# Anti-obesity effects of traditional and standardized *meju* in high-fat diet-induced obese C57BL/6J mice

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The aim of the study was to evaluate the anti-obesity effects of two types of *meju* in diet induced obese C57BL/6J mice. Animals were randomly divided into 4 dietary group (*n* = 10); normal diet, high fat diet with 30% soybean, high fat diet with 30% traditional *meju*, high fat diet with 30% standardized *meju*. After 16 weeks, after animals were sacrificed. It was observed that the high fat diet with 30% traditional *meju* and high fat diet with 30% standardized *meju* significantly reduced body weight gain, epididymal fat weight, serum triglyceride along with serum insulin and leptin levels compared to the high fat diet with 30% soybean. And also, the expression levels of hepatic lipid anabolic genes were significantly decreased in the high fat diet with 30% traditional *meju* and high fat diet with 30% standardized *meju* compared to the high fat diet with 30% soybean. In conclusion, the assessment of all the obesity markers strongly advocate the anti-obesity effect of traditional as well as standardized *meju* in diet induce obesity conditions.

*Key Words*: *meju*, high fat diet, lipid metabolism, C57BL/6J mice, anti-obesity

Obesity is currently a global pandemic, and represents an important health problem both in developed and developing countries.[1] Based on data from 106 countries, 24% of the world's male population is overweight and 8% are obese, with much higher numbers in the established economies.[2] Obesity severely distorts the normal physiology leading to a severe condition well known as metabolic syndrome. The metabolic syndrome is accompanied by insulin resistance, hyperinsulinemia, dyslipidemia, hyperleptinemia, fatty liver etc.[3] If not prevented or treated at the earliest, obesity associated metabolic syndrome can provoke a life threatening situation.

Several approaches are available in managing or preventing obesity, including bariatric surgery, exercise, drugs, vaccines etc. However, natural products like herbal medications, probiotics etc. and fermented foods are considered much safer to use.[4–6] Food plays a vital role in our day to day life, and has higher effect on our health than another factor. Soybean is pulse rich in protein, flavonoid and lipids. Soybeans are consumed in many ways for example as milk, sprouts, tofu etc. In Korea, many fermented food products are prepared using soybean including Kochujang, Doenjang and Ganjang (Korean traditional soy sauce).[7–9] Recently, soybean fermented products showed significant anti-obesity properties.[10] *Meju*, is one of the soybean fermented product most widely used in Korea. During the process of *meju* fermentation, microbial enzymes play a major role affecting the quality of paste due to the production of various types of

peptide,[11] that modulates the nutritional value of *meju*, and also contribute to taste, dissolution and emulsification and many other related parameters.

*Meju* is well known to exert many health promoting activities like anti-cancer, lowering of blood pressure and serum cholesterol, enhancement of immune function and promotion of calcium absorption.[12] The traditionally *meju* are produced by cooked, crushed soybean that are made into blocks of cake followed by outdoor fermentation for 60 to 90 days by microorganisms present in the environment. The *Aspergillus* and *Bacillus* species are the predominant microbes playing major role in fermenting *meju* produced through tradition procedure.[13] Standardization of *meju* fermentation process is difficult because of the fermentation period varies depending on location and the individual preparing it and its industrialization faces tricky problems.[14] It takes 60 days to prepare *meju* using traditional method; however, standardized *meju* can be made in a short duration of 6 days by inoculating with *Bacillus subtilis* and *Aspergillus oryzae*. However, the health benefit of both of these *meju* is not documented. There have been many studies using Korean fermented foods like *Chungkukjang*, *Doenjang* paste and *Kochujang* showing anti-obesity properties. However, *meju* is lacking such studies, elucidating its anti-obesity properties in animal models. It is very necessary to evaluate both the traditional and standard *meju*s in order to find whether the standardized form of *meju* has similar qualities and produces the beneficial effects similar to the traditional *meju*. In case of affirmative results; it would be beneficial to adapt the standardized procedure for large scale production of *meju* due to its relatively short duration of maturation.

Therefore in this present study we compare the anti-obesity properties of the *meju* samples prepared by two different methods; a traditional and a standardized method on diet induced obese C57BL/6J mice.

