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Antioxidative and Hypoglycemic Effects of Silk Fibroin/SericinMixtures in High Fat-Fed Mice

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The effect of dietary feeding of silk fibroin/sericinmixtureson the antioxidative status and glucose metabolism in high fat-fed mice was investigated. The mice weregiven experimental diets for 6 weeks: normal control (NC),high fat (HF) andhigh fat 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 decreased lipid peroxidation, enhancedantioxidant enzymesactivities and lower blood glucose level relative to HF group. The HF-F50 animals exhibited significantly lower insulin level, higher glycogen concentration, enhanced hepatic glucokinaseactivity and reduced glucose-6-phosphate and phosphoenolpyruvatecarboxynaseactivities than the HF ones. The *in vivo* antioxidant activity and hypoglycemic action tended to increase with increased amount of sericin and decreased fibroin content in the diet. These findings demonstrate that silk protein, particularly sericin, may be beneficial in suppressing high fat diet-induced hyperglycemiaand oxidative stress.

Keywords: Silk protein, Oxidative stress, Hypoglycemic effect, Antioxidant enzyme, High fat diet

Introduction

A high intake of dietary fat has been linked with the development of obesity, impaired glucose tolerance, and type 2 diabetes mellitus (Alsaif and Duwaihy, 2004; Paeratakul and Popkin, 2004). Due to sedentary lifestyle and poor eating habit, the incidence of diabetes is increasing in epidemic proportions, with a current estimate of about 285 million people worldwide with diabetes (Shaw *et al.*, 2010). Characterized by hyperglycemia, this metabolic disease is one of the leading causes of death in developed countries (McGill and Felton, 2007). Oxidative stress is regarded as the major factor in the pathogenesis of diabetesmellitus and its associated health disorders. Studies have shown that increased generation of free radicals and reduced antioxidant defense mechanisms could lead to increased lipid peroxidation and development of insulin resistance (Sharma *et al.*, 2010). While a number of oral anti-diabetic medicines are currently available, these drugs have been associated with several side effects and high rates of secondary failures (Inzucchi, 2002). Hence, there is a need for an alternative approach, including the use of an effective and safe natural drug with strong antioxidative effect and hypoglycemic action.

Silk proteins are natural polymers obtained from the cocoons produced by silkworms during their metamorphosis to moths. They have been used for thousands of years in the production of high quality textile. The silk fiber is mainly composed of fibroin and sericin proteins andpossesses a wide range of biological functions. These protein-based fibers were found to have high antioxidant activity, anti-tumor effect, and anti-obesity action (Fan *et al*., 2009; Hong *et al*., 2002; Mondal *et al*., 2007). Sericin was reported to suppress in vitro lipid peroxidation and tyrosinase activity and fibroin was shown to enhance insulin activity and glucose metabolism (Hyun *et al.*, 2004; Kato *et al*., 1998).

In spite of the numerous studiesconducted with regards to the functional properties of both fibroin and sericin, there is still insufficient pharmacological research on silk protein in relation to hyperglycemia and oxidative stress. Moreover, in the previous studies, silk proteins with very

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low molecular weight were used to determine the biological activities of silk. The silk was hydrolyzed to peptide or amino acid level to make it edible. Hence, 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 glucose metabolism and antioxidative activity using mouse model under high fat diet condition has not been studied yet. Thus, this study was conducted to investigate the effect of dietary feeding of silk proteins with different fibroin/sericin compositions on the antioxidant defense status and glucose metabolism in C57BL/6N mice fed with high fat diet.

Materials and Methods

Chemicals

All reagents were of analytical grade and used without further purification. The calcium chloride, ethanol, ketamine-HCl, tricholoroacetic acid, thiobarbituric acid, potassium phosphate buffer, calcium chloride, and magnesium chloride were obtained from Merck KGaA (Darmstadt, Germany). All other chemicals were purchased from Sigma-Aldrich, Inc. (Steinhein, Germany).

