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Antihyperlipidemic and Body Fat-Lowering Effects of Silk Proteins with Different Fibroin/Sericin Compositions in Mice Fed with High Fat Diet

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ABSTRACT: The effect of silk protein with different fibroin/sericin compositions on body weight and lipid metabolism in high fat-fed mice was investigated. The animals were given experimental diets for 6 weeks: normal control (NC), high fat (HF) and high fat diet supplemented with F100 (pure fibroin, HF-F100), F81 (81:19 fibroin/sericin, w/w, HF-F81) or F50 (50:50 fibroin/sericin, w/w, HF-F50). The silk protein-fed mice showed markedly reduced body weight and enhanced lipid profile relative to the HF group. In general, the amount of body fat, triglyceride and total plasma cholesterol levels, atherogenic index and free fatty acid level tended to decrease, while the HDL-cholesterol level increased, with increased amount of sericin in the diet. This hypolipidemic effect was partly due to increased fecal lipid excretion, inhibition of lipogenesis and regulation of adipokine production. These findings illustrate that silk protein, particularly sericin, may be beneficial in the prevention of high fat diet-induced hyperlipidemia and obesity.

KEYWORDS: silk protein, hyperlipidemia, obesity, high fat diet, mice

INTRODUCTION

Silk proteins are biopolymers from the cocoons produced by silkworms, such as *Bombyx mori*, during their metamorphosis to moths.[1] These protein-based fibers have been used for centuries in the production of high quality fabrics due to their characteristic luster and strength. The silk fiber is 98% protein, composed mainly of fibroin and sericin, with minimal impurities such as fat, inorganic salt and waxes.[1,2] Sericin constitutes 25−30% of the total cocoon weight and coats the core structural protein fibroin in successive sticky layers to ensure the cohesion of the cocoon.[3] During harvest of cocoons, the sericin is mostly removed and discarded while the fibroin is processed into raw silk. Scientific studies have shown that silk proteins possess various biological properties. Sericin was reported to have strong antioxidant action by suppressing in vitro lipid peroxidation in rat brain homogenate and inhibiting tyrosinase activity, indicating that it can be a valuable ingredient for food and cosmetics.[4] Fibroin, on the other hand, has antitumor effects and anti-HIV-1 activity and could enhance insulin activity and glucose metabolism.[1,5,6] Its hydrolysate was also found to reduce body weight in obese mice and lower blood pressure in hypertensive rats.[7,8]

Nowadays, obesity is considered one of the leading metabolic diseases in the world due to its increasing prevalence in both developed and developing countries. Sedentary lifestyle and poor eating habits, particularly high dietary fat intake, have led to the rapid increase in the incidence of health problems associated with obesity and hyperlipidemia.[9,10] Chronic consumption of a high fat diet alters the cholesterol and triglyceride levels in plasma and tissues, resulting in a higher risk for cardiovascular diseases.[11−13] Due to the rising trend in the global occurrence of obesity-related health problems, the need for therapeutic measures is becoming more urgent. There is a concerted effort among scientists and researchers to find functional biomaterials capable of regulating lipid metabolism and having antihyperlipidemic action.

While the importance of silk in the textile industry has been well-recognized and the functional properties of both sericin and fibroin have been extensively studied, reports on the physiological activities of silk protein in relation to obesity and hyperlipidemia have been limited. Furthermore, in previous studies, silk proteins with very low molecular weight were used to explore the biological activities of silk. The silk was hydrolyzed to peptide or amino acid level to make it edible. Thus, the biological activities of high molecular weight silk fibroin and sericin have not been investigated. In particular, the effect of fibroin and sericin and their mixture on the lipid profile in a mouse model under high fat diet conditions has not been studied yet. Hence, this study was carried out to evaluate the effect of dietary feeding of silk protein with different fibroin/sericin compositions on the body weight and lipid metabolism in mice fed with a high fat diet.

MATERIALS AND METHODS

Chemicals. Chemicals such as ethanol, calcium chloride, ketamine-HCl, triethanolamine, magnesium chloride, potassium phosphate buffer, and Triton X-100 were purchased from Merck KGaA (Darmstadt, Germany). All other chemicals used were obtained from Sigma-Aldrich, Inc. (Steinhein, Germany).

