sup> Therefore, we believe that, in our study the isoflavones and PGA in the CKB might have been involved in the activation of AMPK that in turn would have resulted in the higher glucose clearance and insulin sensitivity as observed in the CKB group. The effect was also reflected in the lower hepatic TC and TG level in the CKB supplemented group than in the HD group.

The serum TG did not show any significant differences among the group, however the serum TC level was lower in the CKB supplemented group than the HD group. It is possible that the high fat diet was not effective in raising the serum triglyceride. We observed similar results in our earlier studies.<sup>(58–60)</sup> Epidemiologic



studies have shown an inverse correlation between HDL-c levels and the risk of cardiovascular disease.<sup>(61)</sup> But in our study, serum HDL-cholesterol concentration was significantly higher in the HD group than other groups. Hayek *et al.*<sup>(62)</sup> reported that the dietary fat-induced decrease in HDL-c and apo A-1 fractional catabolic rate may have been caused by the increase in HDL particle size in mice, as was suggested by their previous studies in humans. In another study, there was no significant difference observed in serum HDL-cholesterol level between HD group and CK fermented by *B. subtilis*.<sup>(63)</sup>

Our study had certain limitations, while preparing the diet we tried to maintain a similar amount of calorie between HD and CKB group; therefore it was not possible to arrange an equal amount of fat between the two diets. Adding 30% of CK in the high fat diet lowered the total fat in the CKB diet by 5%, though the carbohydrate in the CKB and HD diets remained unchanged. However, we noticed through several publications that at least 12% or more differences in the amount of fat content in the diet are required to produce significant differences in body weight gain between groups fed with different percentage of fat in the diet.<sup>(64–65)</sup> Therefore, we believe that, the difference in fat content between the HD and CKB diet might have some influence on the outcome, however the amelioration of the obesity related parameters in the CKB group was not solely due to 5% lower fat

content in the CKB compared to the HD group.

In conclusion, CK (CKB) fermented with *B. licheniformis*-67 has beneficial effects in metabolic syndrome related biomarkers. This study provided evidence that, CK supplementation prevents gain of body weight, epididymal fat mass; and averts the increase in hepatic TG and TC, along with serum TC; and improves oral glucose tolerance and thus insulin sensitivity. CKB supplementation increased the hepatic ACO and CPT-1 mRNA levels, whereas it lowered the hepatic LXR $\alpha$  mRNA levels. Also, the expression of PPAR $\alpha$ —the regulator of lipid catabolism, was higher in both the fermented as well as unfermented soybean groups. Insulin and leptin levels were significantly lower in the supplemented groups than in the HD group. Our study established the efficacy of CKB as anti-obesity food that mitigates obesity related symptoms, including fatty liver and insulin resistance.

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## Conflict of Interest

No potential conflicts of interest were disclosed.