Preparation of silk protein powder

Silk cocoons from *Bombyxmori* silkworms were provided by Korea Rural Development Administration (RDA, Suwon, Korea). Since the cocoons are composed of 74% fibroin and 26% sericin, different preparation conditions were employed to prepare three types of silk proteins with different fibroin/sericin compositions. The pure silk fibroin was produced by degumming the silk cocoons with sodium oleate (0.6%, on the weight of fiber [o.w.f.]) and sodium carbonate (0.4%, o.w.f.) solutions at boiling temperature for 2 hours to remove the sericin. Forty grams of 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 cut-off = 12,000~14,000) against a 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 hour. 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 hour. The pure silk sericin aqueous solution was then mixed with F81 aqueous solution to prepare 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 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 mice groups were fed with a normal control (NC) and high fat (HF, 17%, w/w) diets, respectively. The other three groups were given with 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 semi-synthetic diet. The mice were fed for 6 weeks and allowed free access to food and water during the experimental period. The body weight gain was measured weekly. At the end of the experimental period, the mice were anaesthetized with ketamine-HCl following a 12-h fast. The blood samples were drawn from the inferior vena cava into a heparin-

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
Cholinbitartrate	0.2	0.2	0.2	0.2	0.2
Mineral mixture[1]	3.5	3.5	3.5	3.5	3.5
Vitamin mixture[2]	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

[1]AIN-76 mineral mixture.
[2]AIN-76 vitamin mixture.

coated tube and centrifuged at 1,000 x g for 15 min at 4°C to obtain the plasma and erythrocyte. The livers and kidneys were removed, rinsed with physiological saline, and stored at −70°C until analysis. The weight of the white adipose tissues was determined. The current study protocol was approved by the Ethics Committee of Kyungpook National University for animal studies.

Determination of lipid peroxidation

The erythrocyte and plasma thiobarbituric acid reactive substances (TBARS) were measured according to the method of Ohkawa *et al*. (1979). Trichloroacetic acid (5%, v/v) and 0.06 M thiobarbituric acid were added to 50 μL of plasma and red blood cell preparation and incubated at 80°C for 90 min. After cooling at room temperature, the mixtures were centrifuged at 2,000 rpm for 25 min and the absorbance of the resulting supernatant was determined at 535 nm. A malondialdehyde (MDA) solution was used as standard. The results were calculated and expressed as nmol MDA/mL plasma or g Hb.

Measurement of antioxidant enzyme activities

The hepatic enzyme source was prepared according to the method developed by Hulcher and Oleson (1973). The superoxide dismutase (SOD) activity was spectrophotometrically measured based from the method of Marklund and Marklund (1974). The SOD was detected on the basis of its ability to inhibit superoxide-mediated reduction. The activity was expressed as unit/mg protein, wherein one unit represents the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The amount of protein was determined using Bradford protein assay (Bradford, 1976).

The catalase (CAT) activity was measured using the method of Aebi (1974). The disappearance of hydrogen peroxide was monitored spectrophotometrically at 240 nm for 5 min. A molar extinction coefficient of 0.041/mM/cm was used to determine the CAT activity. The activity was defined as the μmol decreased H_2O_2 / min/mg protein.

The glutathione peroxidase (GSH-Px) activity was measured according to the method of Paglia and Valentine (1967) with slight modifications. The cytosolic supernatant was added to the reaction mixture (6 mM glutathione, 1.2 mM NADPH, and 1.25 μM H_2O_2 in 20 mM Tris-HCl, pH 7.0), which was pre-warmed at 25°C for 5 min. The mixture was further incubated at 25°C for 5 min and the absorbance was measured at 340 nm. A molar extinction coefficient of 6.22/mM/cm was used to determine the activity, which was expressed as nmol oxidized NADPH / min/mg protein.