Preparation of Silk Protein Powder. Silk cocoons from *Bombyx mori* silkworms were obtained from Korea Rural Development Administration (RDA, Suwon, Korea). Since the cocoons originally consist of

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Table 1. Composition of the Experimental Diets (%)

component	NC	HF	HF-F100	HF-F81	HF-F50
casein	20.0	20.0	20.0	20.0	20.0
DL-methionine	0.3	0.3	0.3	0.3	0.3
sucrose	50.0	50.0	48.0	48.0	48.0
cellulose	5.0	5.0	5.0	5.0	5.0
corn oil	5.0	3.0	3.0	3.0	3.0
choline bitartrate	0.2	0.2	0.2	0.2	0.2
mineral mixture[a]	3.5	3.5	3.5	3.5	3.5
vitamin mixture[b]	1.0	1.0	1.0	1.0	1.0
corn starch	15.0				
lard		17.0	17.0	17.0	17.0
silk protein			2.0	2.0	2.0
total (%)	100.0	100.0	100.0	100.0	100.0

[a] AIN-76 mineral mixture. [b] AIN-76 vitamin mixture.

74% fibroin and 26% sericin, different preparation conditions were employed in order to prepare three types of silk proteins with different fibroin/sericin compositions. Pure silk fibroin was produced by degumming the silk cocoons with sodium oleate (0.6%, on the weight of fiber [owf]) and sodium carbonate (0.4%, owf) solutions at boiling temperature for 2 h to remove the sericin. Forty grams of silk cocoons was used for each extraction, with a solvent to solid material ratio of 25:1. The degummed cocoons were rinsed thoroughly with warm distilled water (60−80 °C) and dissolved in calcium chloride/distilled water/ethanol solution (molar ratio: 1/8/2) for 30 min. The silk fibroin solution was dialyzed in a cellulose tube (molecular weight cutoff = 12,000−14,000) against circulating pure water for 5 days at room temperature. The solution was then freeze-dried to obtain 100% regenerated silk fibroin sponge (F100). In the case of fibroin/sericin mixture, the raw silk cocoons were degummed with distilled water without degumming agent at boiling temperature for 1 h. The degummed silk was composed of 81% fibroin and 19% sericin (F81). The F81 silk protein was obtained using the same dissolution process as mentioned above. For the preparation of silk protein containing 50% fibroin and 50% sericin (F50), 100% silk sericin was extracted from raw silk cocoons by boiling in water for 1 h. The pure silk sericin aqueous solution was then mixed with F81 aqueous solution to prepare the F50 mixture. The solution was freeze-dried to obtain the sponge samples. The sericin and fibroin contents of each silk protein sample were determined by weight change after the degumming process and verified using rheological and turbidity measurements.

Animals and Diet. Forty male C57BL/6N mice of 4 weeks of age, weighing 17 g, were purchased from Orient Inc. (Seoul, Korea). They were individually housed in stainless steel cages in a room maintained at 25 °C with 50% relative humidity and a 12/12 h light/dark cycle and fed with a pelletized chow diet for 2 weeks after arrival. The mice were then randomly divided into 5 dietary groups (n = 8). The first and second mouse groups were fed with normal control (NC) and high fat (HF, 17%, w/w) diets, respectively. The other three groups were given a high fat diet supplemented with either F100 (HF-F100), F81 (HF-F81) or F50 (HF-F50) silk proteins. The composition of the experimental diet (Table 1) was based on the AIN-76 semisynthetic diet. The mice were fed for 6 weeks and allowed free access to food and water during the experimental period. The food consumption and weight gain were measured daily and weekly, respectively. The food efficiency ratio (FER) was calculated based on the average daily body weight gain of mice divided by their food intake. Feces were collected during the final week to measure the level of fecal cholesterol and triglyceride excretion. At the end of the experimental period, the mice were anesthetized with ketamine-HCl following a 12 h fast. Blood samples were drawn from the inferior vena cava into a heparin-coated tube and centrifuged at 1000g for 15 min at 4 °C to obtain the plasma. The body organs (liver, kidney and heart) and adipose tissues (epididymal, perirenal and visceral) were removed, rinsed with physiological saline, weighed and stored at −70 °C until analysis. The current study protocol was approved by the Ethics Committee of Kyungpook National University for animal studies.

