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High-Molecular-Weight Poly-Gamma-Glutamate Protects Against Hypertriglyceridemic Effects of a High-Fructose Diet in Rat

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We studied the effects of 2 different dosages of high-molecular-weight poly-γ-glutamic acid (hm γ-PGA) derived from *Bacillus subtilis chungkookjang* on lipid metabolism in a high-fructose diet-induced hypertriglyceridemic animal model. For 4 weeks, rats were fed either AIN-93 diet (normal control, NC; n = 10) or modified AIN-93 diet in which cornstarch was substituted with 63% fructose (n = 30) to induce hypertriglyceridemia. After 4 weeks, the hypertriglyceridemic rats were treated with daily oral doses of 0 mg (hypertriglyceridemic control, HC), 2.5 mg (hypertriglyceridemic, low hm γ-PGA, HL), or 5 mg·kg·bw^{-1}·d^{-1} (hypertriglyceridemic, high hm γ-PGA, HH) hm γ-PGA for 4 weeks. The HL and HH groups exhibited significantly lower levels of serum triglyceride, total cholesterol, LDL cholesterol, and free fatty acids than the HC group. The administration of hm γ-PGA reduced serum ALT and AST levels. The activities of lipogenic enzymes such as hepatic malic enzyme and glucose-6-phosphate dehydrogenase as well as glucose-6-phosphate dehydrogenase mRNA expression were significantly decreased by hm γ-PGA administration ($p < 0.05$). These results indicate that hm γ-PGA has an anti-hypertriglyceridemic effect in high-fructose diet-induced hypertriglyceridemic rats.

Key words: γ-PGA, triglyceride, lipogenesis, hypertriglyceridemia, rat

Poly-γ-glutamic acid (γ-PGA) is the major component of the viscous mucilage in fermented soybean products such as *natto* or *chungkookjang* produced by *Bacillus subtilis* sp. [39, 33]. *Chungkookjang* and *natto*, traditional Korean and Japanese soybean products, respectively, are widely consumed in normal diets for their popular characteristic tastes and health benefits. Therefore, the consumption of hm γ-PGA through *chungkookjang* and *natto* is safe. γ-PGA is a naturally occurring polyamide containing a repeating unit of glutamic acid, linked *via* γ-amide linkages between the α-amino and γ-carboxyl groups [37]. The molecular mass of PGA can range from 10 kDa to over 10,000 kDa depending on the bacterial strain [37]. *Bacillus subtilis* used in *natto* produces PGA with a molecular mass from 10 to 1,000 kDa, whereas *B. subtilis chungkookjang* produces γ-PGA with a high molecular mass (>1000 kDa) [33, 38]. Sung *et al*. [38] produced pure high-molecular-weight γ-PGA (hm γ-PGA) using *B. subtilis chungkookjang* as a biocatalyst. A previous study using a murine model shows that γ-PGA from *natto* has an inhibitory effect on the oxidation of low-density lipoprotein and lowers the plasma triglyceride (TG) and total cholesterol levels [16]. Previously, we reported that hm γ-PGA (approximately 500 kDa) supplementation into high-fat-induced obese rats decreases body weight gain, visceral fat accumulation, and blood serum cholesterol, and increases HDL cholesterol [34].

The metabolic syndrome, which includes visceral obesity, dyslipidemia, hyperglycemia, and hypertension, has become a major health problem worldwide; it is a combination of medical disorders that increase the risk of developing cardiovascular disease and diabetes mellitus [35]. The prevalence of metabolic syndrome is increasing; it currently affects 22.1% of men and 27.8% of women in Korea [5, 18, 35]. Hypertriglyceridemia has long been associated with an increased risk of cardiovascular disease [5, 18, 35]. A meta-analysis of data from population-based prospective studies demonstrates that hypertriglyceridemia increases

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the risk of cardiovascular disease by 32% and 76% in men and women, respectively [2, 36]. Another prospective study shows that the plasma TG level is an independent risk factor for cardiovascular disease after adjusting for HDL cholesterol and other risk factors [4, 32]. Therefore, dyslipidemia should be a primary target of interventions aiming to reduce the prevalence of the metabolic syndrome.

High-sucrose and high-fructose diets have been used in animal models to induce the metabolic changes characteristic of the metabolic syndrome [29]. Although the underlying mechanisms of the detrimental effects of high-fructose diets in animal models are unclear, observations that dietary fructose facilitates oxidative damage and obesity indirectly support these phenomena. Since fructose feeding stimulates lipid synthesis in tissue, it is suggested that fructose-induced increases in hepatic lipogenesis may elevate blood TG levels in animals [15].