The glutathione reductase (GR) activity was determined using the method of Mize and Langdon (1952).The reaction mixture contained 1 mM EDTA and 1 mM GSSG in a 0.1 M potassium phosphate buffer (pH 7.4). The oxidation of NADPH was monitored at 340 nm and the activity was expressed as nmol oxidized NADPH/min/mg protein.

The paraoxonase (PON) activity was determined based from the method described by Mackness *et al*. (1991). Briefly, 50 μL of serum was added to 1 mLTris/HCl buffer (100 mM, pH 8.0) containing 2 mM $CaCl_2$ and 5.5 mM, paraoxon. The absorbance of the mixture was measured at 412 nm at 25°C to determine the generation rate of 4-nitrophenol. The enzymatic activity was calculated using the molar extinction coefficient 17100/M/cm.

Determination of blood glucose level

The blood glucose level was measured using Accu-Chek Active Blood Glucose Test Strips (Roche Diagnostics GmbH, Germany). The blood samples were drawn from the tail vein of the mice after 2, 4, and 6 weeks of feeding the animals with experimental diets.

Determination of insulin and glycogenlevels

The glycogen concentration in liver was determined using the method described by Seifter *et al*. (1950). Fresh liver (100 mg) was mixed with 30% KOH and heated at 100°C for 30 min. The mixture was then added with 1.5 mL ethanol (95%) and kept overnight at 4°C. The pellet was mixed with 4 mL distilled water. A 500 mL of the mixture was added with 0.2% anthrone (in 95% H_2SO_4) and the absorbance of the sample solution was measured at 620 nm. The results were calculated on the basis of a standard calibration curve of glucose. The insulin content was measured using enzyme-linked immunosorbent assay (ELISA) kits (TMB Mouse Insulin ELISA kit, Sibayagi, Japan).

Measurement of glucose-regulating enzyme activities

The glucokinase (GK) activity was determined based from the method of Davidson and Arion (1987) with slight modification. A 0.98 mL of the reaction mixture containing 50 mM Hepes-NaOH (pH 7.4), 100 mM KCl, 7.5 mM $MgCl_2$, 2.5 mM dithioerythritol, 10 mg/mL albumin, 10 mM glucose, 4 units of glucose-6-phosphate dehydrogenase, 50 mM NAD^+ and 10 mL cytosol was pre-incubated at 37°C for 10 min. The reaction was initiated with the addition of 10 mL of 5 mM ATP and the mixture was incubated at 37°C for 10 min. The change in absorbance at 340 nm was recorded.

The glucose-6-phosphatase (G6pase) activity was measured using the method described by Alegre *et al*. (1988). The reaction mixture contained 765 mL of 131.58 mM

Hepes-NaOH (pH 6.5), 100 mL of 18 mM EDTA (pH 6.5), 100 mL of 265 mM glucose-6-phosphate, 10 mL of 0.2 M $NADP^+$, 0.6 IU/mL mutarotase and 0.6 IU/mL glucose dehydrogenase. After pre-incubation at 37°C for 3 min, the mixture was added with 5 mL microsome and incubated at 37°C for 4 min. The change in absorbance at 340 nm was measured.

The phosphoenolpyruvatecarboxykinase (PEPCK) activity was determined based from the method developed by Bentle and Lardy (1976). The reaction mixture consisted of 72.92 mM sodium Hepes (pH 7.0), 10 mM dithiothreitol, 500 mM $NaHCO_3$, 10 mM $MnCl_2$, 25 mM NADH, 100 mM IDP, 200 mM PEP, 7.2 unit of malic dehydrogenase and 10 mL cytosol. The enzyme activity was determined based from the decrease in the absorbance of the mixture at 350 nm at 25°C.

