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Differential Effects of Powdered Whole Soy Milk and Its Hydrolysate on Antiobesity and Antihyperlipidemic Response to High-Fat Treatment in C57BL/6N Mice

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ABSTRACT: This study was performed to investigate the beneficial effects of powdered whole soy milk and its hydrolysate, compared to the processed soy milk and its hydrolysate, on the alteration of lipid metabolism and their possible effects on antiobesity in C57BL/6N mice fed a high-fat and -cholesterol diet. The mice were divided into a control group (20% casein) and four test groups for 5 weeks: soy milk (SM, 20% soy milk protein), soy milk hydrolysate (SMH, 20% hydrolyzed soy milk protein), whole soy milk (WSM, 20% whole soy milk protein), and whole soy milk hydrolysate (WSMH, 20% whole soy milk hydrolysate protein). The body weight and adipose tissue weights were significantly lowered in SMH, WSM, and WSMH groups compared to the control group despite providing an isoenergetic diet. Plasma lipid concentrations and hepatic fatty acid synthase (FAS) and glucose-6-phosphate dehydrogenase (G6PD) activities were significantly lowered in all soy milk groups; however, the hepatic lipid contents and malic enzyme (ME) activity were only significantly lowered in the WSM and WSMH groups, compared to the control group. Data suggest that powdered WSM or WSMH appears to be more beneficial than SM or SMH in overall antiobesity and antihyperlipidemic properties following in the order WSMH/WSM, SMH, SM, and casein.

KEYWORDS: antiobesity, antihyperlipidemic response, whole soybean milk, whole soybean milk hydrolysate

INTRODUCTION

Modern lifestyle, with an abundant nutrient supply and reduced physical activity, has resulted in dramatic increases in metabolic syndrome-related diseases including obesity, dyslipidemia, diabetes, and cardiovascular disease.[1] Dietary fat is one of the most important environmental factors associated with the obesity that results in accumulation of excess body fat.[2] In the body, white adipose tissue (WAT) is a major site of energy storage and is important for energy homeostasis; it stores energy in the form of triglycerides during calorie abundance and releases it as free fatty acids (FFAs) during deprivation.[3]

Various dietary interventions to control excess body weight and dyslipidemia have included low-energy and low-fat diets and the consumption of vegetables, fruits, and grains, foods with high fiber and antioxidant contents. In recent years, increased attention also has been directed toward the role of dietary protein intake in obesity and hyperlipidemia. Soy protein appears to be one of the good choices for inclusion in a weight-loss regimen. Plant proteins can be more beneficial for health promotion than animal protein sources[4] with regard to improving hyperlipidemia. Consumption of the phytoestrogen-rich soybean has been shown to have a beneficial effect by improving serum lipids, obesity, and diabetes.

Numerous investigators have shown that foods containing phytochemicals with antioxidant potential have strong protective effects against the risk of cancer and cardiovascular diseases.[5] Increasing evidence from nutritional intervention studies in animals and humans indicates that dietary soy protein has beneficial effects on obesity;[6] however, the functions of whole soy milk or its hydrolyzed product have not been reported in comparison with regular soy milk. We prepared whole soy milk and its hydrolysate in an attempt to modify the amounts of isoflavone aglycones or possibly molecule sizes of soy peptides present in the soy milk. Accordingly, the objective of this study was to investigate the supplementary effects of the soy milks we prepared [soy milk (SM), soy milk hydrolysate (SMH), whole soy milk (WSM), and whole soy milk hydrolysate (WSMH)] and their possible actions on body fat-lowering and alteration of lipid metabolism in C57BL/6N mice. These effects also were compared with that of casein as an animal protein source.

MATERIALS AND METHODS

Preparation of Soy Milk and Its Hydrolysate and Analysis of Crude Composition. Soybean used in this study was cultivated in the Sangju region of Kyungpook province in 2008. As the enzymes for the hydrolysis of soy milk, the enzyme produced by KMF-G (70000 PU/g; optimal temperature, 55 °C; pH range, 6.0−8.0; KMFoodex. Co., Ltd., Korea) composed by fungus originated protease and bacterium originated glucoamylase was used. SM, WSM, SMH, and WSMH were

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Table 1. Proximate Composition of the Powdered Soybean Milk Including Isoflavones[a]

	SM	SMH	WSM	WSMH
composition		percent		
moisture	5.18	5.03	5.19	5.35
crude protein	41.44	44.54	37.48	38.10
crude fat	24.78	25.88	23.35	21.17
ash	5.71	5.92	4.31	5.93
crude fiber	0.45	0.24	3.49	2.64
carbohydrate	22.44	18.39	26.18	26.81
total	100	100	100	100
isoflavones content		μg/g of powdered soy milk		
daidzin	578 ± 23	547 ± 29	750 ± 32	712 ± 25
genistin	681 ± 22	657 ± 34	881 ± 27	845 ± 32
daidzein	24 ± 8	21 ± 9	27 ± 6	25 ± 5
genistein	32 ± 9	31 ± 7	40 ± 7	38 ± 6
total	1313 ± 47	1254 ± 45	1692 ± 55	1623 ± 156