## Materials and Methods

**Preparation of traditional and standardized *meju*.** The two *meju* samples used in our study were prepared by the following method (Fig. 1); soybeans were washed and soaked in water for 12 h at 15°C and boiled for 30 min at 120°C. For the traditional *meju*, cooked soybeans were crushed and formed into blocks and fermented outdoors for 60 days by micro-organisms present in the environment. For making standardized *meju*; the cooked soybeans were inoculated with *Bacillus subtilis*, dried

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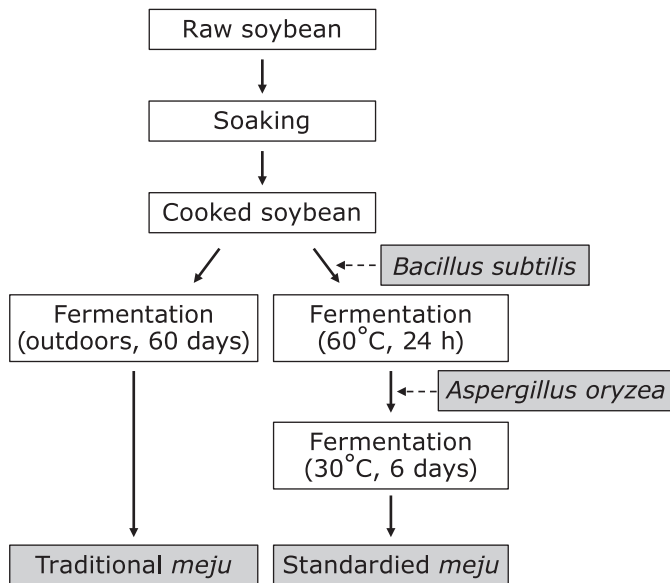E-mail: cha8@jbnu.ac.kr

doi: 10.3164/jcbn.13-52
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J. Clin. Biochem. Nutr. | January 2014 | vol. 54 | no. 1 | 45–50

**Fig. 1.** Manufacturing process of traditional and standardized *meju*.

**Table 1.** Proximate composition (% dry basis) of the *meju* samples

| Composition | SS[1] | KMJ[2] | MJ[3] |
|---|---|---|---|
| Carbohydrate | 33.11 | 34.27 | 29.82 |
| Crude protein | 41.25 | 42.63 | 44.28 |
| Crude fat | 16.91 | 14.70 | 16.62 |
| Moisture | 3.64 | 3.11 | 3.21 |
| Ash | 5.09 | 5.29 | 6.07 |

[1]SS, soybean; [2]KMJ, 60 days traditional *meju*; [3]MJ, 6 days standardized *meju*.

**Table 2.** Composition of experimental diets

| Ingredient (g) | ND[1] | HD | | |
| | | SS[2] | KMJ[3] | MJ[4] |
|---|---|---|---|---|
| Casein, lactic | 18.96 | 31.65 | 32.05 | 32.73 |
| L-cystine | 0.28 | 0.27 | 0.27 | 0.27 |
| Corn starch | 29.86 | 10.88 | 11.23 | 9.86 |
| Maltodextrin | 3.32 | 11.31 | 11.31 | 11.31 |
| Sucrose | 33.17 | 6.22 | 6.22 | 6.22 |
| Cellulose | 4.74 | 4.52 | 4.52 | 4.52 |
| Soybean oil | 2.37 | 7.82 | 7.07 | 7.76 |
| Lard | 1.90 | 22.16 | 22.16 | 22.16 |
| Mineral mix | 0.95 | 0.90 | 0.90 | 0.90 |
| Dicalcium phosphate | 1.23 | 1.18 | 1.18 | 1.18 |
| Calcium carbonate | 0.52 | 0.50 | 0.50 | 0.50 |
| Potassium citrate | 1.56 | 1.49 | 1.49 | 1.49 |
| Vitamin mix | 0.95 | 0.90 | 0.90 | 0.90 |
| Choline bitarrate | 0.19 | 0.18 | 0.18 | 0.18 |
| Total (g) | 100 | 100 | 100 | 100 |

[1]AIN-93 modified diet with 4% fat (10% fat calories) content. [2]AIN-93 modified high fat diet with 35% fat (60% fat calories) containing soybean 30%. [3]AIN-93 modified high fat diet containing 60 days traditional *meju* 30%. [4]AIN-93 modified high fat diet containing 6 days standardized *meju* 30%.