Recent studies indicate that the reversal or regression of cardiovascular lesions can be achieved by aggressive lipid lowering or drug treatment [11, 31]. Dietary regulation to prevent post-prandial lipemia is a cornerstone of dyslipemia management. Dietary interventions aiming to ameliorate lipid disturbances include soybean and/or soybean products. From this perspective, many studies have investigated the effects of fermented soybean products such as *natto* and *chungkookjang* on lipid metabolism [3, 16]. Another study reported that hm γ-PGA from *chungkookjang* or *natto* could be a new material that shares many properties with mucilages, which are secondary plant compounds that have a high water-holding capacity [9]. The cholesterol-lowering effect of flax seed is attributed to its mucilage [22]. Moreover, a previous study shows that hm γ-PGA intake prevents visceral fat accumulation and decreases blood cholesterol level [34]. However, despite these promising results, the effect of hm γ-PGA on hypertriglyceridemic conditions remains unclear.

Rats fed high-fructose diets exhibit metabolic changes observed the metabolic syndrome. In the present study, we used a 63% high-fructose diet to induce hypertriglyceridemia and administered γ-PGA orally. Thus, the present study assessed the effects of hm γ-PGA administration on lipid metabolism and related mechanisms in hypertriglyceridemic rats.

Materials and Methods

Preparation and Molecular Weight Determination of γ-PGA

Hm γ-PGA were derived from *Bacillus subtilis chungkookjang* in a pilot-scale plant (BioLeaders Corporation, Daejeon, Korea) as described previously [38]. A culture solution of *Bacillus subtilis (chungkookjang)* (KCTC 0697BP) was inoculated into preparative basic medium of γ-PGA [GS basic medium with 5% L-glutamic acid:glucose 5%, $(NH_4)_2SO_4$ 1%, KH_2PO_4 0.27%, $Na_2HPO_4 \cdot 12H_2O$ 0.42%, NaCl 0.05%, pH 6.8], and cultured with stirring at 150 rpm, an aeration rate of 1 vvm, at 37°C for 36 h. After culturing, a filter press was used to eliminate microorganisms, yielding a solution containing γ-PGA. Then, after adding 2 N hydrochloric acid to the above solution containing γ-PGA, the solution was left to stand at 10°C for 12 h to sediment γ-PGA. After cleaning with a sufficient amount of reverse osmosis (RO) water, γ-PGA was obtained using a Nutsche filter. The γ-PGA had a molecular mass of 1–15,000 kDa, and separate experiments were carried out on subfractions with a range of molecular masses. To get γ-PGA of K salt, γ-PGA was solubilized by adding KOH.

The molecular mass of γ-PGA was determined by gel permeation chromatography (GPC). Briefly, PGA solution was diluted with 0.1 M $NaNO_3$, and injected into the GPC equipped with a ViscoGel $GMPW_{XL}$ column (7.8 mm × 30 cm; Viscotech, Houston, TX, USA), which had been equilibrated with 0.1 M $NaNO_3$, at 40°C and a flow rate of 0.8 ml/min. γ-PGA was detected with a Viscotek LR25 Laser Refractometer. Polyacrylamide was used as a standard material for molecular mass determination.

Experimental Animals and Diets

The experiment was conducted with forty 8-week-old male Wistar rats with an initial body weight of 281 ± 2.6 g purchased from Orient-Bio Laboratory Animal Research Center Co., Ltd. (Seongnam, Korea). The animals were housed in individual stainless-steel wire cages in a controlled environment of 22°C ± 2°C, 65% ± 5% relative humidity, and a 12 h light/dark cycle (lights on at 9 a.m.). After a week of acclimation, the rats were randomly divided into 2 groups and fed either the AIN-93 diet [34] as the control group (NC, n = 10) or the modified AIN-93 diet in which cornstarch was substituted with 63% fructose to induce hypertriglyceridemia for 4 weeks. Hypertriglyceridemia was defined as serum TG level > 300 mg/dl [7, 41] by a LipidoCare Biosensor (MedicalSK, Inc., Daegu, Korea). Subsequently, the hypertriglyceridemic rats were randomly divided into 3 subgroups (n = 10 each) according to the serum TG levels and administered oral doses of 0 mg (hypertriglyceridemic control, HC), 2.5 mg (hypertriglyceridemic low γ-PGA, HL), or 5 $mg \cdot kg \cdot bw^{-1} \cdot d^{-1}$ (hypertriglyceridemic high γ-PGA, HH) hm γ-PGA for 4 weeks. The ingredients used in this study were as follows: casein (Daejung Chemical and Metal Co., Gyenoggi-do, Korea); cellulose, mineral mix, and vitamin mix (G-Bio Co., Gyenoggi-do, Korea); cornstarch (Daesang Co., Seoul, Korea); sucrose (Samyang Co., Ulsan, Korea); fructose (Tate and Lyle, Decatur, IL, US); soybean oil (Cheiljedang Co., Seoul, Korea); and choline bitartrate and *tert*-butylhydroquinone (Sigma Aldrich, St. Louis, MO, USA). All procedures were performed with the approval of the Animal Ethics Committee of Kookmin University (Approval No. 201202).