Statistical analysis

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

Results

Body weight gain

Prior to feeding the animals with the experimental diets, there was no significant difference in the body weight among the mice groups. The daily food intake was also similar in all groups throughout the study. At the end of the experimental period, the HF and HF-F100 animals exhibited a marked increase in the body weight relative to the control mice (data not shown). Moreover, the weights of epididymal, perirenal, and visceral white adipose tissues were highest in HF and HF-F100 groups. On the other hand, the HF-F81 and HF-50 groups showed significantly lower final body weights and reduced amount of body fatsthan the HF mice, indicating that both F81 and F50 silk proteins could suppress the weight gain inmice under high fat diet condition.

Plasma and erythrocyte lipid peroxides

High fat feeding resulted in a significant increase in the levels of erythrocyte TBARS (Table 2). Addition of silk proteins F81 and F50 in the diet markedly decreased both the plasma and erythrocyte TBARS concentrations. Diet supplementation of F100 reduced the erythrocyte TBARS to normal level, but no significant effect on the plasma TBARS.

Table 2. Plasma and erythrocyte TBARS levels in mice fed with high fat diet supplemented with silk protein

Dietary group	Plasma TBARS (nmol/mL)	Erythrocyte TBARS (nmol/g Hb)
NC	13.76 ± 0.48^{bc}	4.95 ± 0.67^{b}
HF	14.62 ± 0.63^{c}	6.51 ± 0.72^{c}
HF-F100	13.62 ± 0.43^{bc}	4.64 ± 0.77^{ab}
HF-F81	12.71 ± 0.19^{b}	3.33 ± 0.24^{a}
HF-F50	11.28 ± 0.43^{a}	3.16 ± 0.12^{a}

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

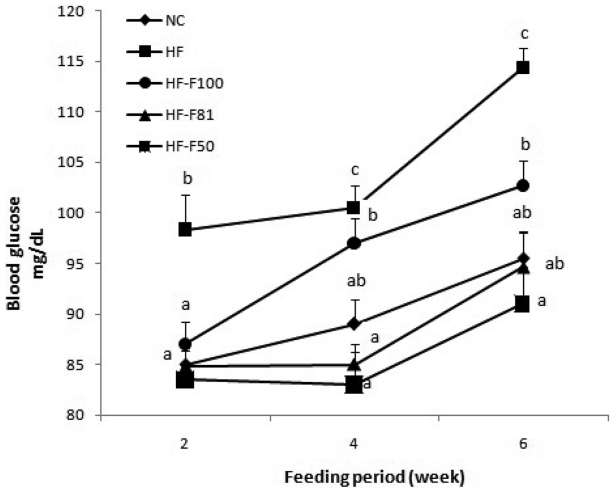

Fig. 1. Effect of silk protein supplementation on the blood glucose level in high fat fed-mice. Means not sharing a common superscript are significantly different at $p<0.05$ (n=8).

Antioxidant enzyme activities

High fat feeding did not significantly change the activities of antioxidant enzymes (Table 2). On the other hand, diet supplementation of F50 resulted in enhanced activities of all the hepatic and erythrocyte antioxidants enzymes analyzed relative to the control and high fat groups. The mice fed with F100 showed significantly higher hepatic CAT, GSH-Px, GR, and erythrocyte SOD and CAT enzymes activities than the HF group. The HF-F81 groups also showed increased activities of hepatic antioxidant enzymes, as well as erythrocyte SOD and CAT.

Blood glucose levels

After 2 weeks of feeding the mice with experimental diets, only the HF group showed a significant elevation in the blood glucose level (Fig. 1). On the 4th week, an increase in the glucose concentration was also observed in

Table 3. Antioxidant enzyme activityin mice fed with high fat dietsupplemented with silk protein