[a] Data were provided from KMFOODEX. Mean ± SE. n = 3. SM, soy milk (20% soy milk protein); SMH, soy milk hydrolysate (20% soy milk hydrolysate protein); WSM, whole soybean milk (20% whole soy milk protein); WSMH, whole soy milk hydrolysate (20% whole soy milk hydrolysate protein).

prepared by following the procedure described below. One hundred grams of whole bean was washed three times, soaked for about 7−8 h, and then heated at 100 °C for 20 min. After removal of the seed coat of soybeans, 700 mL of water was added and ground for 10 min by using a homogenizer (HF-93, SMT Co., Japan) at the speed of 15000 rpm, and the soluble solid content was adjusted to 10% (w/v) to prepare WSM. SM was obtained by removing soy milk cake residues from the WSM. To both SM and WSM was added 0.2% (w/w) KMF-G, and the mixture was hydrolyzed for 1 h at 50 °C; SMH and WSMH were obtained following inactivation of the enzymes at 100 °C for 10 min. All samples were freeze-dried by using a vacuum freeze-dryer (SFD SM24L, Samwon, Korea) and ground for less than 150 μm particle size for the final use. The general composition was determined, and isoflavone contents were analyzed with the freeze-dried samples by using HPLC (Table 1).[7]

Animals and Diets. Four-week-old, male C57BL/6N mice were purchased from Orient Inc. (Seoul, Korea) and maintained under standard light (12 h light/dark), temperature (22 ± 2 °C), and humidity (40 ± 10%) conditions. Fifty mice were fed a pelletized commercial chow diet for a period of 2 weeks after arrival and then were randomly divided into five groups (n = 10) and housed individually. Thereafter, the control group of C57BL/6N mice was fed a 20% high-fat diet (corn oil/lard = 1:1) with 0.1% cholesterol, whereas the other four groups were fed experimental diets in which casein was replaced with various types of soy milk originated proteins for 5 weeks: SM, SMH, WSM, or WSMH (Table 2).

Each soy milk protein given as powdered soy milk is equivalent to 20 g of proteins per 100 g of diet to keep isoenergetic and isonitrogenous conditions as in the control diet (Table 2). The animals were given food and distilled water ad libitum. Food consumption and weight gain were measured daily and weekly, respectively. At the end of the experimental period, the mice were anesthetized with ketamine after withholding food for 12 h, and blood samples were taken from the inferior vena cava to determine the plasma biomarkers. Also, the liver and adipose tissues including the epididymal WAT, perirenal WAT, and brown adipose tissue (BAT) were removed after blood collection, rinsed with a physiological saline solution, weighed, and immediately stored at −70 °C. The mice were treated in accordance with Kyungpook National University guidelines for the care and use of laboratory animals.

Plasma and Hepatic Lipid Levels. The plasma FFA concentration was measured using an enzymatic nonesterified fatty acid kit (Wako, Osaka, Japan). Meanwhile, the plasma triglyceride and total cholesterol concentrations were measured spectrophotometrically using a commercial kit (Sigma Chemical Co., St. Louis, MO). The hepatic lipids were extracted using the method of Folch et al.,[8] and the levels of triglyceride and cholesterol in the liver were analyzed with the same commercial kit as used in the plasma analysis.

Preparation of Enzyme Sources. The hepatic enzyme source was prepared according to the method developed by Hulcher and Oleson[9] with a slight modification. Liver and adipocyte tissues were homogenized in a 5-fold weight of a buffer containing 0.1 mol/L of triethanolamine, 0.02 mol/L of EDTA, and 2 mmol/L of dithiothreitol, pH 7.0, and centrifuged at 600g for 10 min at 4 °C to discard any cell debris, and then the supernatant was centrifuged at 10000g for 20 min followed by 12000g for 20 min at 4 °C to remove the mitochondrial pellet. Thereafter, the supernatant was ultracentrifuged twice at 100000g for 60 min at 4 °C to obtain the cytosolic supernatant. The resulting mitochondrial and microsomal pellets were then redissolved in 800 μL of a homogenization buffer, and the protein content was determined according to the method of Bradford[10] using bovine serum albumin as the standard.