Blood and Tissue Sample Processing

After 8 weeks of the experimental period, the rats were sacrificed by decapitation after 16 h of starvation. The liver, epididymal, and perirenal fat pad were removed quickly, weighed, and stored at −70°C until biochemical analysis. Blood samples were collected and centrifuged at 3,000 rpm at 4°C for 15 min, and the supernatants were stored at −70°C for lipid profile analysis.

Lipid Profile Analysis

The total TG concentration in the liver was measured by an Ultrospec 2100 Pro UV/Visible Spectrophotometer (Amersham BioScience, Buckinghamshire, UK) at Δ540 nm using the method of Folch *et al.*

Table 1. Sequences of primers for G6PD mRNA gene analysis in liver.

	Primer	Sequence
β-Actin	Forward	5'-CCC GCG AGT ACC TTC T-3'
	Reverse	5'-CGT CAT CCA TGG CGA ACT-3'
G6pdx	Forward	5'-GCA AAC AGA GTG AGC CCT TC-3'
	Reverse	5'-TGG CTG TTG AGG TGC TTG-3'

[8]. Levels of TG, total cholesterol, and HDL cholesterol concentrations in the serum were measured by the enzymatic colorimetric method using a commercial ASAN Reagents assay kit (L701-231 TG, L701-212 T-CHO, and HDL-CHO; Asanpharm Co., Gyenoggi-do, Korea). LDL cholesterol levels were calculated using the Friedewald formula [10].

The serum concentrations of free fatty acids (FFAs) were analyzed using a commercial assay kit (EFFA-100; Bio-Assays Systems, CA, USA) according to the manufacturer's instructions.

Enzyme Activity Analysis

The serum concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed using commercial kits (Asanpharm Co., Gyenoggi-do, Korea).

The liver, epididymal, and perirenal fat pad were homogenized in 0.25 M sucrose/Tris-HCl (0.01 M, pH 8) with a homogenizer (OMNI-INC, Marietta, USA) as described previously [41]. The homogenates were centrifuged at 40,000 ×*g* for 10 min, and the supernatants were used to analyze the enzyme activity. The assay medium for malic enzyme consisted of 100 mM Tris buffer (pH 7.4), 100 µl of 100 mM sodium malate, 50 µl of 20 mM, $NADP^+$, and 0.75 ml of 20 mM $MnCl_2$. The final volume of the assay mixtures was 3 ml in all cases. Enzyme activity was evaluated according to the increase in NADPH produced by the reaction catalyzed by malic enzyme [42]. Glucose-6-phosphate dehydrogenase (G6PD) activity was measured as described by Glock and McLean [12]. The protein contents of tissues were quantified by the SMART BCA Protein Assay Kit (21071; Intronbio, Korea) according to the method of Lowry *et al*. [23].

Analysis of G6PD mRNA Expression in the Liver

Total liver mRNA was isolated using the NucleoSpin RNAII kit (Macherey-Nagel, Duren, Germany). Liver tissues were immediately frozen in liquid nitrogen and pulverized for mRNA extraction. The extracted mRNA was used for cDNA synthesis; cDNA was synthesized by first-strand cDNA synthesis using the GoScript Reverse Transcription System kit (Promega, USA). The final volume reaction was 20 µl, containing 10 µl of Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 4 µl of 0.1% DEPC-treated water (Ambion, USA), 0.5 µl of 0.05 µM G6pdx forward and reverse primers, and 5 µl of 50-fold-diluted cDNA. G6PD mRNA expression was measured by real-time RT-PCR (IQ5; Bio-Rad, Hercules, USA). The Bio-Rad IQ5 2.0 software was programmed to run an initial polymerase activation step at 95°C for 10 min, followed by 40 cycles of denaturation (95°C for 15 s) and extension (60°C for 1 min); product synthesis was monitored at the end of the extension step of each cycle. G6PD expression levels were normalized to those of 0.05 µM rat β-actin. The G6PD mRNA gene sequences are presented in Table 1.

Statistical Analysis

The data are expressed as means ± standard error of the means. All data were analyzed using SPSS ver. 19.0 (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was performed, and Duncan's multiple range test was used to determine the significance of differences between groups ($p < 0.05$).