Determination of Lipid Profile in Plasma and Feces. The concentrations of plasma total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol were determined using a commercial kit (Asan Pharmaceutical, Seoul, Korea). The fecal lipids for total cholesterol and triglyceride analyses were extracted and purified using the method described by Folch et al.[14] The cholesterol and triglyceride levels in feces were measured using the same enzymatic kit used in the plasma analysis.

Determination of Free Fatty Acid Concentration in Plasma. The plasma free fatty acid was measured using commercial free fatty acid assay kit (Enzychrom, Bio-Assays Systems, CA, USA) following the instruction manual.

Measurement of Glutamate Oxaloacetate (GOT) and (Glutamate Pyruvate Transaminase (GPT) Levels. The GOT and GPT concentrations were determined using a commercial kit (Sigma Chemical Co., MO, USA) based on the method of Reitman and Frankel.[15]

Determination of Lipid Regulating Enzymes and Hepatic β-Oxidation Activities. The liver was homogenized following the method described by Hulcher et al.[16] Briefly, the tissue was homogenized in 3−5 mL of 0.1 M triethanolamine, 0.02 M EDTA, and 0.002 M dithiothreitol (DTT) per gram of tissue and centrifuged at 1000g at 4 °C for 15 min. The pellet was then removed, and the supernatant was centrifuged again at 10000g at 4 °C for 15 min. After centrifugation, the pellets were resuspended in the same buffer used in homogenization and analyzed for carnitine palmitoyl transferase (CPT) and β-oxidation activities, and the supernatant was further centrifuged at 105000g at 4 °C for 1 h. The resulting supernatant was measured for glucose-6-phosphate dehydrogenase (G6PD) activity, while the pellets were resuspended in the same buffer containing triethanolamine, EDTA, and DTT and analyzed for fatty acid synthase (FAS) and malic enzyme (ME) activities. The protein content, on the other hand, was determined using Bradford protein assay.[17]

The G6PD activity was measured based on the reduction of 6 mM NADP+ by G6PD in the presence of glucose-6-phosphate.[18] The enzyme activity was determined by monitoring the increase in absorption of the reaction mixture at 340 nm at 37 °C.

The ME activity was determined using the method of Ochoa.[19] The reaction mixture contained 0.4 M triethanolamine (pH 7.4), 30 mM malic acid, 0.12 M $MgCl_2$, and 3.4 mM NADP. The absorbance was measured at 340 nm at 27 °C.

The FAS activity was measured using the spectrophotometric method of Gibson and Hubbard.[20] The assay mixture contained 125 mM potassium phosphate buffer (pH 7.0), 10 mM EDTA, 10 mM β-mercaptoethanol, 33 μM acetyl-CoA, 100 μM malonyl-CoA, and 100 μM NADPH. The mixture was added with malonyl CoA and the change in absorbance at 340 nm at 30 °C was recorded. The activities of G6PD, ME, and FAS were expressed as μmol or nmol reduced NADPH/min/mg protein.

The CPT activity was determined based from the method developed by Bieber et al.[21] The assay mixture was composed of 116 mM Tris-HCl (pH 8.0), 1.1 mM EDTA, 2.50 mM L-carnitine, 0.5 mM 5,5-dithiobis-2-nitrobenzoic acid, 75 mM palmitoyl-CoA, 0.2% Triton X-100. The reaction was initiated by the addition of 50 μL of cytosol and incubated at 25 °C for 2 min. The change in absorbance at 412 nm was measured, and the activity was expressed as μmol or nmol of CoASH or oxidized CoA/min/mg protein.

The hepatic β-oxidation activity was measured from the final product of NADH by palmitoyl substrates.[22] The composition of the assay

Table 2. Body Weight Gain and Food Efficiency Ratio in Mice Fed with High Fat Diet Supplemented with Silk Protein

	NC	HF	HF-F100	HF-F81	HF-F50
initial wt[a] (g)	19.72 ± 0.29 a	19.57 ± 0.26 a	19.55 ± 0.45 a	19.65 ± 0.36 a	19.60 ± 0.45 a
final wt (g)	30.20 ± 0.68 a	37.03 ± 0.32 c	35.90 ± 0.90 c	32.72 ± 0.82 b	31.42 ± 0.85 ab
body wt gain (g/day)	0.18 ± 0.01 a	0.31 ± 0.01 c	0.30 ± 0.02 c	0.23 ± 0.02 b	0.21 ± 0.02 ab
food intake (g/day)	3.23 ± 0.05 a	3.19 ± 0.03 a	3.29 ± 0.07 a	3.25 ± 0.06 a	3.26 ± 0.05 a
FER[b]	0.06 ± 0.01 a	0.10 ± 0.00 b	0.09 ± 0.01 b	0.07 ± 0.01 a	0.06 ± 0.01 a