Lipid Regulating Enzyme Activities in Liver and Epididymal Adipose Tissues. The fatty acid synthase (FAS) activity was measured according to the method of Carl et al.[11] by monitoring the malonyl-CoA-dependent oxidation of NADPH at 340 nm, where the activity was represented by the oxidized NADPH nmol/min/mg of protein. The glucose-6-phosphate dehydrogenase (G6PD) activity was assayed by spectrophotometric methods according to the procedures described by Pitkanen et al.,[12] where the activity was expressed as the reduced NADPH nmol/min/mg of protein. The malic enzyme (ME) activity was measured according to the method of Ochoa[13] by monitoring the production of NADPH at 340 nm, where the activity was represented by the formation of NADPH nmol/min/mg of protein.

Histological Analysis of Liver and White Adipose Tissue. Liver and epididymal WAT were removed from the mice and fixed in a buffer solution of 10% formalin. Fixed tissues were processed routinely for paraffin embedding, and 4 μm sections were prepared and stained with hematoxylin eosin (H&E); stained areas were viewed using an optical microscope (Zeiss Axioscope, Germany) with a magnifying power of ×200, and epididymal adipocyte size were measured by using Leica Application Suite software ver. 2.8.1 (Leica, Bensheim, Germany).

Statistical Analysis. All data are presented as the mean ± SE. Significant differences among the groups were determined by one-way ANOVA using SPSS (version 12.0, Chicago, IL). Duncan's multiple-range test was performed when the F test was significant ($p < 0.05$).

RESULTS

Composition of Soy Milk and Its Hydrolysate. According to the analysis of these four types of soy milk (Table 1), SM and SMH contained less dietary fiber than WSM or WSMH. Four isoflavones were analyzed from the powdered SM and WSM and each respective hydrolysate prepared. Among isoflavones, the amount of daidzin and genistin was much greater than each respective aglycone, daidzein and genistein, for all types of soy milk prepared. The concentration of each or total isoflavones was highest in the WSM, followed by WSMH, SM, and SMH in that order. Hydrolysis of WSM and SM with protease mixed with glucoamylase resulted in the reduction of the concentration of all isoflavones in WSMH and SMH.

Body Weight Gain, Food Efficiency Ratio, Organ Weight, and Adipose Tissue Weight. The food intake of the WSM group was significantly lower than those of the other groups

Table 2. Composition of Experimental Diets Based on Powdered Soybean Milk or Whole Soybean Milk

	dietary group[a]								
		SM		SMH		WSM		WSMH	
composition	control	A	B	A	B	A	B	A	B
casein (soy protein)	20	20		20		20		20	
DL-methionine	0.3		0.3		0.3		0.3		0.3
sucrose	49.9	10.83	39.07	8.26	41.64	13.97	35.93	14.07	35.83
cellulose	5	0.22	4.78	0.11	4.89	1.86	3.14	1.39	3.61
fat									
corn oil + lard (1:1)	20		8.04		8.38		7.54		8.89
from soybean milk origin		11.96		11.62		12.46		11.11	
cholesterol	0.1		0.1		0.1		0.1		0.1
mineral mix[b]	3.5	2.76	0.74	2.66	0.84	2.30	1.20	3.11	0.39
vitamin mix[c]	1		1		1		1		1
cholinebitartrate	0.2		0.2		0.2		0.2		0.2
total[d]	100	100		100		100		100	

[a] SM, soy milk (20% soy milk protein); SMH, soy milk hydrolysate (20% soy milk hydrolysate protein); WSM, whole soybean milk (20% whole soy milk protein); WSMH, whole soy milk hydrolysate (20% whole soy milk hydrolysate protein); A, composition derived from soybean milk origin; B, composition derived from control diet origin. [b] AIN-76 mineral mixture. [c] AIN-76 vitamin mixture. [d] A + B = 100.

Table 3. Supplementary Effects of the Powdered Soybean and Whole Soybean Milk and Their Hydrolysates on Food Intake and Organ Weight in C57BL/6N Mice Fed a High-Fat and -Cholesterol Diet[a]

group	control	SM	SMH	WSM	WSMH
food intake (g/day)	4.15 ± 0.09 b	4.41 ± 0.04 b	4.22 ± 0.11 b	3.95 ± 0.18 a	4.21 ± 0.12 b
body weight gain (g/day)	0.33 ± 0.02 c	0.33 ± 0.02 c	0.26 ± 0.02 b	0.20 ± 0.02 a	0.27 ± 0.02 b
FER[b]	0.08 ± 0.005 c	0.07 ± 0.005 bc	0.06 ± 0.005 ab	0.05 ± 0.004 a	0.06 ± 0.005 ab
organ weight (mg/g of BW)					
liver	36.11 ± 0.80 ns	35.81 ± 0.48	34.90 ± 0.62	35.60 ± 0.77	35.69 ± 0.56
kidney	10.03 ± 0.28 a	10.35 ± 0.21 ab	10.99 ± 0.24 bc	11.79 ± 0.31 d	11.52 ± 0.27 cd
muscle	10.17 ± 0.66 ab	9.84 ± 0.50 a	11.34 ± 0.45 bc	12.90 ± 0.56 c	11.89 ± 0.79 bc
adipose tissue (mg/g of BW)					
epididymal WAT	55.84 ± 2.25 c	52.96 ± 1.99 bc	46.78 ± 2.46 ab	38.58 ± 3.07 a	40.35 ± 4.03 a
perirenal WAT	26.48 ± 0.74 c	24.14 ± 1.39 bc	21.32 ± 1.17 ab	18.48 ± 1.24 a	20.60 ± 1.27 a
interscapular WAT	42.91 ± 1.77 d	37.29 ± 1.79 cd	31.84 ± 1.93 bc	22.79 ± 2.60 a	29.16 ± 2.31 b
interscapular BAT	9.26 ± 0.45 c	7.89 ± 0.70 bc	7.38 ± 0.46 ab	6.04 ± 0.59 a	7.34 ± 0.35 ab
total AT	129.41 ± 6.60 d	116.99 ± 6.51 cd	105.98 ± 5.52 bc	85.89 ± 7.09 a	96.11 ± 6.34 ab