RESULTS

Body Weight, Feed Intake, and Food Efficiency Ratio

The final body weight, daily feed intake, and food efficiency ratio of the groups over 4 weeks are shown in Table 2. Fructose in the diet did not affect feed intake. Whereas fructose-fed rats and control-diet-fed rats gained weight similarly (482.7 *vs* 487.9), the hm γ-PGA-treated rats of the HL and HH groups had lower weight gain (final weight 462.9 or 464.9, $p < 0.05$). After sacrifice, the liver, epididymal, and perirenal fat pad weights of the hm γ-PGA-treated HL and HH groups were significantly lower than that of the HC group (Table 3).

Table 2. Body weight, weight gain, feed intake, and feed efficiency ratio (FER).

Group	Initial body weight (g)	Final body weight (g)	Weight gain (g/day)	Feed intake (g/day)	FER
NC	317.3 ± 6.6 ^{NS}	482.7 ± 9.2 ^{ab}	2.8 ± 0.2 ^{b}	24.1 ± 1.0 ^{NS}	0.11 ± 0.02 ^{NS}
HC	318.2 ± 3.4	487.9 ± 4.2 ^{b}	2.5 ± 0.1 ^{ab}	22.0 ± 0.5	0.12 ± 0.00
HL	317.9 ± 4.2	462.9 ± 9.5 ^{ab}	2.1 ± 0.2 ^{a}	23.5 ± 0.7	0.10 ± 0.01
HH	317.3 ± 6.1	464.9 ± 6.7 ^{a}	2.2 ± 0.1 ^{a}	23.2 ± 0.6	0.10 ± 0.00

Values are expressed as the mean ± SEM, n = 10 rats per group. Measurement were made at initial and after the introduction of the fructose diet. Values with different superscripts in the same column are significantly different ($p < 0.05$) as assessed by one-way ANOVA and the Duncan's multiple range test. NS: Not significant. Abbreviations: NC, Normal diet control; HC, high fructose control; HL, hypertriglyceridemic rats with low hm γ-PGA intake; HH, hypertriglyceridemic rats with high hm γ-PGA intake. FER: Weight gain (g) / feed intake (g).

Table 3. The weights of liver, epididymal fat, and perirenal fat tissue.

Group		NC	HC	HL	HH
Liver	Total wt(g)	11.2 ± 0.4^{a}	13.7 ± 0.1^{c}	12.3 ± 0.5^{b}	12.1 ± 0.3^{ab}
	g/100 g bw	2.4 ± 0.06^{a}	2.8 ± 0.04^{c}	2.7 ± 0.06^{bc}	2.6 ± 0.06^{b}
EFP	Total wt(g)	9.1 ± 0.7^{ab}	10.5 ± 0.5^{b}	8.1 ± 0.7^{a}	8.5 ± 0.6^{a}
	g/100 g bw	1.9 ± 0.2^{NS}	2.2 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
PFP	Total wt(g)	10.2 ± 1.2^{ab}	12.1 ± 1.1^{b}	7.2 ± 1.2^{a}	8.1 ± 0.8^{a}
	g/100 g bw	2.0 ± 0.3^{NS}	2.3 ± 0.3	1.7 ± 0.3	1.8 ± 0.2

Values are expressed as the mean ± SEM, n = 10 rats per group. Values with different superscripts in the same row are significantly different ($p < 0.05$) as assessed by one-way ANOVA and the Duncan's multiple range test. NS: Not significant. Abbreviations: NC, Normal diet control; HC, high fructose control; HL, hypertriglyceridemic rats with low hm γ-PGA intake; HH, hypertriglyceridemic rats with high hm γ-PGA intake. EFP: epididymal fat pad; PFP: perirenal fat pad.

Serum Triglyceride Levels During hm γ-PGA Administration

The changes in serum TG levels during the experimental period are shown in Fig. 2. After 4 weeks of hypertriglyceridemia induction, the high fructose-fed groups had significantly higher serum TG levels than the NC group. After oral hm γ-PGA treatment, the serum levels of TGs decreased dramatically in the HL and HH groups. The TG levels over 4 weeks decreased significantly in the HL and HH groups. In particular, the TG levels in the HH and HL groups were similar to those of the NC group at the final fourth week.

Serum Lipid Profiles, and AST and ALT Levels

The serum lipid profiles, and AST and ALT levels are shown in Table 4. It was observed that hm γ-PGA administration significantly decreased the lipid profiles of total cholesterol, LDL cholesterol, and FFAs. The oral supplementation of hm γ-PGA had significantly higher HDL cholesterol levels than the NC group. The AST and ALT levels, which represent liver function, were significantly higher in the HC group than that in the NC group. The enzyme activity levels of the HH group were similar to those of the NC group, indicating that the ALT or AST levels, elevated by high fructose intake, were alleviated by feeding γ-PGA.