[a] Values are means ± SE ($n = 8$). Means in the same row not sharing a common letter are significantly different at $p < 0.05$. [b] Food efficiency ratio = body weight gain/food intake.

mixture was as follows: 50 mM Tris-HCl (pH 8.0) 20 mM NAD^+, 0.33 M DTT, 1.5% BSA (1.5 g/100 mL), 2% Triton X-100 (2 g/100 mL), 10 mM CoA, 1 mM FAD, 100 mM KCN and 5 mM palmitoyl-CoA. The reaction was initiated by addition of 10 μL of cytosol and incubated at 37 °C for 5 min. The change in absorbance at 340 nm was measured.

Determination of Plasma Adipokine Concentrations. The leptin level in plasma was measured using an enzyme immunoassay (EIA) kit (Spi-Bio, Montigny le Bretonneux, France), and the concentrations of resistin, adinopectin and TNF-α were determined using enzyme-linked immunosorbent assay (ELISA) kits (Shibayagi Co., Gunma, Japan).

Statistical Analysis. All data are presented as the mean ± SE. The data was evaluated by one-way ANOVA using a Statistical Package for Social Sciences software program (SPSS Inc., Chicago, IL, USA), and the differences between the means were assessed using Duncan's multiple range test. Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Body Weight Gain. The initial body weights of mice, prior to feeding with experimental diets, did not significantly differ, and the daily food intake was similar in all the animal groups (Table 2). However, at the end of the experimental period, a marked increase in the body weight of HF and HF-F100 mice groups was observed relative to that of the NC group. On the other hand, the HF-F81 and HF-F50 mice exhibited lower body weight than the HF animals. Accordingly, the average weight gain and food efficiency ratio (FER) were highest in HF and HF-F100 groups. Dietary feeding of F50 silk protein significantly decreased the final body weight and average weight gain to normal level in high fat-fed mice. Addition of F81 in the diet also resulted in a significant decrease in the body weight. These indicate that diet supplementation with silk protein, specifically F81 and F50, could suppress the high fat diet-induced body weight gain in mice.

Organ Weights. The weights of liver and heart were not significantly different among the animal groups (Table 3). The kidney weight was slightly but significantly higher in HF mice compared with that of the HF-F50 group. The weights of epidymal, perirenal and visceral adipose tissues were lowest in the control mice. A considerable increase in the body fat was observed in HF mice. However, supplementation of F81 and F50 in the diet significantly reduced the amount of adipose tissue. On the other hand, it appears that dietary feeding of F100 had a reducing effect on the amount of visceral fat, but not on the epididymal and perirenal adipose tissues in high fat-fed mice. The lower body weights observed in the HF-F81 and HF-F50 groups seemed to be due to the decreased amount of fat in mice. Interestingly, both the body and white adipose tissue weights tended to decrease with the decreased amount of fibroin and increased amount of sericin in silk protein, indicating that sericin is more effective than fibroin in reducing the high fat diet-induced body fat in mice.

Plasma and Fecal Lipids. High fat feeding resulted in a significant increase in the plasma triglyceride and total cholesterol levels in mice (Table 4). Earlier studies have also shown that dietary fat caused considerable elevation in plasma total cholesterol and triglyceride contents in various laboratory animals.[23,24] However, dietary feeding of silk protein resulted in a marked decrease in the plasma total cholesterol in high fat-fed mice. The animals fed with silk proteins F81 and F50 also exhibited lower plasma triglyceride concentration. Moreover, they showed the highest concentration of HDL-cholesterol and HDL-cholesterol/total cholesterol ratio (HTR). Higher HDL-cholesterol level signifies lower risk for heart disease. The HDL particles have antiatherogenic properties because they facilitate the translocation of cholesterol from peripheral tissues to liver for catabolism.[25] Accordingly, the atherogenic index (AI), which measures the coronary heart disease risk, was lowest in HF-F81 and HF-F50 groups and highest in the control and HF groups, indicating that F81 and F50 could reduce the risk of atherosclerosis. Among the silk protein-fed mice groups, only the HF-F50 animals showed significantly lower free fatty acid and GOT levels than that of the HF group. The amount of GPT, on the other hand, did not significantly differ among the groups. The plasma enzymes GOT and GPT are specific markers of liver damage.[26] The reduced activities of these enzymes indicate decreased hepatic oxidative stress under a high fat diet condition.