	NC	HF	HF-F100	HF-F81	HF-F50
Liver					
SOD (unit/mg protein)	1.56 ± 0.14^{ab}	1.38 ± 0.13^{a}	2.03 ± 0.30^{abc}	2.27 ± 0.24^{bc}	2.45 ± 0.38^{c}
CAT (μmol H_2O_2/min/mg protein)	1.17 ± 0.03^{ab}	1.14 ± 0.02^{a}	1.24 ± 0.03^{bc}	1.28 ± 0.05^{cd}	1.36 ± 0.02^{d}
GSH-Px (nmol NADPH/min/mg protein)	4.03 ± 0.13^{ab}	3.85 ± 0.04^{a}	4.34 ± 0.19^{b}	4.43 ± 0.11^{bc}	4.52 ± 0.11^{c}
GR (nmol NADPH/min/mg protein)	16.13 ± 0.67^{ab}	14.83 ± 0.91^{a}	17.13 ± 0.66^{b}	18.34 ± 0.45^{bc}	19.49 ± 0.54^{c}
PON (nmol p-nitrophenol/min/mg protein)	2.48 ± 0.30^{a}	2.26 ± 0.14^{a}	2.62 ± 0.20^{ab}	3.16 ± 0.27^{b}	3.27 ± 0.32^{b}
Erythrocyte					
SOD (unit/mg Hb)	4.87 ± 0.33^{a}	4.30 ± 0.11^{a}	6.01 ± 0.46^{b}	6.09 ± 0.22^{b}	6.40 ± 0.45^{b}
CAT (μmol H_2O_2/min/g Hb)	0.37 ± 0.01^{b}	0.33 ± 0.01^{a}	0.37 ± 0.01^{b}	0.39 ± 0.02^{b}	0.40 ± 0.02^{b}
GSH-Px(nmol NADPH/min/g Hb)	2.15 ± 0.15^{ab}	1.84 ± 0.11^{a}	2.11 ± 0.17^{ab}	2.19 ± 0.17^{ab}	2.41 ± 0.19^{b}
GR (μmol NADPH/min/g Hb)	0.27 ± 0.02^{ab}	0.24 ± 0.01^{a}	0.27 ± 0.01^{ab}	0.27 ± 0.03^{ab}	0.32 ± 0.03^{b}

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

Table 4. Insulin and glycogen concentrations in mice fed with high fat diet supplemented with silk protein

Dietary group	Insulin (ng/mL)	Glycogen (mg/g)
NC	8.95 ± 0.30^{ab}	4.87 ± 0.56^{ab}
HF	9.77 ± 0.96^{b}	4.64 ± 0.53^{a}
HF-F100	9.56 ± 0.65^{ab}	5.04 ± 0.34^{ab}
HF-F81	8.97 ± 0.71^{ab}	5.80 ± 0.50^{ab}
HF-F50	8.34 ± 0.97^{a}	6.29 ± 0.79^{b}

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

the NC and HF-F100 mice, but the levels were considerably lower than that of the HF group. While all the animal groups exhibited increased glucose level on the final week, the levels in silk protein fed-mice groups were not significantly different with that of the normal group. The HF-F50 showed the lowest concentration of blood glucose.

Insulin and glycogen concentrations

Among the silk protein-fed mice groups, only the HF-F50 animals showed a significant change in the insulin and glycogen concentrations (Table 4). The insulin level was considerably lower, while the glycogen level was higher, in these animals compared with that of the HF ones. It was also noted that the insulin and glycogen concentrations tended to decrease and increase, respectively, with decreased concentration of fibroin and increased amount of sericin in the diet.

Glucose-regulating enzymeactivities

No significant change in the activities of glucose-regulating enzymes was found in HF and HF-F100 groups (Table 5). The HF-F81 group showed significantly lower activities of kidney G6pase and PEPCK compared with

Table 5. Glucose-regulating enzyme activity in mice fed with high fat diet supplemented with silk protein