Liver Triglyceride Concentration and Fecal Lipid Excretion

The TG contents of the whole liver and fecal lipid excretion are shown in Fig. 2 and Fig. 3, respectively. Fecal lipid excretion did not differ significantly among groups (Fig. 3). However, the liver TG level was significantly lower in the HL and HH groups than that in the HC group (Fig. 2).

Malic Enzyme Activity

The activities of malic enzyme in the liver, epididymal, and perirenal fat pad are presented in Fig. 4. Malic enzyme

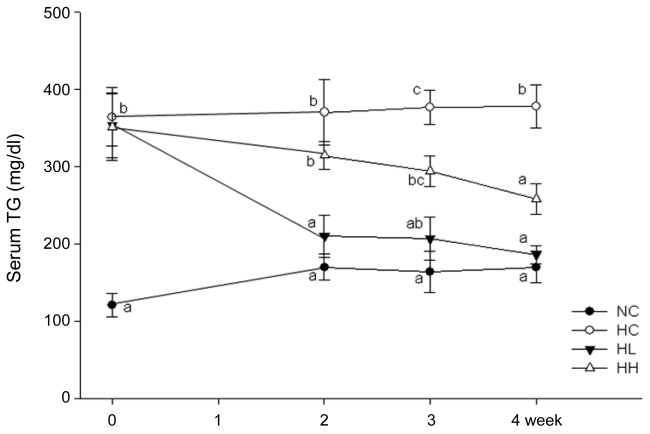

Fig. 1. Serum triglyceride concentration during γ-PGA administration.
Bars are expressed as the mean ± SEM, n = 10 rats per group. Values with different superscripts in the same week are significantly different ($p < 0.05$) as assessed by one-way ANOVA and the Duncan's multiple range test. Abbreviations: NC, Normal diet control; HC, high fructose control; HL, hypertriglyceridemic rats with low hm γ-PGA intake; HH, hypertriglyceridemic rats with high hm γ-PGA intake.

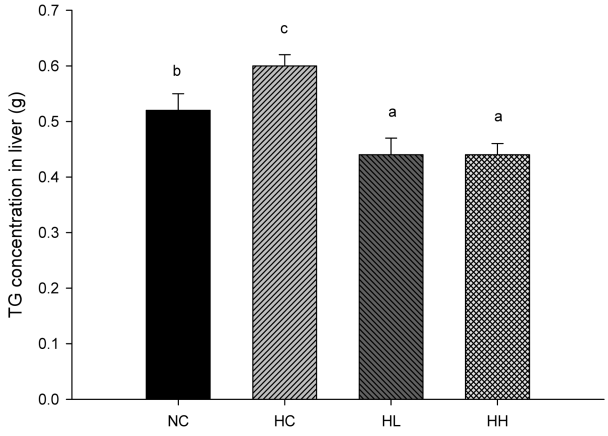

Fig. 2. Triglyceride concentration in liver.
Bars represent the mean ± SEM, n = 10 rats per group. Values with different superscripts are significantly different ($p < 0.05$) as assessed by one-way ANOVA and the Duncan's multiple range test. Abbreviations: NC, Normal diet control; HC, high fructose control; HL, hypertriglyceridemic rats with low hm γ-PGA intake; HH, hypertriglyceridemic rats with high hm γ-PGA intake.

Table 4. Serum lipid profiles, and AST and ALT levels.

	Groups			
	NC	HC	HL	HH
TC (mg/dl)	76.5 ± 4.8^{a}	103.2 ± 3.7^{c}	84.0 ± 2.3^{ab}	88.2 ± 2.9^{b}
LDL-C (mg/dl)	38.7 ± 2.8^{ab}	46.9 ± 4.6^{b}	30.5 ± 2.6^{a}	34.2 ± 2.2^{a}
HDL-C (mg/dl)	33.8 ± 6.3^{a}	41.8 ± 5.0^{b}	42.0 ± 3.5^{b}	45.3 ± 6.9^{b}
FFA (μM/ml)	441.2 ± 45.1^{ab}	490.4 ± 42.3^{b}	318.7 ± 40.5^{a}	390.9 ± 34.2^{ab}
AST (U/L)	182.6 ± 6.6^{a}	243.6 ± 16.4^{b}	209.4 ± 13.6^{ab}	194.9 ± 12.4^{a}
ALT (U/L)	46.5 ± 3.2^{a}	67.2 ± 3.1^{c}	57.8 ± 3.4^{bc}	54.8 ± 3.7^{ab}

Values are expressed as the mean ± SEM, n = 10 rats per group. Values with different superscripts in the same row are significantly different ($p < 0.05$) as assessed by one-way ANOVA and the Duncan's multiple range test. Abbreviations: NC, Normal diet control; HC, high fructose control; HL, hypertriglyceridemic rats with low γ-PGA intake; HH, hypertriglyceridemic rats with low γ-PGA intake.

activities in the liver and epididymal fat pad were significantly higher in the HC group than those in the NC group. The HL and HH groups showed significantly lower malic enzyme activities in liver and fat tissues than those in the same tissues of the HC group. The malic enzyme activities of the epididymal and perirenal fat pad from the HL and HH groups were lower than those of the same tissues from the NC group.