## References

- Kopelman PG. Obesity as a medical problem. *Nature* 2000; **404**: 635–643.
- Després JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006; **444**: 881–887.
- Hennige AM, Burks DJ, Ozcan U, *et al.* Upregulation of insulin receptor substrate-2 in pancreatic beta cells prevents diabetic. *J Clin Invest* 2003; **12**: 1521–1532.
- Schreyer SA, Wilson DL, LeBoeuf RC. C57BL/6 mice fed high fat diets as models for diabetes-accelerated atherosclerosis. *Atherosclerosis* 1998; **136**: 17–24.
- Kwon DY, Jang JS, Lee JE, Kim YS, Shin DW, Park S. The isoflavonoid aglycone-rich fractions of Chungkookjang, fermented unsalted soybeans, enhance insulin signaling and peroxisome proliferator-activated receptor-g activity *in vitro*. *BioFactors* 2006; **26**: 245–258.
- Koh JB. Effects of cheonggukjang on lipid metabolism in hyperlipidemia female rats. *Korean J Nutr* 2006; **39**: 331–337.
- Kim JI, Kang MJ, Kwon TW. Antidiabetic effect of soybean and chongkukjang. *Korea Soybean Digest* 2003; **20**: 44–52.
- Kang MJ, Kim JL, Kwon TW. Effect of chongkukjang on blood glucose and lipid profile in neonatal streptozotocin-induced diabetic rats. *Food Sci Biotechnol* 2003; **12**: 544–547.
- Kwon DY, Hong SM, Lee JE, Sung SR, Park S. Long-term consumption of fermented soybean-derived Chungkookjang attenuates hepatic insulin resistance in 90% pancreatectomized diabetic rats. *Horm Metab Res* 2007; **39**: 752–757.
- Kwon DY, Jang JS, Hong SM, *et al.* Long-term consumption of fermented soybean derived Chungkookjang enhances insulinotropic action unlike soybeans in 90% pancreatectomized diabetic rats. *Eur J Nutr* 2007; **46**: 44–52.
- Yang HJ, Kwon DY, Moon NR, *et al.* Soybean fermentation with *Bacillus licheniformis* increases insulin sensitizing and insulinotropic activity. *Food Funct* 2013; **4**: 1675–1684.
- Sarkar PK, Hasenack B, Nout MJ. Diversity and functionality of *Bacillus* and related genera isolated from spontaneously fermented soybeans (Indian Kinema) and locust beans (African Soumbala). *Int J Food Microbiol* 2002; **77**: 175–186.
- Kim GM, Lee AR, Lee KW, *et al.* Characterization of a 27 kDa fibrinolytic enzyme from *Bacillus amyloliquefaciens* CH51 isolated from cheonggukjang. *J Microbiol Biotechnol* 2009; **19**: 997–1004.
- Kwon GH, Lee HA, Park JY, *et al.* Development of a RAPD-PCR method for identification of *Bacillus* species isolated from Cheonggukjang. *Int J Food Microbiol* 2009; **129**: 282–287.
- Yoon SH, Do JH, Lee SY, Chang HN. Production of poly- $\gamma$ -glutamic acid by fed-batch culture of *Bacillus licheniformis*. *Biotechnol Lett* 2000; **22**: 585–588.
- Park JH, Choi JC, Sung MH, Kang JH, Chang MH. High molecular weight poly- $\gamma$ -glutamic acid regulates lipid metabolism in rats fed a high-fat diet and humans. *J Microbiol Biotechnol* 2011; **21**: 766–775.
- Lee EH, Son WC, Lee SE, Kim BH. Anti-obesity effects of poly- $\gamma$ -glutamic acid with or without isoflavones on high-fat diet induced obese mice. *Biosci Biotechnol Biochem* 2013; **77**: 1694–1702.
- Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959; **37**: 911–917.
- Rossmesl M, Rim JS, Koza RA, Kozak LP. Variation in type 2 diabetes-related traits in mouse strains susceptible to diet-induced obesity. *Diabetes* 2003; **52**: 1958–1966.
- West DB, Boozer CN, Moody DL, Atkinson RL. Dietary obesity in nine inbred mouse strains. *Am J Physiol* 1992; **262** (6 Pt 2): R1025–R1032.
- Kim JH, Hahm DH, Yang DC, Kim JH, Lee HJ, Shim I. Effect of crude saponin of Korean red ginseng on high-fat diet-induced obesity in the rat. *J Pharmacol Sci* 2005; **97**: 124–131.
- Winzell MS, Ahrén B. The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. *Diabetes* 2004; **53** Suppl 3: S215–S219.
- Kwon SH, Lee KB, Im KS, Kim SO, Park KY. Weight reduction and lipid lowering effects of Korean traditional soybean fermented products. *J Korean Soc Food Sci Nutr* 2006; **35**: 1194–1199.
- Soh JR, Shin DH, Kwon DY, Cha YS. Effect of *Cheonggukjang* supplementation upon hepatic acyl-CoA synthase, carnitine palmitoyltransferase I, acyl-CoA oxidase and uncoupling protein 2 mRNA levels in C57BL/6J mice fed with high fat diet. *Genes Nutr* 2008; **2**: 365–369.
- Soret MG, Kupiecki FP, Wyse BM. Epididymal fat pad alterations in mice with spontaneous obesity and diabetes and with chemically induced obesity. *Diabetologia* 1974; **10** Suppl: 639–648.
- Suzuki M, Ding Q, Muranaka S, *et al.* Correlation between body weight (epididymal fat) and permeation rate of serum leptin through the blood-brain barrier (BBB) in male rats aged 8 months. *Exp Anim* 2008; **57**: 485–488.
- Guan HP, Goldstein JL, Brown MS, Liang G. Accelerated fatty acid oxidation in muscle averts fasting-induced hepatic steatosis in SJL/J mice. *J Biol Chem* 2009; **284**: 24644–24652.
- Braissant O, Foufelle F, Scotto C, *et al.* Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR $\alpha$ ,  $\beta$ , and  $\gamma$  in the adult rat. *Endocrinology* 1996; **137**: 354–366.
- Takahashi Y, Ide T. Effects of soy protein and isoflavone on hepatic fatty acid synthesis and oxidation and mRNA expression of uncoupling proteins and peroxisome proliferator-activated receptor gamma in adipose tissues of rats. *J Nutr Biochem* 2008; **19**: 682–693.
- Mezei O, Li Y, Mullen E, Ross-Viola JS, Shay NF. Dietary isoflavones