**Table 3.** Sequences of primers for PCR amplification

| | | Primer sequence |
|---|---|---|
| SREBP-1c | F: | 5'-GCGGAGCCATGGATTGCAC-3' |
| | R: | 5'-CTCTTCCTTGATACCAGGCCC-3' |
| ACC | F: | 5'-CCAACATGAGGACTATAACTTCCT-3' |
| | R: | 5'-TACATACGTGCCGTCAGGCTTCAC-3' |
| PPARγ | F: | 5'-GCTGTTATGGGTGAAACTCTG-3' |
| | R: | 5'-ATAAGGTGGAGATGCAGGTTC-3' |
| CPT-1 | F: | 5'-AAAGATCAATCGGACCCTAGACA-3' |
| | R: | 5'-CAGCGAGTAGCGCATAGTCA-3' |
| PPARα | F: | 5'-GGATGTCACACAATGCAATTCGCT-3' |
| | R: | 5'-CAGCGAGTAGCGCATAGTCA-3' |
| β-actin | F: | 5'-GTGGGGCGCCCCAGGCACCAG-3' |
| | R: | 5'-CTCCTTAATGTCACGCACGATTTC-3' |

and stored in blocks, the temperature of surface for drying was maintained at 60°C for 24 h. After that the *meju* were inoculated with of *Aspergillus oryzae* and left in fermentation chamber at 30°C for 6 days. After 6 days period the *meju* samples were lyophilization at −70°C and mixed with AIN-93 modified rodent diet. Their proximate compositions are shown in Table 1.

**Animals and diets.** Male C57BL/6J mice, aged 4 weeks, were purchased from Charles River Laboratories (Tokyo, Japan). The animals were maintained on a pellet diet (Research Diets, New Brunswick, NJ) for 1 week, and then randomly divided into four groups: normal diet (ND), high fat diet with 30% soybean (SS), high fat diet with 30% traditional *meju* (KMJ), high fat diet with 30% standardized *meju* (MJ). Each group consisted of ten mice. The compositions of the experimental diets (Research Diets) are shown in Table 2. The animals were housed in a temperature-controlled environment with a 12 h light/dark cycle and were given pair fed feed and *ab libitum* water daily during the entire experimental period of 16 weeks. The feed intake and body weight were measured daily and weekly, respectively. The experimental protocol was approved by the Animal and Use Committee of Chonbuk National University (CBU 2008-025).

**Collection of serum and tissue samples.** After 12 h of overnight fasting, blood was collected by orbital vein puncture and kept on ice for 1 h. Serum was separated from the blood by centrifugation at 1,100 × g for 15 min at 4°C (Micro 17R, Hanil Science In Co. Ltd., Gangneung, Korea). Tissues were removed, weighed and quickly frozen in liquid nitrogen. Both tissue and serum samples were stored at −80°C until analysis.

**Analysis of serum and hepatic lipids profile.** Serum and hepatic triglyceride (TG), total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C) were measured using commercial kit (Asan Pharmaceutical Co., Seoul, Korea).

**Analysis of insulin and leptin.** Serum insulin and leptin level were measured using commercially available Kits (Shibayagi, Shibukawa, Japan) and Quantikine® Immunoassay kit (R&D system, Minneapolis, MN).

**Quantitative real-time polymerase chain reaction (PCR) analysis.** Total RNA was extracted from liver by Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA) and the concentration was measured spectrophotometrically. The extracted RNA was reverse transcribed into cDNA using 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 SYBER Green PCR Master Mix (Applied Biosystems, Woolston, Warrington, UK) and the 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA) according to the manufacture's protocol. Gene specific primers used are given in Table 3. Relative quantification of gene expression with real-time PCR data was calculated relative to β-actin.

**Statistical analysis.** The data were analyzed by one-way ANOVA using SPSS ver. 16.0 program. Values are expressed as mean ± SD. The differences among groups assessed using Duncan's multiple range tests. Statistical significance was considered at $p<0.05$.

## Results

**Feed intake, body weight and epididymal adipose tissue weight.** The changes in feed intake and body weight are shown in Table 4. There was no significant difference in feed intake between the groups. However, a significant difference in the quantity of energy intake was observed. All the three HD groups had a significant higher energy intake compared to the ND group. But, animals in the KMJ and MJ group showed a significantly lesser weight gain compared to the SS group. Therefore, the final body weight of animals in KMJ and MJ groups were significantly lower compared to SS group, and resembled the ND group. To examine the changes in the adipose tissue mass, we measured the epididymal fat pad weight. The epididymal adipose tissue weight was significantly lower in the KMJ and MJ groups than the SS group (Fig. 2).