G6PD Activity

Hepatic G6PD activity is presented in Fig. 5. The activity was similar to that of malic enzyme. Hepatic G6PD activity was significantly higher in the HC group than that in the NC group. The HL and HH groups exhibited significantly lower hepatic G6PD activities than NC groups, respectively. However, the higher dosages of hm γ-PGA did not cause any further antilipogenic response with respect to lipogenic enzyme activities.

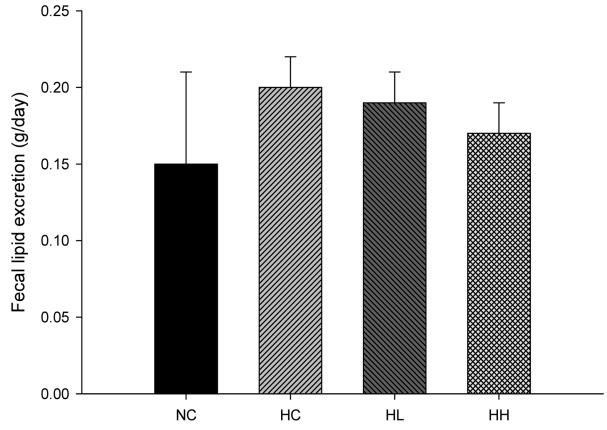

Fig. 3. Fecal lipid excretion.
Bars represent the mean ± SEM, n = 10 rats per group. NS: Not significant. Abbreviations: NC, Normal diet control; HC, high fructose control; HL, hypertriglyceridemic rats with low γ-PGA intake; HH, hypertriglyceridemic rats with high γ-PGA intake.

Expression of mRNA in the Liver

The HC group fed with the high-fructose diet exhibited higher liver G6PD mRNA expression levels than the NC group fed with the normal diet (Fig. 6). However, the oral administration of hm γ-PGA affected G6PD mRNA expression; its expression was 2-fold higher in the HC group than that in the NC group. G6PD mRNA expression in the HL and HH groups was similar to that in the NC group.

DISCUSSION

The metabolic syndrome is strongly associated with cardiovascular diseases. Dyslipidemia is regarded as one of the independent causes of metabolic syndrome [32]. In

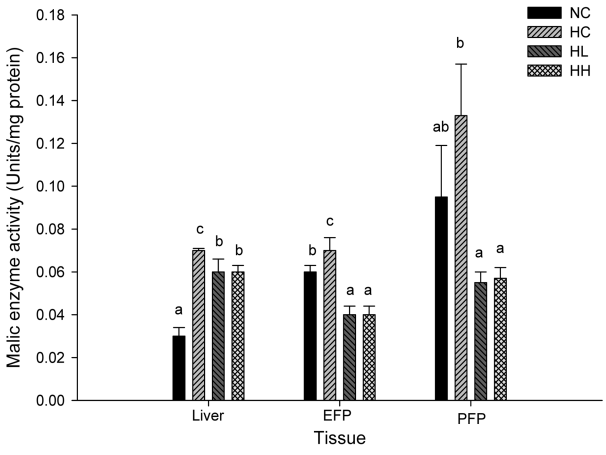

Fig. 4. Malic enzyme activity in the liver, EFP, and PFP.
Bars represent the mean ± SEM, n = 10 rats per group. Values with different superscripts in the same tissue are significantly different ($p < 0.05$) as assessed by one-way ANOVA and the Duncan's multiple range test. Abbreviations: NC, Normal diet control; HC, high fructose control; HL, hypertriglyceridemic rats with low hm γ-PGA intake; HH, hypertriglyceridemic rats with high hm γ-PGA intake. EFP: epididymal fat pad; PFP: perirenal fat pad.