[a] Mean ± SE, $n = 10$. Means not sharing a common letter in the same row are significantly different among groups at $p < 0.05$. SM, soy milk (20% soy milk protein); SMH, soy milk hydrolysate (20% soy milk hydrolysate protein); WSM, whole soybean milk (20% whole soy milk protein); WSMH, whole soy milk hydrolysate (20% whole soy milk hydrolysate protein); BW, body weight; AT, adipose tissue; WAT, white adipose tissue; BAT, brown adipose tissue. [b] FER (food efficiency ratio) = body weight gain/food intake.

(Table 3). Values of average daily weight gain for 5 weeks were significantly lower in the SMH, WSM, and WSMH groups than in the control group (0.26 ± 0.02, 0.20 ± 0.02, and 0.27 ± 0.02 vs 0.33 ± 0.02 g/day). Similarly, weekly changes in body weight over 5 weeks also exhibited lower values in the WSM and WSMH groups (Figure 1). Among the various types of soy milk fed groups, WSM supplement particularly exhibited greater response for suppressing both food intake and weight gain (Table 3). Accordingly, the food efficiency ratio (FER) was lower in the SMH, WSM, and WSMH groups than in the control group (Table 3).

The supplementation of SMH, WSM, and WSMH resulted in significant decrease in the fat pad weights in epididymal, perirenal, and interscapular adipose tissues (Table 3). The total adipose tissue weight was also markedly lower in the WSM group compared to SM or SMH. However, supplementation of WSM significantly increased in the kidney and muscle weights compared to the control or SM group (Table 3).

Plasma and Hepatic Lipids. The supplementation of SM, SMH, WSM, and WSMH significantly lowered the plasma concentrations of total cholesterol, triglyceride, and FFA compared to the control group (Table 4). In particular, plasma total cholesterol concentration in the WSM group was even significantly lower than in the SM or SMH group. Different from plasma lipid, the hepatic triglyceride and cholesterol contents were significantly lower only in the WSM and WSMH groups than in the control group (Table 4).

Lipid-Regulating Enzyme Activities in Liver and Epididymal Tissues. The activities of hepatic FAS and G6PD were significantly lower in the SM, SMH, WSM, and WSMH groups

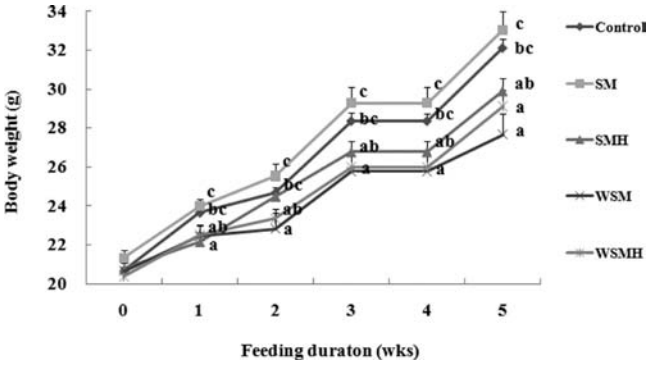

Figure 1. Supplementary effects of the powdered soybean and whole soybean milk and their hydrolysates on body weight in C57BL/6N mice fed a high-fat and -cholesterol diet. Mean ± SE, n = 10. Means not sharing the same letter are significantly different among groups at $p < 0.05$. SM, soy milk (20% soy milk protein); SMH, soy milk hydrolysate (20% soy milk hydrolysate protein); WSM, whole soybean milk (20% whole soy milk protein); WSMH, whole soy milk hydrolysate (20% whole soy milk hydrolysate protein).

than in the control group (Figure 2). The hepatic FAS activity was lower by 30, 39, 51, and 48%, whereas the hepatic G6PD activity was lowered by 58, 49, 47, and 55%, in the SM, SMH, WSM, and WSMH groups, respectively, compared to the control group (Figure 2). However, in the epididymal adipose tissue, the FAS activity was not significantly different between the groups, whereas its G6PD activity was significantly lowered by the SMH, WSM, and WSMH supplements compared to the control group. The hepatic ME activity was significantly lowered only by WSM and WSMH supplement, and epididymal ME activity was significantly lowered by WSMH supplement (Figure 2).