**Serum and hepatic lipid profile.** The serum and hepatic lipid profile are shown in Table 5. The KMJ and MJ groups showed a significant decrease in serum triglyceride compared to the ND group. The serum TG level in SS, KMJ and MJ group were not significant, however a KMJ and MJ showed a lower TG level compared to the SS group. The serum TC level was not significantly different between the groups; however, the serum TC in the MJ group was lower than SS and KMJ groups. The HDL-C level was significantly increased in the KMJ group compared to rest of the groups. HDL-C/TC ratio was significantly higher in the KMJ group than the MJ group. The hepatic TG level was significantly decreased in the KMJ group than the SS group. There was no significant difference in the hepatic TC level among the group, however, the MJ group showed a lower hepatic TG level than the SS group.

**Serum insulin and leptin levels.** Changes in serum insulin and leptin levels are shown in Fig. 3. Insulin and leptin level in serum were significantly decreased in the KMJ and MJ groups than the SS group.

**Hepatic mRNA expression levels.** The mRNA expression levels of lipid metabolic genes are shown in Fig. 4. The regulators for fatty acid synthesis: SREBP-1c, PPARγ and ACC mRNA expression levels were significantly down-regulated in both the KMJ and MJ groups compared with the SS group. Moreover, KMJ group showed lower expression level than the MJ group. The expression level of PPARα, the transcriptional regulator of β-
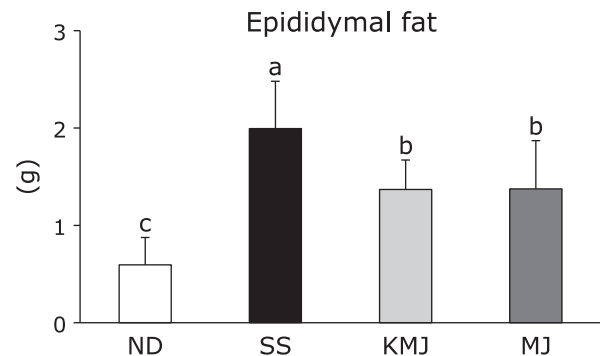


**Fig. 2.** Epididymal fat weight of mice fed experimental diets for 16 weeks. ND, AIN-93 modified diet with 4% fat (10% fat calories) content; SS, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing soybean 30%; KMJ, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing 60 days traditional *meju* 30%; MJ, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing 6 days standardized *meju* 30%. All values are mean ± SD. Values with different superscripts are significantly different among high fat diet groups by ANOVA with Duncan's multiple range test at *p*<0.05.

**Table 4.** Body weight gain and body composition between groups

| Groups | ND[1] | HD | | |
| --- | --- | --- | --- | --- |
| | | SS | KMJ | MJ |
| Initial body weight (g)[2] | 20.38 ± 0.73 | 20.54 ± 1.16 | 20.07 ± 1.18 | 19.53 ± 1.59 |
| Final body weight (g) | 31.24 ± 1.66[b] | 37.96 ± 3.81[a] | 33.64 ± 1.34[b] | 31.57 ± 1.86[b] |
| Body weight gain (g) | 11.44 ± 2.16[b] | 18.64 ± 2.61[a] | 13.80 ± 1.46[b] | 11.68 ± 2.22[b] |
| Feed intake (g/day) | 2.63 ± 0.01 | 2.46 ± 0.04 | 2.37 ± 0.06 | 2.39 ± 0.08 |
| Energy intake (kcal/day) | 10.12 ± 0.04[b] | 12.78 ± 0.19[a] | 12.31 ± 0.31[a] | 12.40 ± 0.40[a] |

[1]ND, AIN-93 modified diet with 4% fat (10% fat calories) content; SS, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing soybean 30%; KMJ, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing 60 days traditional meju 30%; MJ, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing 6 days standardized meju 30%. [2]All values are mean ± SD. Values with different superscripts are significantly different among high fat diet groups by ANOVA with Duncan's multiple range test at *p*<0.05.