- supplementation modulates lipid metabolism via PPAR alpha-dependent and -independent mechanisms. *Physiol Genomics* 2006; **26**: 8–14.
- 31 Morifuji M, Sanbongi C, Sugiura K. Dietary soya protein intake and exercise training have an additive effect on skeletal muscle fatty acid oxidation enzyme activities and mRNA levels in rats. *Br J Nutr* 2006; **96**: 469–475.
- 32 Motojima K. Peroxisome proliferator-activated receptor (PPAR): structure, mechanisms of activation and diverse functions. *Cell Struct Funct* 1993; **18**: 267–277.
- 33 Yu GS, Lu YC, Gulick T. Co-regulation of tissue-specific alternative human carnitine palmitoyltransferase I beta gene promoters by fatty acid enzyme substrate. *J Biol Chem* 1998; **273**: 32901–32909.
- 34 Minnich A, Tian N, Byan L, Bilder G. A potent PPARalpha agonist stimulates mitochondrial fatty acid beta-oxidation in liver and skeletal muscle. *Am J Physiol Endocrinol Metab* 2001; **280**: E270–E279.
- 35 Louet JF, Chatelain F, Decaux JF, et al. Long-chain fatty acids regulate liver carnitine palmitoyltransferase I gene (L-CPT I) expression through a peroxisome-proliferator-activated receptor alpha (PPARalpha)-independent pathway. *Biochem J* 2001; **354** (Pt 1): 189–197.
- 36 Duplus E, Forest C. Is there a single mechanism for fatty acid regulation of gene transcription? *Biochem Pharmacol* 2002; **64**: 893–901.
- 37 Le May C, Catizac M, Diradourian C, et al. Fatty acids induce L-CPT I gene expression through a PPARalpha-independent mechanism in rat hepatoma cells. *J Nutr* 2005; **135**: 2313–2319.
- 38 Steffensen KR, Gustafsson JA. Putative metabolic effects of the liver X receptor (LXR). *Diabetes* 2004; **53**: S36–S42.
- 39 Tovar AR, Torre-Villalvazo I, Ochoa M, et al. Soy protein reduces hepatic lipotoxicity in hyperinsulinemic obese Zucker fa/fa rats. *J Lipid Res* 2005; **46**: 1823–1832.
- 40 Ronis MJ, Chen Y, Badeaux J, Badger TM. Dietary soy protein isolate attenuates metabolic syndrome in rats via effects on PPAR, LXR, and SREBP signaling. *J Nutr* 2009; **139**: 1431–1438.
- 41 Nazian SJ. Leptin secretion from the epididymal fat pad is increased by the sexual maturation of the male rat. *J Androl* 2001; **22**: 491–496.
- 42 Nogalska A, Stelmanska E, Sledzinski T, Swierozynski J. Surgical removal of perirenal and epididymal adipose tissue decreases serum leptin concentration and increases lipogenic enzyme activities in remnant adipose tissue of old rats. *Gerontology* 2009; **55**: 224–228.
- 43 Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* 2006; **444**: 847–853.
- 44 Rus VI, Dombay E, Catoi C, Gal A, Baba AI. Impaired glucose tolerance and adipose tissue inflammation in nutritional induced obesity in rat. *Lucrări Stiintifice Medicină Veterinară* 2008; **41**: 248–254.
- 45 Cerf ME, Chapman CS, Louw J. High-fat programming of hyperglycemia, hyperinsulinemia, insulin resistance, hyperleptinemia, and altered islet architecture in 3-month-old wistar rats. *ISRN Endocrinol* 2012; **2012**: 627270.
- 46 Davis J, Iqbal MJ, Steinle J, et al. Soy protein influences the development of the metabolic syndrome in male obese ZDFxSHHF rats. *Horm Metab Res* 2005; **37**: 316–325.
- 47 Mezei O, Banz WJ, Steger RW, Peluso MR, Winters TA, Shay N. Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW 264.7 cells. *J Nutr* 2003; **133**: 1238–1243.
- 48 Ishihara K, Oyaizu S, Fukuchi Y, et al. A soybean peptide isolate diet promotes postprandial carbohydrate oxidation and energy expenditure in type 2 diabetic mice. *J Nutr* 2003; **133**: 752–757.