Morphological Changes in Hepatocytes and Epididymal Adipocytes. The supplementation of SM, SMH, WSM, and WSMH reduced the hepatic lipid droplets and average epididymal adipocyte size compared to the control group. Interestingly, lipid droplets were not observed in the livers of the WSM and WSMH groups, and average epididymal adipocyte size was smaller in the WSMH group than in other groups (Figure 3).

DISCUSSION

Plant proteins are generally more hypolipidemic than animal protein sources. The health-promoting effects of soybean have been attributed to its contents of polyphenol and peptide molecules. In recent years, research has mainly focused on the effects of soybean on the prevention of obesity,[4] cardiovascular disease,[14−16] and cancer[17,18] as well as its antioxidative properties.[19] Soy protein is considered to be a complete protein because it contains ample amounts of all the essential amino acids plus several other macronutrients with a nutritional value roughly equivalent to that of animal protein of high biological value.[20] Recently, various soy peptides have been produced via enzymatic hydrolysis of soy protein into lower molecular weight peptide.[21,22] An increasing body of literature suggests that soy proteins and isoflavones may have beneficial roles in obesity.

The present study reveals that four types of powdered soy milk supplemented to a high-fat diet significantly lowered the concentration of plasma lipids, total cholesterol, triglyceride, and free fatty acids, compared to casein as protein source of the control group, in high-fat-fed C57BL/6N mice. Although rats fed soy protein exhibited lower hepatic lipids compared to the casein-fed group in other's finding,[23] in the present study, the hepatic triglyceride and cholesterol contents were only significantly lower in the WSM and WSMH groups than in the casein group. In a study with genetically obese mice, Aoyama et al.[24] reported that soy protein isolate and its hydrolysate were more effective than whey protein isolate or its hydrolysate in reducing body weight and perirenal fat pad weight. The reduction in body fat by soy protein isolate and its hydrolysate compared with casein was also observed in genetically obese yellow KK mice and in rats fed a high-fat diet.[25] Several nutritional intervention studies in animals and humans indicate that consumption of soy protein reduces body weight and fat mass in addition to lowering plasma cholesterol and triglycerides concentration.[4] However, according to the very recent scientific opinion by the European Food Safety Authority, a health claim related to soy protein and reduction of blood cholesterol concentration is still a complex situation as a cause and effect relationship has not been established between the consumption of soy protein and the reduction of LDL-cholesterol concentration.[26] It is well-known that an elevation in serum free fatty acid leads directly to insulin resistance in the liver and muscle as well as adipose tissue,[27] and hypertriglyceridemia responsible for vascular complications is a common finding in patients with diabetes.[28] Changes in the synthesis of hepatic fatty acids also modify the plasma triglyceride concentration, because it alters the hepatic triglyceride synthesis and, in turn, affects the production of very low density lipoproteins by the liver.[29] In fact, hepatic FAS activity was markedly increased in the liver of casein-fed mice compared to the soy milk fed mice, which is correlated with the increased plasma triglyceride concentration. There also appeared a marked decrease of hepatic triglyceride content with a simultaneous decrease of hepatic lipid droplet accumulation in the mice supplemented with WSM and WSMH compared to the casein group. Interestingly, among the four types of soy milk that revealed plasma lipid-lowering property, SM and SMH did not alter the hepatic lipid level. Nevertheless, the activities of hepatic lipogenic enzymes, FAS and ME, were also lowered by these four types of soy milk. The WSM and WSMH ameliorated fatty liver and markedly reduced hepatic cholesterol and triglyceride content. ME is involved in supplying NADPH for fatty acid biosynthesis. Thus, lower hepatic ME activities can limit the availability of the long-chain fatty acids required for hepatic triglyceride synthesis.[30] The present results seemed to suggest that down-regulation of hepatic lipogenesis by the four types soy milk, in particular WSM and its hydrolysate, possibly contribute to the suppression of hepatic steatosis. Overall, WSM and WSMH were more effective for lipid-lowering action than SM or SMH. It is plausible that the high isoflavone content in WSM and WSMH could be attributable to this beneficial effect.