**Table 5.** Lipid concentrations in serum and liver

| Groups | ND[1] | HD | | |
| --- | --- | --- | --- | --- |
| | | SS | KMJ | MJ |
| Serum (mg/dl)[2] | | | | |
| Triglyceride | 98.44 ± 15.87[a] | 91.74 ± 22.93[ab] | 64.53 ± 28.18[b] | 57.98 ± 25.75[b] |
| Total cholesterol | 117.87 ± 18.53 | 110.76 ± 18.88 | 111.74 ± 12.49 | 112.13 ± 14.97 |
| HDL-cholesterol | 48.24 ± 14.24[b] | 63.74 ± 12.98[b] | 82.68 ± 18.01[a] | 63.92 ± 15.08[b] |
| HDL-c/TC (%) | 41.14 ± 10.61[b] | 58.62 ± 16.93[b] | 76.74 ± 22.85[a] | 51.57 ± 11.63[b] |
| Liver (mg/g) | | | | |
| Triglyceride | 32.60 ± 5.69 | 32.80 ± 5.22 | 25.23 ± 5.32 | 27.72 ± 6.65 |
| Total cholesterol | 5.03 ± 1.57 | 5.55 ± 1.87 | 4.26 ± 1.99 | 3.82 ± 1.06 |

[1]ND, AIN-93 modified diet with 4% fat (10% fat calories) content; SS, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing soybean 30%; KMJ, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing 60 days traditional meju 30%; MJ, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing 6 days standardized meju 30%. [2]All values are mean ± SD. Values with different superscripts are significantly different among high fat diet groups by ANOVA with Duncan's multiple range test at *p*<0.05.
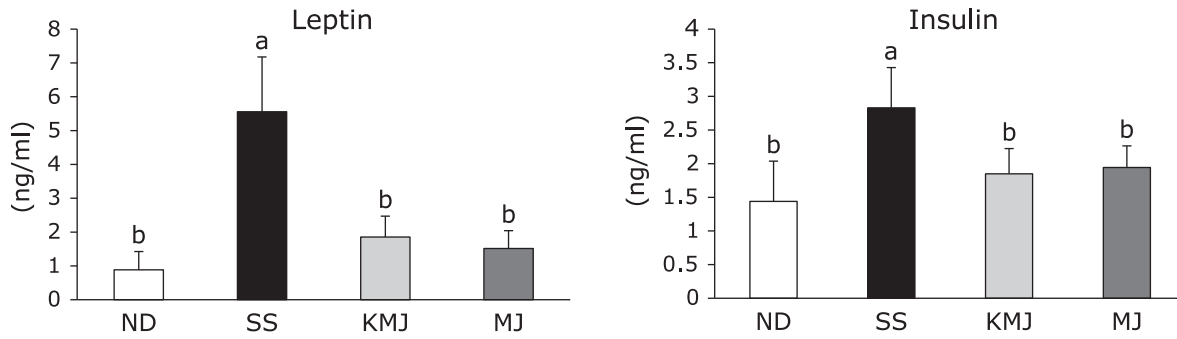
**Fig. 3.** Concentrations leptin and insulin in serum. ND, AIN-93 modified diet with 4% fat (10% fat calories) content; SS, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing soybean 30%; KMJ, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing 60 days traditional *meju* 30%; MJ, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing 6 days standardized *meju* 30%. All values are mean ± SD. Values with different superscripts are significantly different among high fat diet groups by ANOVA with Duncan's multiple range test at *p*<0.05.
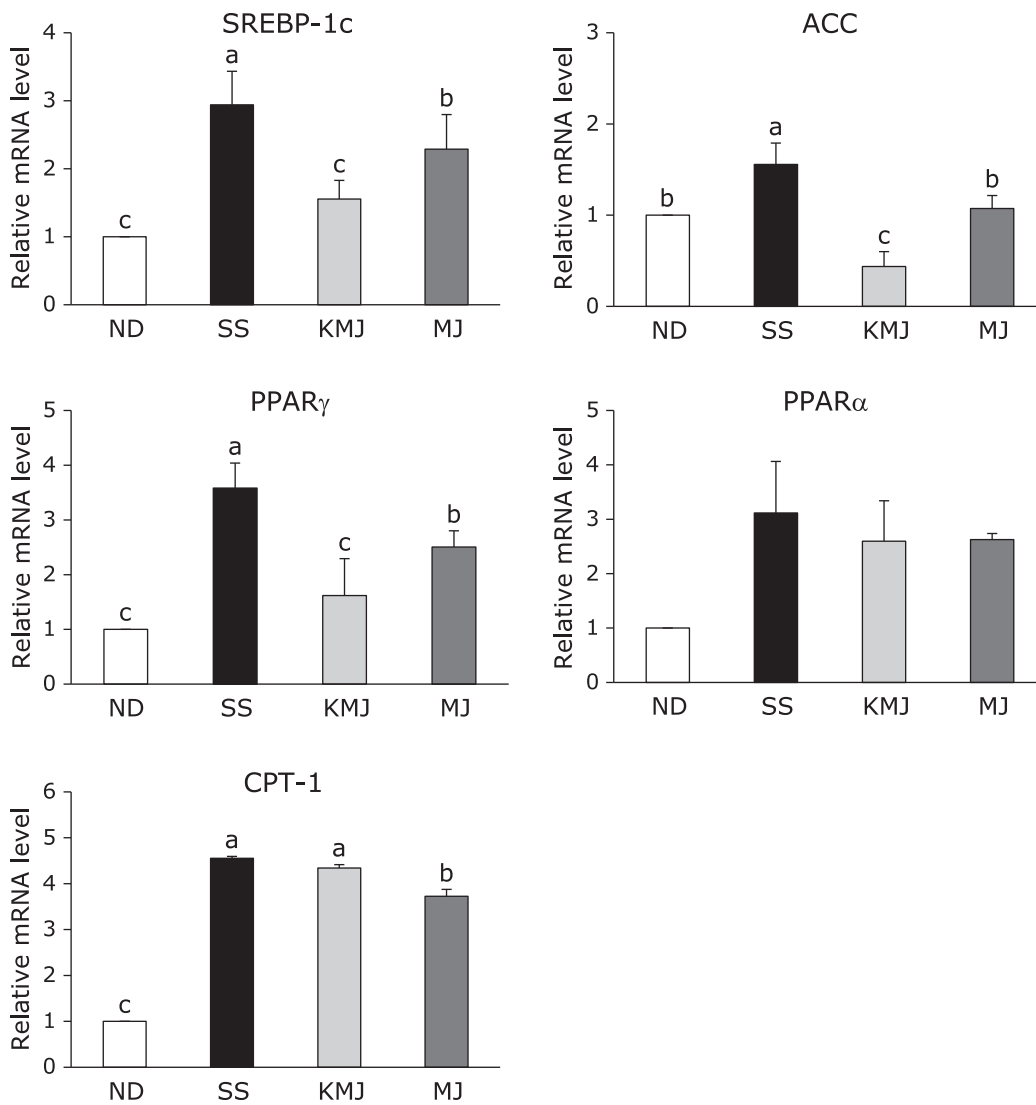