	NC	HF	HF-F100	HF-F81	HF-F50
			Liver (nmol/min/mg protein)		
GK	2.09 ± 0.14^{a}	1.99 ± 0.13^{a}	2.05 ± 0.13^{a}	2.15 ± 0.15^{a}	2.90 ± 0.21^{b}
G6pase	107.86 ± 7.33^{ab}	117.53 ± 9.36^{b}	109.05 ± 7.65^{ab}	103.85 ± 6.54^{ab}	91.50 ± 6.04^{a}
PEPCK	3.16 ± 0.31^{a}	3.67 ± 0.72^{a}	3.21 ± 0.49^{a}	3.10 ± 0.75^{a}	2.67 ± 0.84^{a}
			Kidney (nmol/min/mg protein)		
G6Pase	125.20 ± 3.23^{bc}	128.25 ± 4.43^{c}	122.52 ± 2.82^{bc}	115.20 ± 2.38^{b}	75.09 ± 5.78^{a}
PEPCK	10.05 ± 0.50^{bc}	11.19 ± 0.72^{c}	9.64 ± 0.51^{bc}	8.63 ± 0.36^{ab}	7.48 ± 0.80^{a}

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

the HF animals. Higher GK activity and lower kidney G6pase and PEPCK activities were observed in HF-F50 group than that of the NC and HF groups. Moreover, the mice fed with F50 exhibited significantly lower hepatic G6pase than the high fat-fed mice.

Discussion

The development of hyperglycemia is associated with oxidative stress resulting from increased generation of free radicals and reduced antioxidant defense mechanisms. The TBARS concentration has been widely used an indicator of lipid peroxidation and oxidative stress in laboratory animals. In the present study, a significant reduction in the plasma and erythrocyte TBARS levels was found in mice fed with silk proteins compared with that of the high fat-fed mice, indicating a decreased rate of lipid peroxidation. To control oxidative stress and regulate the destructive potential of free radicals, the cells have developed a highly complex antioxidant protection system including antioxidant enzymes that catalyze free radicals-quenching reactions. In general, higher activities of antioxidant enzymes SOD, CAT, GSH-Px, GR, and PON were observed in mice fed with silk proteins compared with that of the control- and high fat-fed animals. These reduced levels of plasma and erythrocyte TBARS and enhanced activities of hepatic and erythrocyte antioxidant enzymes in silk protein-fed mice suggest a marked improvement in the *in vivo* antioxidative system of the mice, making them less susceptible to peroxidative damage under high fat diet condition. A study conducted by Kato *et al*. (1998) provided the first scientific evidence of the antioxidant action of silk protein, in which sericin inhibited *in vitro* lipid peroxidation. Dietary sericin was also shown to suppress colonic oxidative stress in rats and mice (Zhaorigetu *et al*., 2001; Zhaorigetu *et al*., 2007). The protective effect of silk protein against high fat-induced lipid peroxidation and oxidative stress could be partly attributed to a mechanism involving the elevation of the activities of antioxidant enzymes. The SOD protects cells from oxidative damage by converting superoxide radicals into hydrogen peroxides, which in turn are utilized and degraded by CAT and GSH-Px to non-toxic products (Reiter *et al*., 2002). The PON hydrolyzes biologically active oxidized phospholipids and destroys lipid hydroperoxides (Ng *et al*., 2005). The enhanced antioxidant enzymes activities in the liver and erythrocyte may have a protective role against reactive oxygen species (ROS), thereby suppressing the formation of hydrogen peroxide and lipid peroxidation and preventing hepatic and erythrocyte damage.Interestingly, it was found that the TBARS level and enzyme activities tended to decrease and increase, respectively, with reduced amount of fibroin and increased sericin content in the diet, indicating that sericin may be more effective than fibroin in suppressing high fat diet-induced oxidative stress and enhancing the antioxidative defense status in mice.