The biological actions of certain constituents of soybean, particularly conglycinin, soyasaponins, phospholipids, and isoflavones, have been reported regarding hypolipidemic and anti-obesity properties, although they are not entirely clear. Because soybean or soy milk contains many bioactive compounds or nutrients that may have synergistic interactions, it is difficult in nutritional intervention trials to differentiate or speculate on the effect of any one constituent on lipid or body fat reduction. There are, however, In Vivo studies in which the effects of an isolated component or a single compound of soy protein on lipids have been examined. The soybean β-conglycinin peptone suppresses food intake and gastric emptying[31] and lowers serum triglycerides, glucose, and insulin levels in normal and genetically obese (KK-Ay) mice.[32] WSM supplement exhibited the reduction of

Table 4. Supplementary Effects of the Powdered Soybean and Whole Soybean Milk and Their Hydrolysates on Plasma and Hepatic Lipids Concentration in C57BL/6N Mice Fed a High-Fat and -Cholesterol Diet[a]

	control	SM	SMH	WSM	WSMH
plasma (mmol/L)					
total cholesterol	3.24 ± 0.07 c	2.59 ± 1.10 b	2.56 ± 0.13 b	2.12 ± 0.09 a	2.35 ± 0.06 ab
triglyceride	1.36 ± 0.06 b	1.12 ± 0.07 a	1.13 ± 0.08 a	1.04 ± 0.07 a	1.10 ± 0.05 a
FFA	0.91 ± 0.04 b	0.78 ± 0.02 a	0.77 ± 0.04 a	0.77 ± 0.04 a	0.76 ± 0.05 a
liver (mmol/g liver)					
cholesterol	4.04 ± 0.25 c	3.80 ± 0.22 c	3.64 ± 0.15 bc	2.60 ± 0.27 a	2.97 ± 0.28 ab
triglyceride	1.46 ± 0.09 b	1.38 ± 0.07 b	1.30 ± 0.09 ab	1.08 ± 0.09 a	1.04 ± 0.09 a

[a] Mean ± SE, $n = 10$. Means not sharing the same letter in the same row are significantly different among groups at $p < 0.05$. SM, soy milk (20% soy milk protein); SMH, soy milk hydrolysate (20% soy milk hydrolysate protein); WSM, whole soybean milk (20% whole soy milk protein); WSMH, whole soy milk hydrolysate (20% whole soy milk hydrolysate protein); FFA, free fatty acid.