**Fig. 4.** Expression of lipid metabolism-related gene in the liver. ND, AIN-93 modified diet with 4% fat (10% fat calories) content; SS, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing soybean 30%; KMJ, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing 60 days traditional *meju* 30%; MJ, AIN-93 modified high fat diet with 35% fat (60% fat calories) containing 6 days standardized *meju* 30%. All values are mean ± SD. Values with different superscripts are significantly different among high fat diet groups by ANOVA with Duncan's multiple range test at *p*<0.05.

oxidation, was not significantly changed among the entire groups. Whereas the expression level of CPT-1 in the SS and KMJ groups were significantly higher than the MJ group.

## Discussion

Anti-obesity effects of Korean fermented soybean foods have been reported by several investigators.[15,16] *Meju* is the principle ingredient of many Korean traditional fermented soybean food items like *kochujang, doenjang, ganjnag* (soy sauce). These traditional foods shows anti-obesity effect in diet induced obese animals, and significantly reduces body weight gain and triglyceride compared to the control group.[17,18] In our present study, KMJ and MJ groups showed lesser body weight gain compared to the SS group (Table 3). The prevention of weight gain in KMJ and MJ group was due to decreased epididymal fat mass gain (Fig. 2). A recent study suggested that soy isoflavone genistein shows anti-obesity effect and prevents weight gain and visceral fat accumulation.[19–21] *Meju*, being a soy product is rich is genistein; fermentation process initiates cleavage of the β-glycosyl bond of genistin by microbial enzymes,[22] leading to further increases of genistein amount in fermented soy product comparing to non-fermented counterpart.[23] Therefore, in our study the body weight and epididymal fat mass gain could be prevented due to the presence of higher genistein content in KMJ and MJ compared to SS samples.