Although the pure fibroin silk protein F100 significantly reduced the total cholesterol level and improved the HTR and AI values, it was apparent that the levels of plasma total cholesterol, triglyceride, free fatty acid, GOT, GPT and AI tended to decrease, while that of the HDL-cholesterol and HTR increased, with reduced fibroin and increased sericin concentrations in the diet. This suggests that sericin may be more effective than fibroin in improving the lipid metabolism in mice under high fat diet conditions. A recent study conducted by Okazaki et al.[27] provided the first evidence of the hypolipidemic effect of sericin. It was reported that dietary feeding of sericin resulted in reduced serum levels of triglyceride and cholesterol in high fat-fed rats. Likewise, Limpeanchob et al.[28] accounted that administration of sericin solution suppressed the blood cholesterol concentrations in high cholesterol-fed rats. Sericin has low digestibility due to its protease-resistant property and functions as dietary fiber,[29] which would account for its antihyperlipidemic effect. In the present study, the HF-F50 mice group showed a substantial increase in the fecal triglyceride and total cholesterol compared with the NC and HF groups. The HF-F100 mice also exhibited higher fecal cholesterol concentration than the HF animals. The increase in fecal triglyceride and total cholesterol contents could probably be due to the changes in the cholesterol

Table 3. Weights of Organs and Adipose Tissues in Mice Fed with High Fat Diet Supplemented with Silk Protein

	NC	HF	HF-F100	HF-F81	HF-F50
			Organ Weight (g)[a]		
liver	4.44 ± 0.16 a	4.59 ± 0.19 a	4.39 ± 0.21 a	4.12 ± 0.21 a	4.31 ± 0.13 a
heart	0.43 ± 0.01 a	0.43 ± 0.01 a	0.46 ± 0.02 a	0.45 ± 0.01 a	0.46 ± 0.01 a
kidney	1.28 ± 0.02 ab	1.30 ± 0.02 b	1.26 ± 0.01 ab	1.27 ± 0.02 ab	1.24 ± 0.02 a
			White Adipose Tissue Weight (g)		
epididymal	4.66 ± 0.28 a	6.88 ± 0.25 c	6.74 ± 0.26 c	5.74 ± 0.16 b	5.48 ± 0.47 b
perirenal	1.89 ± 0.16 a	3.17 ± 0.17 c	2.98 ± 0.22 c	2.71 ± 0.17 bc	2.44 ± 0.18 b
visceral	3.67 ± 0.15 a	5.30 ± 0.18 c	4.59 ± 0.15 b	4.28 ± 0.12 b	4.18 ± 0.22 b

[a] Values are means ± SE (n = 8). Means in the same row not sharing a common letter are significantly different at $p < 0.05$.

Table 4. Plasma and Fecal Lipid Profiles and GOT and GPT Levels in Mice Fed with High Fat Diet Supplemented with Silk Protein

	NC	HF	HF-F100	HF-F81	HF-F50
			Plasma		
triglyceride[a] (mg/dL)	159.82 ± 5.26 b	175.75 ± 7.36 c	171.99 ± 4.72 bc	162.03 ± 2.77 b	147.18 ± 2.25 a
total cholesterol (mg/dL)	156.81 ± 6.48 a	179.72 ± 8.92 b	163.97 ± 4.42 a	154.33 ± 8.34 a	149.01 ± 9.63 a
HDL-cholesterol (mg/dL)	83.33 ± 2.00 a	89.00 ± 2.13 ab	92.99 ± 0.95 bc	96.37 ± 3.54 c	98.06 ± 1.67 c
HTR[b] (%)	53.13 ± 1.79 ab	50.07 ± 2.46 a	56.84 ± 1.02 b	62.41 ± 1.54 c	66.00 ± 1.84 c
AI[c]	0.87 ± 0.06 cd	1.02 ± 0.11 d	0.76 ± 0.03 bc	0.60 ± 0.04 ab	0.52 ± 0.04 a
free fatty acid (mmol/L)	1.24 ± 0.10 ab	1.59 ± 0.27 b	1.60 ± 0.22 b	1.14 ± 0.08 ab	0.76 ± 0.06 a
GOT (karmen/mL)	74.18 ± 9.56 ab	88.86 ± 5.79 b	85.77 ± 5.45 b	79.59 ± 6.15 ab	67.22 ± 7.45 a
GPT (karmen/mL)	35.70 ± 4.25 ab	40.54 ± 5.98 ab	43.45 ± 6.30 b	36.19 ± 2.04 ab	27.96 ± 4.72 a
			Feces		
triglyceride (mg/g)	4.97 ± 0.26 a	4.81 ± 0.33 a	5.38 ± 0.23 ab	5.51 ± 0.12 ab	5.94 ± 0.46 b
total cholesterol (mg/g)	6.39 ± 0.14 ab	5.88 ± 0.09 a	6.51 ± 0.10 b	6.47 ± 0.04 ab	8.38 ± 0.40 c