- 49 Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor-beta. *Endocrinology* 1998; **139**: 4252–4263.
- 50 Sathyapalan T, Manuchehri AM, Thatcher NJ, et al. The effect of soy phytoestrogen supplementation on thyroid status and cardiovascular risk markers in patients with subclinical hypothyroidism: a randomized, double-blind, crossover study. *J Clin Endocrinol Metab* 2011; **96**: 1442–1449.
- 51 Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. *Obstet Gynecol* 1996; **87** (5 Pt 2): 897–904.
- 52 Kwon DY, Daily JW 3rd, Kim HJ, Park S. Antidiabetic effects of fermented soybean products on type 2 diabetes. *Nutr Res* 2010; **30**: 1–13.
- 53 Taniguchi A, Yamanaka-Okumura H, Nishida Y, et al. Natto and viscous vegetables in a Japanese style meal suppress postprandial glucose and insulin responses. *Asia Pac J Clin Nutr* 2008; **17**: 663–668.
- 54 Kwon DY, Hong SM, Ahn IS, Kim YS, Shin DW, Park S. Kochujang, a Korean fermented red pepper plus soybean paste, improves glucose homeostasis in 90% pancreatectomized diabetic rats. *Nutrition* 2009; **25**: 790–799.
- 55 Cederroth CR, Vinciguerra M, Gjinovci A, et al. Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism. *Diabetes* 2008; **57**: 1176–1185.
- 56 O'Neill HK. AMPK and exercise: glucose uptake and insulin sensitivity. *Diabetes Metab J* 2013; **37**: 1–21.
- 57 Okabe Y, Shimazu T, Tanimoto H. Higher bioavailability of isoflavones after a single ingestion of aglycone-rich fermented soybeans compared with glucoside-rich non-fermented soybeans in Japanese postmenopausal women. *J Sci Food Agric* 2011; **91**: 658–663.
- 58 Park JA, Tirupathi Pichiah PB, Yu JJ, Oh SH, Daily JW 3rd, Cha YS. Anti-obesity effect of kimchi fermented with *Weissella koreensis* OK1-6 as starter in high-fat diet-induced obese C57BL/6J mice. *J Appl Microbiol* 2012; **113**: 1507–1516.
- 59 Pichiah PB, Cha YS. *Salicornia herbacea* prevents weight gain and hepatic lipid accumulation in obese ICR mice fed a high-fat diet. *J Sci Food Agric* 2015; **95**: 3150–3159.
- 60 Pichiah PB, Moon HJ, Park JE, Moon YJ, Cha YS. Ethanolic extract of seabuckthorn (*Hippophae rhamnoides* L.) prevents high-fat diet-induced obesity in mice through down-regulation of adipogenic and lipogenic gene expression. *Nutr Res* 2012; **32**: 856–864.
- 61 Brewer HB Jr. High-density lipoprotein: a new potential therapeutic target for the prevention of cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2004; **24**: 387–391.
- 62 Hayek T, Ito Y, Azrolan N, et al. Dietary fat increases high density lipoprotein (HDL) levels both by increasing the transport rates and decreasing the fractional catabolic rates of HDL cholesterol ester and apolipoprotein (Apo) A-I. Presentation of a new animal model and mechanistic studies in human Apo A-I transgenic and control mice. *J Clin Invest* 1993; **91**: 1665–1671.
- 63 Park JH, Lee KW, Cho KD, et al. Effects of Korean traditional Cheonggukjang added with Job's Tears on body weight gains and lipid metabolism in rats fed high-fat diet. *J Korean Soc Food Sci Nutr* 2011; **40**: 409–415.
- 64 Boozer CN, Schoenbach G, Atkinson RL. Dietary fat and adiposity: a dose-response relationship in adult male rats fed isocalorically. *Am J Physiol* 1995; **268** (4 Pt 1): E546–E550.
- 65 Verschoyle RD, Greaves P, Cai H, Edwards RE, Steward WP, Gescher AJ. Evaluation of the cancer chemopreventive efficacy of rice bran in genetic mouse models of breast, prostate and intestinal carcinogenesis. *Br J Cancer* 2007; **96**: 248–254.