A lower level of serum TG levels was observed in KMJ and MJ groups than SS group (Table 4). Our data is in agreement with the study by Kim *et al.*[24] and Park *et al.*[25] showing the serum triglyceride and total cholesterol lowering effect of *meju*.

Serum leptin and insulin levels are increased in obesity condition making them an important marker for assessing the severity of obesity. Leptin is adipose tissue derived hormone positively correlated with the extent of the triglyceride stores in adipocytes.[26,27] Serum leptin concentrations are directly proportional to the amount of adipose tissue mass.[28,29] Earlier reports suggests that higher intake of high fat diet, raises serum leptin level leading to a condition known as leptin resistance.[30] In our study, serum leptin levels decreased in the KMJ and MJ groups compared to the SS group. The reason for lower leptin levels in KMJ and MJ groups could be due to reduced epididymal tissue mass when compared to the HD group. Insulin is an anabolic hormone and involves in many function including glucose uptake by muscles, liver and adipose tissues; triglyceride synthesis in liver and adipose tissue. In a comparison between, soy protein and casein, the author reported that, soy protein were more effective in reducing the serum insulin concentrations comparing to casein.[31] Recent studies showed that, both the traditional as well as standardized *meju* and *chungkookjang* increases β-cell mass and improve insulinotropic activity and insulin sensitivity. It was also noticed that, comparing to steamed soybean, *meju*, and *chungkookjang* have a greater effect in improving insulin sensitivity and increasing β-cell mass.[32,33] As a result of fermentation, *meju* have increased level of isoflavonoid aglycones comparing to soybean, thus *meju* intake could facilitate insulin sensitivity by lowering the level of serum insulin. In our study, the lower serum insulin (Fig. 3) in KMJ and MJ group might be due to the isoflavonoid aglycones.

To gain insight into the molecular mechanism underlying the anti-obesity effects of KMJ and MJ described above, we analyzed the expression patterns of genes involved in lipid metabolism.

Our results showed the hepatic expression level of SREBP-1c, PPARγ and ACC mRNA were reduced in KMJ and MJ groups than the SS group (Fig. 4). SREBP-1c is regarded as a key transcription factors to regulate the expression of lipogenic enzymes. [34] PPARγ is a nuclear transcription factor that regulates key lipogenic genes including the ACC. In animal models with high fat diet induced obesity the expression level of PPARγ is up-regulated and consequently activates the lipogenic target genes,[35] suggesting that SREBP-1c and PPARγ are mainly responsible for lipogenic gene expression and promotes fatty acid synthesis in the liver.[36] On the other hand, CPT-1 is rate-limiting enzyme regulated by PPARα, it acts as lipid transporter for mitochondria-mediated lipid β-oxidation.[37]

In this study the hepatic PPARα mRNA expression level was increased in SS, KMJ and MJ groups than the ND group. However, no significant differences were observed among the experimental groups. The CPT-1 mRNA expression significantly increased in SS and KMJ groups than the MJ group. In a study, Tovar *et al.*[38] reported that soybean diet increased PPARα gene expression in the liver and is associated with a higher content of CPT-1 mRNA. It was suggested that SS and KMJ increased the activity of CPT-1 more than MJ group.

In conclusion, the results of this study demonstrated that both the *meju* fermented either by tradition procedure or standardized procedure decreases the amount of epididymal fat, and improved the lipid profile through inhibition of hepatic lipogenesis in high fat fed mice. Korean traditional *meju* and standardized *meju* significantly enhances its physiological effect that was comparable with that of soybean. Therefore, Korean traditional and standardized *meju* can be used as functional food with preventive therapy against high fat diet induced obesity.

## Acknowledgments

## Abbreviations

| | |
|---|---|
| ACC | acetyl-CoA carboxylase |
| CPT-1 | carnitine palmitoyltransferase-1 |
| HDL-C | high density lipoprotein-cholesterol |
| KMJ | high fat diet with 30% traditional *meju* |
| MJ | high fat diet with 30% standardized *meju* |
| ND | normal diet |
| PCR | polymerase chain reaction |
| PPARα | peroxisome proliferator-activated receptor α |
| PPARγ | peroxisome proliferator-activated receptor γ |
| SS | high fat diet with 30% soybean |
| SREBP-1c | sterol regulatory element-binding protein-1c |
| TC | total cholesterol |
| TG | hepatic triglyceride |

## Conflict of Interest

No potential conflicts of interest were disclosed.

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