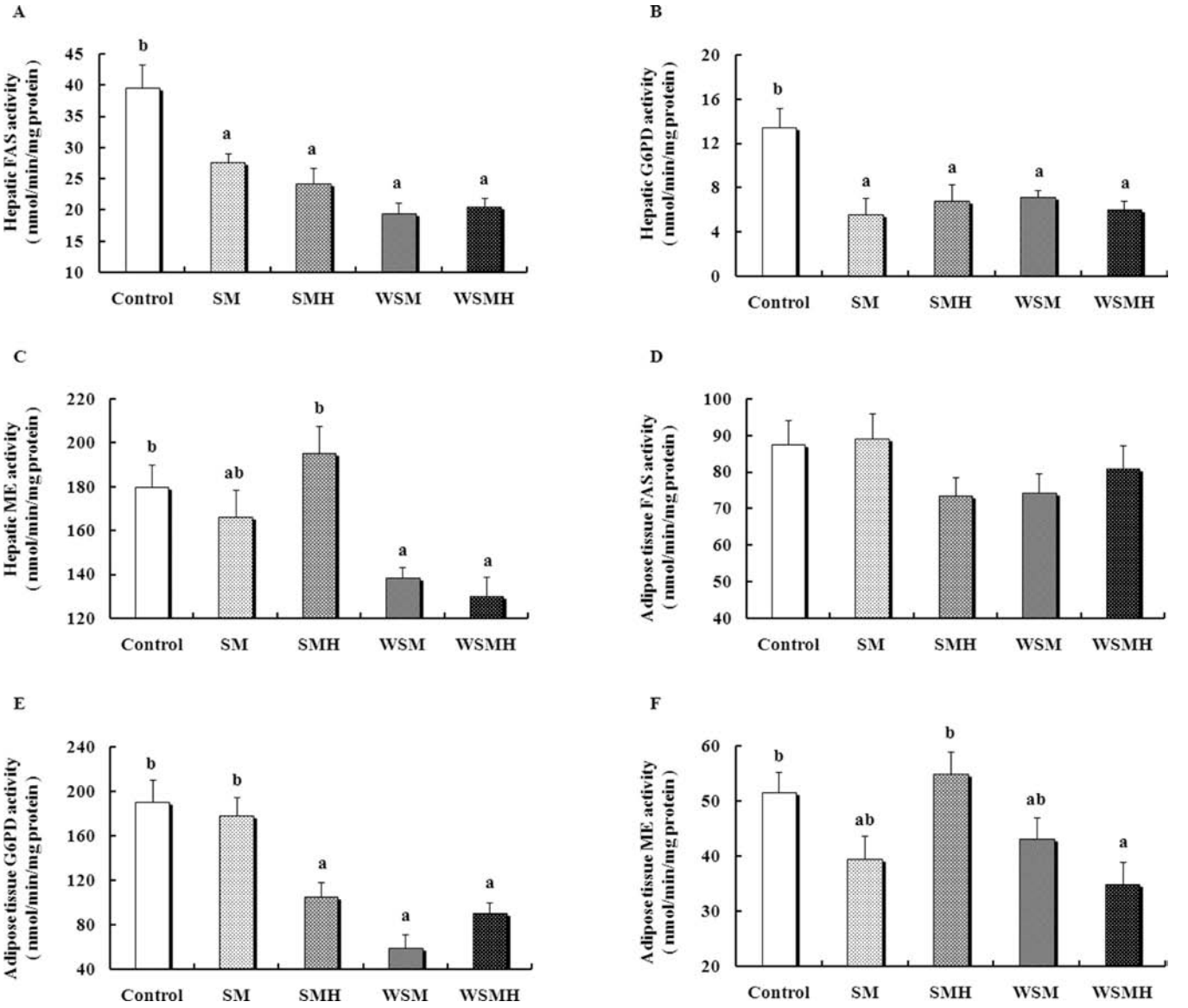

Figure 2. Supplementary effects of the powdered soybean and whole soybean milk and their hydrolysates on lipid metabolic enzyme activities in C57BL/6N mice fed a high-fat and -cholesterol diet. Mean ± SE, $n = 10$. Means not sharing the same letter are significantly different among groups at $p < 0.05$. SM, soy milk (20% soy milk protein); SMH, soy milk hydrolysate (20% soy milk hydrolysate protein); WSM, whole soybean milk (20% whole soy milk protein); WSMH, whole soy milk hydrolysate (20% whole soy milk hydrolysate protein); FAS, fatty acid synthase; G6PD, glucose-6-phosphate dehydrogenase; ME, malic enzyme.