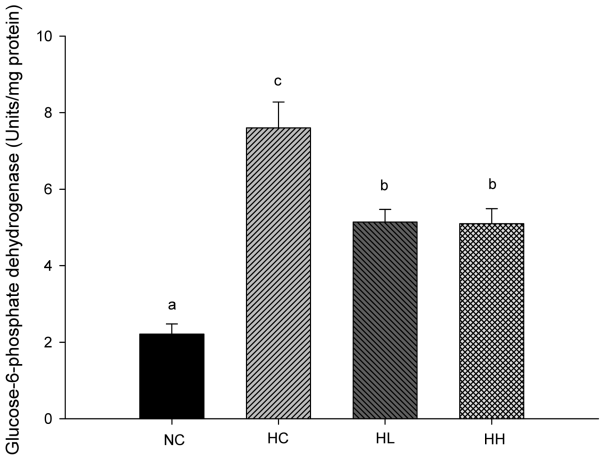

Fig. 5. G6PD enzyme activity in liver.
Bars represent the mean ± SEM, n = 10 rats per group. Values with different superscripts are significantly different (p <0.05) as assessed by one-way ANOVA and the Duncan's multiple range test. Abbreviations: NC, Normal diet control; HC, high fructose control; HL, hypertriglyceridemic rats with low hm γ-PGA intake; HH, hypertriglyceridemic rats with high hm γ-PGA intake.

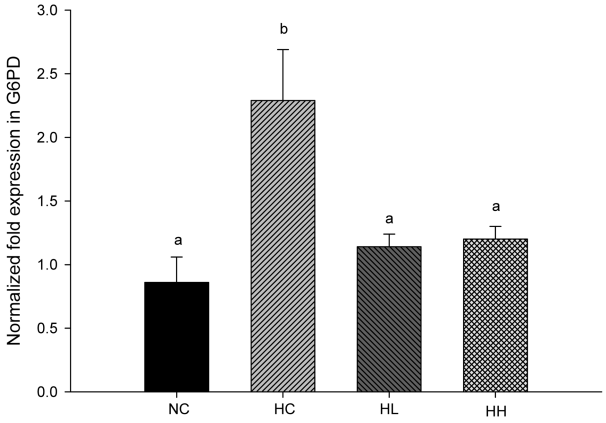

Fig. 6. G6PD mRNA expression in liver.
Bars represent the mean ± SEM, n = 10 rats per group. The G6PD mRNA expression levels of experimental groups were normalized to the level of the normal control group. Values with different superscripts are significantly different (p < 0.05) as assessed by one-way ANOVA and the Duncan's multiple range test. Abbreviations: NC, Normal diet control; HC, high fructose control; HL, hypertriglyceridemic rats with low hm γ-PGA intake; HH, hypertriglyceridemic rats with high hm γ-PGA intake.

particular, hypertriglyceridemia is the most typical metabolic abnormality associated with the metabolic syndrome among Koreans [5, 18, 29, 35]. Although reports on the association between hypertriglyceridemia and atherosclerosis are not completely consistent, the emerging trend is that hypertriglyceridemia can be a significant risk factor of cardiovascular disease [5, 18, 26, 35]. Moreover, feeding high-fructose diets to laboratory animals precipitates the development of hypertriglyceridemia [29]. Blood TG-lowering interventions are reported to significantly reduce coronary heart disease [27].

Our results are concordant with previous data and indicate that the consumption of a high-fructose diet increases the liver and plasma TG levels [11]. The increased blood TG levels are attributable to the overproduction of TG in the liver. In addition, hypertriglyceridemia was accompanied by increases in the levels of serum total cholesterol in the present study.

The administration of hm γ-PGA at 2.5 mg·kg·bw^{-1}·d^{-1} to rats with hypertriglyceridemia induced by a high-fructose diet lowered the serum TG levels and prevented TG accumulation in the liver. However, no further decreases were noted at higher dosages. The present experiment addressed the question of the dose effect of γ-PGA administration. We compared two different dose levels of hm γ-PGA (2.5 mg·kg·bw^{-1} *vs* 5.0 mg·kg·bw^{-1}). The changes in serum triglyceride levels indicated a significant difference between high fructose-fed control and γ-PGA-treated groups (p < 0.05). There was no obvious significant difference between the two different dosage groups. We expected the additive improvement of dyslipidemia of hm PGA in the higher-dose-treated group. Looking for similarities between the two groups, the results seem to indicate that the higher level of dosage was not enough or too high to induce further additive changes. Lim *et al.* [22] reported that there were no difference in the improvement of diabetic parameters between 500 mg/kg/day fermented soybean extracts-treated group and 1,000 mg/kg/day treated groups. Although the administration of γ-PGA may modify lipid metabolism in the hypertriglyceridemic condition, the question regarding optimal effective dose at the physiological level needs further study.

