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 mRNA expression were significantly decreased by hm γ-PGA treatment (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

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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...
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 dyslipidemia 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 hypertriglyceridermic conditions remains unclear.

Rats fed high-fructose diets exhibit metabolic changes observed in the metabolic syndrome. In the present study, we used a 63% high-fructose diet to induce hypertriglyceridermia and administered γ-PGA orally. Thus, the present study assessed the effects of hm γ-PGA administration on lipid metabolism and related mechanisms in hypertriglyceridermic 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%, (NH₄)₂SO₄ 1%, KH₂PO₄ 0.27%, Na₂HPO₄·12H₂O 0.42%, NaCl 0.05%, pH 6.8], and cultured with stirring at 150 rpm, an aeration rate of 1vvm, 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 NaNO₃, and injected into the GPC equipped with a ViscoGel GMPW₃₀ column (7.8 mm × 30 cm; Viscotech, Houston, TX, USA), which had been equilibrated with 0.1 M NaNO₃ at 40°C and a flow rate of 0.8 ml/min. γ-PGA was detected with a Visocetk LR25 Laser Refractometer. Polycrylamide 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 hypertriglyceridermia for 4 weeks. Hypertriglyceridermia was defined as serum TG level > 300 mg/dl [7, 41] by a LipidoCare Biosensor (MedicalSK, Inc., Daegu, Korea). Subsequently, the hypertriglyceridermic rats were randomly divided into 3 subgroups (n = 10 each) according to the serum TG levels and administered oral doses of 0 mg (hypertriglyceridermic control, HC), 2.5 mg (hypertriglyceridermic low γ-PGA, HL), or 5 mg·kg·bw⁻¹·d⁻¹ (hypertriglyceridermic high γ-PGA, HH) hm γ-PGA for 4 weeks. The ingredients used in this study were as follows: casein (Daejang Chemical and Metal Co., Gyenoggi-do, Korea); cellulose, mineral mix, and vitamin mix (G-Bio Co., Gyenoggi-do, Korea); choline bitartrate and 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 Ultraspec 2100 Pro UV/Visible Spectrophotometer (Amersham BioScience, Buckinghamshire, UK) at 540 nm using the method of Folch et al.