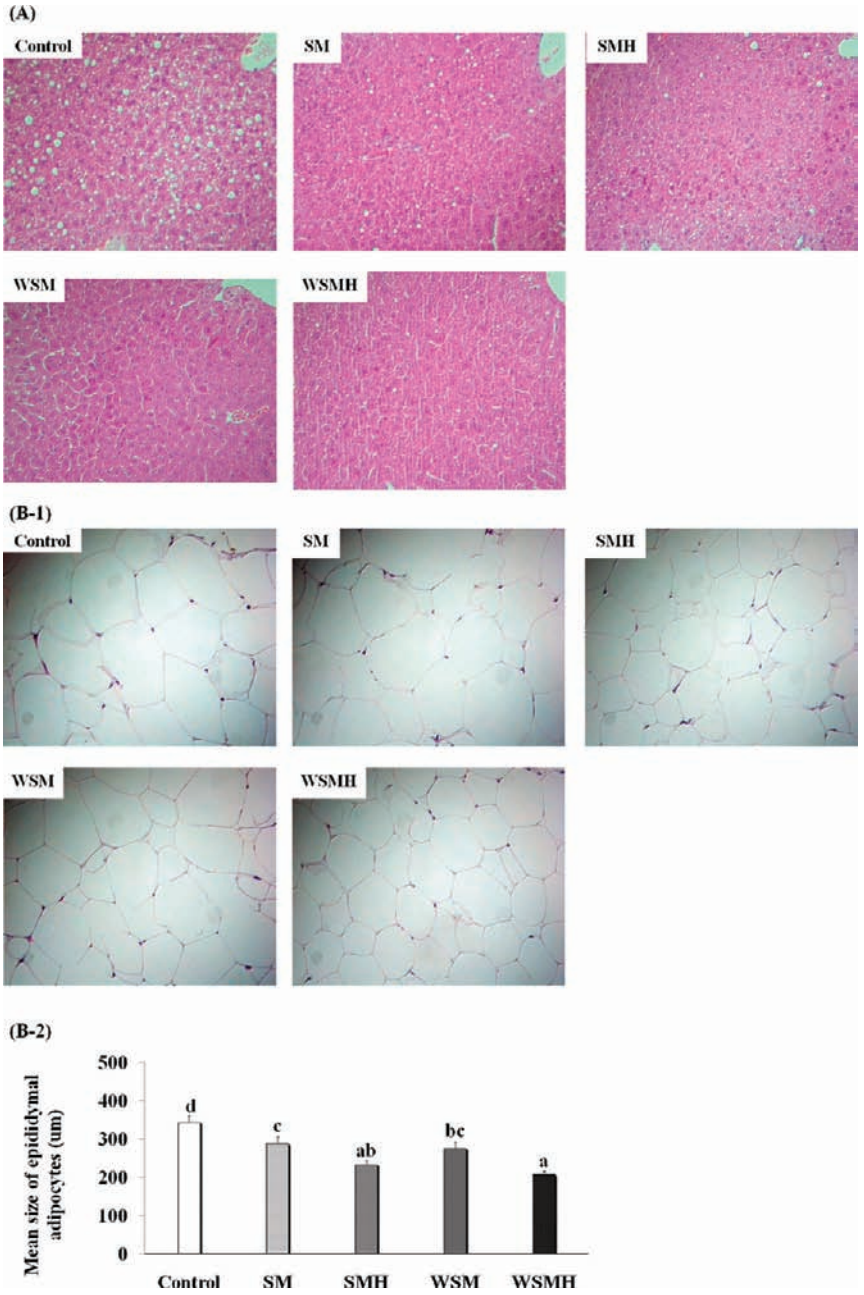

Figure 3. Supplementary effects of the powdered soybean and whole soybean milk and their hydrolysates on hepatic tissue (A) and epididymal adipocyte (B) morphologies in C57BL/6N mice fed a high-fat and -cholesterol diet. Mean ± SE, $n = 10$. Means not sharing the same letter are significantly different among groups at $p < 0.05$. SM, soy milk (20% soy milk protein); SMH, soy milk hydrolysate (20% soy milk hydrolysate protein); WSM, whole soybean milk (20% whole soy milk protein); WSMH, whole soy milk hydrolysate (20% whole soy milk hydrolysate protein). Original magnification ×200.

food intake, although it requires further clarification. The hepatic G6PDH, FAS, and ME activities were reduced in the WSM group, which could contribute to body fat-lowering reduction.

One can speculate on the lipid-lowering function of soy proteins on the basis of the composition of the soy milk prepared. SM and SMH contained less fiber and isoflavones than WSM or WSMH. The hypocholesterolemic effect of soy protein may partly be attributed to its protein per se as suggested by Huang et al.,[33] although this claim is still complex.

Simultaneously, part of the antiobesity effect of soy protein could be due to the presence of isoflavones, because soy isoflavones have been shown to decrease fat accumulation in some animal models of obesity.[34−36] Among four isoflavones, genistein and daidzein and their β-glucoside conjugates, genistin and daidzin, in soy milk samples could exert some influence on lipid lowering or body fat reduction as shown in data from soy milk fed mice. Cell culture studies reported that genestein and daidzein up-regulated PPARγ-mediated gene expression.[6] Contents of isoflavones analyzed in four soy milks indicate that all isoflavones were highest in WSM, which was followed by WSMH, SM, and SMH in that order. In general, soy milk made from whole soybeans had higher total isoflavone contents that

those made with soy protein isolates.[37] WSM was greater in daidzin and genistein contents than SM. By enzymatic hydrolysis of SM and WSM, each isoflavone content was slightly reduced. Genistein can be absorbed in the upper small intestine,[38] whereas the β-glucoside conjugate, genistin, must be converted to an aglycone through the action of a β-glucosidase produced by intestinal bacteria before being absorbed. When rats were supplemented with isoflavones, smaller adipocyte size in epididymal and retroperitoneal fat pads was observed.[39] Soy isoflavones may synergistically affect the lipid-lowering effect of soy milk in the current study, although their exact mode of action has not been elucidated yet. A diet containing soybean phospholipids also markedly decreased the hepatic mRNA levels of enzymes involved in fatty acid synthesis.[40]

The present study indicates that the coordinated action of lowering the circulating FFAs and hepatic FAS activities in high-fat-fed mice seemed to be responsible for the lipid-lowering effects of the soy milk prepared, leading to improved liver tissue morphology, regardless of its hydrolysis status or fiber content. Taken together, the WSM and WSMH may have greater influence on lipogenesis in the liver of diet-induced obesity models because these two types of soy milk reduced the concentrations of triglycerides in plasma and liver, particularly in liver. Their effects are plausibly associated with the reduced lipogenic enzyme activities, FAS, G6PD, and, particularly, ME in the WSM and WSMH groups. Soyasaponins are possibly another component that is responsible for cholesterol-lowering action of soybean products. They reduce serum cholesterol concentration,[41] but their role in fatty acid metabolism is unknown.

In conclusion, we found that the powdered soy milk prepared improves hyperlipidemia and fatty liver via partial inhibition of hepatic lipogenic enzymes in C57BL/6N mice, a model for diet-induced obesity animals. WSM and WSMH exhibited the hepatic lipid-lowering effect, which is not due to their additional fiber content. Hydrolysis of WSM maintained the hypocholesterolemic property of soy milk and the hepatic lipid-lowering effect without altering food intake. Overall, WSMH among different types of soy milks seemed to be most desirable as a health-promoting agent for improving dyslipidemia and hepatic and/or body fat accumulation associated with diet-induced obesity mice.

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