Beneficial changes in the serum total cholesterol and LDL cholesterol levels were observed after the intake of hm γ-PGA. The TG-lowering effect of hm γ-PGA may be related to a reduction of liver fatty acid synthesis. The FFA levels of the HL group were significantly lower than that of the HC group; a similar trend was observed for the HH group. FFAs are released from visceral adipose TG stores by lipolysis into circulation. Elevated FFA levels increase hepatic TG production [21]. Because the FFAs of adipocytes drain into the portal vein, the FFAs are delivered to the liver [7]; in turn, this stimulates gluconeogenesis, increasing TG synthesis, ultimately resulting in the development of dyslipidemia [1]. The administration of hm γ-PGA decreased visceral obesity by decreasing the weight of the perirenal fat pad; it might also have led to the reduction of serum FFA levels in the HL and HH groups in the present study (Tables 3 and 4). However, no significant effect of hm γ-PGA on the food efficiency ratio was observed.

Several studies suggest that soybean and fermented soybean products effectively improve lipoprotein lipid metabolism and prevent diabetes mellitus [20], oxidative

stress [19], and carcinogenesis [28]. Maki *et al*. [25] report that soy protein exerts hypocholesterolemic effects and that fecal bile acid excretion is elevated in humans with moderate hypercholesterolemia.

During soybean fermentation with *B. subtilis* (*i.e*., *natto*) or *B. subtilis chungkookjang*, γ-PGA is synthesized from glutamate monomers. The hm γ-PGA obtained from the culture filtrate of *B. subtilis chungkookjang* is very viscous in aqueous conditions. Viscous soluble dietary components are capable of decreasing the hepatic accumulation of TGs in both normal and hyperlipidemic rats [34]. In the present study, serum TG, total cholesterol, LDL cholesterol, and FFA levels were lower in the hypertriglyceridemic rats administered hm γ-PGA than that in the controls. Therefore, hm γ-PGA improves lipid metabolism and hypertriglyceridemia.

To understand the effect of hm γ-PGA on hepatic TG levels and visceral adipose tissue weight, the activities of enzymes related to lipid metabolism were evaluated. Gupte *et al*. [14] report that G6PD expression is 117% higher in the liver and hepatocytes of Zucker fa/fa rats compared with that in lean rats. They also report that G6PD activity is significantly higher in the liver (400%) and hepatocytes (160%) of Zucker fa/fa rats compared with that in age-matched lean rats.

The present results show that hm γ-PGA reduced the hepatic activities of lipogenic enzymes such as G6PD and malic enzyme. The activities of malic enzyme in epididymal and perirenal fat pad tissues decreased after hm γ-PGA treatment. This result suggests that reduced hepatic lipogenesis in hm γ-PGA-administered groups might lead to decreased hepatic TG level and fat pad weight. Hepatic G6PD mRNA expression was inhibited by hm γ-PGA treatment. However, the higher dosages of hm γ-PGA did not reduce the hepatic lipogenic enzyme activity or G6PD mRNA expression to a greater extent in high-fructose-fed rats. The present results suggest a possible mechanism involving the increased activities of 2 key players, G6PD and malic enzyme, which seem to be activated simultaneously in the hepatic tissue prior to the onset of hypertriglyceridemia and operate synergistically to upregulate lipogenesis by feeding a high-fructose diet to the rats. This possibly contributes to the development of hypertriglyceridemia-associated hepatic complications. Changes in the synthesis of hepatic fatty acids due to the reduction of lipogenic enzymes also affect the plasma TG levels, because they alter hepatic TG synthesis, which in turn affects the production of very-low-density lipoproteins by the liver [13]. The decreased hepatic lipogenic enzyme activities due to hm γ-PGA treatment were similar to those caused by a soy protein diet [40].

Deibert *et al*. [6] report that soy protein-based meal replacement significantly reduces body weight in overweight or obese patients with normal renal function without altering lean body mass. Fermented soy products are known to be effective for weight reduction [3]. In the present study, among the bioactive components of fermented soybean products such as *chungkookjang*, hm γ-PGA seems to positively affect dyslipidemia in rats with normal body weight.

In summary, we observed that administration of hm γ-PGA from *chungkookjang* may affect the blood lipid profiles of hypertriglyceridemic rats because it decreases the serum levels of total cholesterol, TG, LDL cholesterol, and FFAs. In addition, the findings of our study indicate that hm γ-PGA causes a hypotriglyceridemic effect by regulating G6PD and malic enzyme activities of the liver or fat tissue as well as liver G6PD gene expression in hypertriglyceridemic rats. These results encourage further investigation of hm γ-PGA in clinical trials to determine whether it can prevent hypertriglyceridemia and decrease the risk of cardiovascular disease. Furthermore, this study provides the rationale for improving *chungkookjang* fermentation to better understand the biochemical and enzymatic (*i.e*., biotechnological) basis for the release of functional bioactive materials responsible for the hypolipidemic effect. Therefore, hm γ-PGA administration is recommended for the alleviation of dyslipidemia.

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