[a] Values are means ± SE (n = 8). Means in the same row not sharing a common letter are significantly different at $p < 0.05$. [b] HTR = (HDL-cholesterol/total cholesterol) × 100. [c] Atherogenic index = (total cholesterol − HDL-cholesterol)/HDL-cholesterol.

Table 5. Lipid-Regulating Enzyme and Hepatic β-Oxidation Activities in Mice Fed with a High Fat Diet Supplemented with Silk Protein

	NC	HF	HF-F100	HF-F81	HF-F50
			Liver		
G6PD[a] (nmol of NADPH/min/mg of protein)	7.20 ± 0.51 c	6.74 ± 0.52 bc	5.67 ± 0.53 ab	5.01 ± 0.21 a	4.52 ± 0.51 a
ME (nmol of NADPH/min/mg of protein)	31.92 ± 1.92 b	30.64 ± 1.75 b	31.35 ± 2.23 b	20.00 ± 2.41 a	21.26 ± 1.81 a
FAS (nmol of NADPH/min/mg of protein)	18.42 ± 1.45 a	26.79 ± 1.97 b	17.68 ± 1.24 a	14.27 ± 2.31 a	13.52 ± 1.85 a
CPT (nmol of CoASH/min/mg of protein)	18.76 ± 0.99 a	18.79 ± 1.08 a	18.88 ± 0.71 a	19.97 ± 0.81 a	22.95 ± 1.40 b
hepatic β-oxidation (nmol of NADPH/min/mg of protein)	2.85 ± 0.14 a	2.83 ± 0.19 a	3.04 ± 0.35 a	3.11 ± 0.16 a	3.91 ± 0.28 b
			Adipocyte		
G6PD (μmol of NADPH/min/mg of protein)	128.83 ± 9.36 a	140.22 ± 6.68 a	132.40 ± 8.87 a	119.57 ± 14.64 a	111.91 ± 7.62 a
ME (μmol of NADPH/min/mg of protein)	309.73 ± 16.41 bc	343.31 ± 19.44 c	319.91 ± 13.73 c	276.29 ± 6.86 ab	252.43 ± 12.47 a
FAS (μmol of NADPH/min/mg of protein)	57.51 ± 5.62 a	62.41 ± 5.42 a	60.08 ± 6.26 a	56.33 ± 5.65 a	47.21 ± 7.90 a
CPT (μmol of CoASH/min/mg of protein)	54.62 ± 9.24a	60.27 ± 9.89 ab	62.73 ± 9.10 ab	82.46 ± 6.94 b	85.75 ± 10.16 b

[a] Values are means ± SE (n = 8). Means in the same row not sharing a common letter are significantly different at $p < 0.05$.

and triglyceride absorption in the intestines. Past studies revealed that fiber proteins, including sericin, could decrease the cholesterol absorption in Caco-2 cell culture model.[28,30] It was suggested that the undigested protein might have influenced the gut absorptive function by either blocking the absorption or sequestering the cholesterol, thereby enhancing the excretion of cholesterol via feces. Nevertheless, this elevation in the excretion of fecal lipids partly explains the reducing effect of silk protein on the plasma triglyceride and total cholesterol levels in high fat-fed mice.