High fat feeding resulted in a significant increase in the blood glucose level in mice, whereas diet supplementation of silk proteins caused a marked reduction in the glucose concentration. All animal groups exhibited an increasing trend in the glucose level through time, which could probably be due to the aging of the mice. It was previously reported that C57BL/6 mice fed with standard food pellets showed elevated blood glucose from 4 to 6 weeks of age (Amrani *et al*., 1998). The insulin and glycogen levels were not significantly affected with the high fat diet, but the F50-fed mice showed significantly lower insulin level and higher glycogen concentration than the high fat-fed animals. Past studies on the pharmacological effects of silk protein revealed that the soluble fibroin could increase glucose uptake and metabolism and its hydrolysate could decrease both the blood glucose and insulin concentrations in mice (Hyun *et al*., 2004; Nahm and Oh, 1995). Similarly, dietary feeding of silk sericinwas recently found to suppress the increase in plasma glucose and insulin secretion after an intraperitoneal glucose injection in high fat-fed rats (Okazaki *et al*., 2010). Of the 3 silk proteins analyzed, the F50 appeared to be the most effective in lowering the glucose level. While the dietary feeding of pure fibroin F100 caused a significant decrease in the glucose concentration relative to the high fat-fed mice, diet supplementation with F50 containing 50% sericin and 50% fibroin resulted in a considerably lower concentration of blood glucose in mice compared with that of the HF-F100 group. Moreover, the glycogen and insulin levels were highest and lowest, respectively, in HF-F50 group. It was also noted that the insulin level tended to decrease, while the glycogen level tended to increase, with decreased amount of fibroin and increased amount of sericin in the diet. These findings suggest that sericin may have a greater hypoglycemic effect than fibroin.

The hypoglycemic action of silk protein, particularly F50, is probably associated with the marked enhancement in the activities of hepatic GK enzyme and inhibition of G6pase and PEPCK activities in the liver and kidney. The hepatic GK enzyme is involved in the regulation of glucose homeostasis. Elevated expression of GK, as observed in HF-F50 animals, could cause an increase in blood glucose utilization for energy production or glycogen storage in the liver, resulting in decreased level of blood glucose (Coope*et al*., 2006). Accordingly, enhanced rate of glycogenesis was found in F50-fed mice, as manifested by a marked increase in the

hepatic glycogen concentration. The G6pase and PEPCK enzymes, on the other hand, are involved in the regulation of gluconeogenesis and glucose output in the liver (Friedman *et al*., 1997). Hence, decreased G6pase and PEPCK activities signify reduced hepatic glucose production. The enhancement of glucose metabolism in silk protein-fed mice is possibly linked with the *in vivo* antioxidative status-improving effect of silk protein. It has been shown that a high fat diet impairs glucose metabolism and the regulation of blood glucose is essential in preventing the development of diabetes. Hyperglycemia, the hallmark of diabetes, promotes the formation of ROS that causes cellular injury, and an increase in ROS is related to the damage in hepatic glucose-regulating enzymes (Hong *et al*., 2009; Lelli *et al*., 2005). Since diabetes is a free radical-mediated disease, antioxidantscould reduce blood glucose levels by protecting the cells against the toxic effects of ROS under hyperglycemic condition (Kaneto *et al*., 1999). The strong antioxidant activity *in vivo* of silk protein may be partly responsible for its hypoglycemic effect in mice fed with high fat diet.

Results of this study illustrate that silk protein could suppress oxidative stress and improve the blood glucose metabolism in high fat high fat-fed C57BL/6N micethrough enhancement of the antioxidant enzymes activities, activation of GK enzyme, and inhibition of G6pase and PEPCK enzymes. Furthermore, sericin was found to have greater *in vivo* antioxidant activity and hypoglycemic action than fibroinsince the pure fibroin F100 exhibited only marginal effect while the F50, which has the highest sericin and lowest fibroin contents among the silk protein analyzed, markedly enhanced the antioxidant defense system and improved the glucose metabolism. The silk protein may be useful as a biomaterial in the development of functional food or therapeutic agent against hyperglycemia. Further studies are needed, however, on the effect of pure sericin on the antioxidative status and glucose metabolism to have a better understanding on the therapeutic potential of silk protein against high fat-induced oxidative stress and hyperglycemia.

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