Lipid-Regulating Enzymes and Hepatic β-Oxidation Activities. The activities of hepatic G6PD and ME enzymes

Table 6. Plasma Adipokines Concentrations in Mice Fed with a High Fat Diet Supplemented with Silk Protein

adipokines	NC	HF	HF-F100	HF-F81	HF-F50
leptin[a] (ng/mL)	1.43 ± 0.10 ab	1.83 ± 0.15 c	1.74 ± 0.12 bc	1.52 ± 0.08 abc	1.38 ± 0.13 a
resistin (ng/mL)	15.44 ± 0.21 ab	16.54 ± 0.11 c	15.93 ± 0.24 b	15.68 ± 0.14 ab	15.35 ± 0.27 a
adiponectin (μg/mL)	1.37 ± 0.11 a	1.40 ± 0.28 a	1.38 ± 0.16 a	2.38 ± 0.13 b	2.76 ± 0.28 b
TNF-α (ng/mL)	1.28 ± 0.04 b	1.49 ± 0.03 c	1.46 ± 0.05 c	1.40 ± 0.05 c	1.17 ± 0.03 a

[a] Values are means ± SE (n = 8). Means in the same row not sharing a common letter are significantly different at $p < 0.05$.

significantly decreased in HF-F81 and HF-F50 mice relative to that of the NC and HF groups (Table 5). The hepatic FAS activity increased with high fat feeding, but the activity was reduced to normal level with the addition of silk protein in the diet. Similarly, the activity of adipocyte ME markedly decreased in HF-F81 and HF-F50 groups. The activities of the adipocyte enzymes G6PD and FAS in mice fed with F81 and F50 silk proteins remained at the level of the NC group. On the other hand, dietary feeding of F100 did not significantly change the adipocyte enzymes activities. The lipogenic enzymes are essential for the biosynthesis of fatty acid and cholesterol.[31] Decreased activities of these enzymes could reduce the availability of fatty acids necessary for the synthesis of hepatic triglycerides. Moreover, a marked increase in the hepatic CPT and β-oxidation activities was found in HF-F50. Previous studies have shown that an increase in CPT activity could lead to an increase in the fatty acid oxidation, thereby reducing the accumulation of triglycerides.[32] The adipocyte CPT activity significantly increased in HF-F81 and HF-50 groups. These findings indicate that the silk proteins F81 and F50 were able to reduce the cholesterol and triglyceride levels in high fat-fed mice by enhancing fatty acid oxidation and partly suppressing the lipogenesis in liver and adipocytes via regulation of the lipogenic enzymes activities.

Plasma Adipokine Concentrations. Adipose tissues produce various protein hormones called adipokines that are involved in the regulation of glucose and lipid metabolism.[33] Studies have shown that the expression, production and release of adipokines, like leptin, resistin and TNF-α, increased with obesity.[34] Adiponectin, on the other hand, declined in obesity and was found to have antiatherosclerotic, anti-inflammatory and hepatoprotective functions.[35] In the present study, a marked increase in the leptin, resistin and TNF-α levels was observed in the mice fed with a high fat diet relative to the control group (Table 6). However, dietary feeding of silk protein F50 counteracted the elevation in the adipokine concentrations. The F100 and F81 also significantly decreased the resistin level in mice. Both the HF-F50 and HF-F81 mice groups showed considerably higher amount of adiponectin compared with the NC and HF groups. These results suggest that the decrease in plasma leptin, resistin and TNF-α and increase in the plasma adiponectin by silk protein may have partly contributed to the antihyperlipidemic effect in mice under high fat diet conditions. The results also illustrate that among the silk proteins analyzed, the F50 was the most effective, while F100 was the least effective, in regulating the production of plasma adipokines, which further implies that sericin may have a greater antiobesity effect than fibroin.

The present study demonstrates that dietary feeding of silk protein significantly suppressed body weight gain, reduced the amount of body fat and improved the lipid metabolism in high fat-fed mice. The hypolipidemic effect was partly due to the inhibition of hepatic and adipocyte lipogenesis and regulation of adipokine production. Moreover, sericin was found to be more effective than fibroin in suppressing high fat-induced hyperlipidemia and obesity since the pure fibroin F100 showed only a marginal effect while the F50, which has the highest sericin and lowest fibroin contents among the silk proteins analyzed, markedly enhanced the lipid profile. The silk protein may be useful as a biomaterial in the development of functional food or therapeutic agent against obesity and its associated diseases. However, further studies on the effect of pure sericin on lipid metabolism and body fat content are needed to better understand the beneficial effects of silk protein on high fat diet-induced hyperlipidemia and obesity.

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