High Molecular Weight Poly-Gamma-Glutamic Acid Regulates Lipid Metabolism in Rats Fed a High-Fat Diet and Humans

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We investigated the effect of high molecular weight poly-gamma-glutamic acid (hm γ-PGA) on adiposity and lipid metabolism of rats in the presence of an obesity-inducing diet. Thirty-two Sprague-Dawley rats were fed either a normal-fat (11.4% kcal fat, NFC) or high-fat (51% kcal fat, HFC) diet. After 5 weeks, half of each diet-fed group was treated with hm γ-PGA (NFP or HFP) for 4 weeks. The HFC group had significantly higher body weight, visceral fat mass, fasting serum levels of total cholesterol, LDL cholesterol, and leptin, and lower serum HDL cholesterol level compared with those of the NFC group (p < 0.05). Treatment with hm γ-PGA decreased body weight gain and perirenal fat mass (p<0.05), fasting serum total cholesterol, and mRNA expression of glucose-6-phosphate dehydrogenase (G6PD), regardless of dietary fat contents (p < 0.01). However, hm γ-PGA increased serum HDL cholesterol in the HFC group (p < 0.05).

In vitro, 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase activity was suppressed by the addition of hm γ-PGA. In agreement with observations in animal study, the supplementation of hm γ-PGA (150 mg/day) to 20 female subjects in an 8-week double-blind, placebo-controlled study resulted in a tendency to decrease total cholesterol and LDL cholesterol concentrations. We thus conclude that dietary supplementation of hm γ-PGA may act as a hypocholesterolemic agent, secondary to its inhibitor effect on HMG-CoA reductase, and decrease abdominal adiposity by decreasing hepatic lipogenesis. The present study is an important first step in establishing the effect of hm γ-PGA on cholesterol levels in rats and humans.

Keywords: γ-PGA, adiposity, cholesterol, hepatic G6PD, rats, pilot human study

In recent decades, obesity has become an epidemic in many countries worldwide and is closely related to the development of diabetes, dyslipidemia, hypertension, and other metabolic diseases [15]. Atherosclerosis is one of the major risk factors for cardiovascular disease, and cholesterol may play an important role in its development [7]. Lipid metabolism, a complicated process that is highly regulated by various factors such as hormones and peptides, is influenced by diet [20, 31]. Numerous natural substances have been proposed as effective ways to regulate lipid metabolism in animal models and humans [1, 11, 14].

In recent years, there has been a considerable interest in the effects of soybean and soy-based products on human health [9, 33]. Chungkookjang and natto, traditional Korean and Japanese soybean products, respectively, are prepared by fermenting steamed soybeans with Bacillus species for 2 or 3 days without salt or other seasoning. During fermentation, Bacillus strains produce extracellular viscous materials in the stationary phase, along with the development of spores [16]. Studies of the bioactive effects of chungkookjang components have reported that chungkookjang increases insulin sensitivity, antioxidative capacity [22, 24, 25], and fibrinolytic activity, and possesses anti-diabetic, antioxidative, and hypocholesterolemic properties [24, 26, 38]. The main source of the beneficial effects associated with chungkookjang and other fermented soybean foods is thought to be isoflavones. Glycoside isoflavones in soybeans are known to be hydrolyzed by β-glycosidase produced by microorganisms, thereby increasing aglycone isoflavones during fermentation [18]. Another study showed that poly-gamma-glutamic acid (γ-PGA) from water-soluble fractions of chungkookjang
and natto has antioxidative functions [17]. Bacillus subtilis (natto) and B. subtilis subsp. chungkookjang have been used as the starter strains of natto and chungkookjang and produce different sizes of γ-PGA depending on the bacterial strains used. The former produces the predicted molecular weight product, which ranges widely from 10 kDa to 200 kDa, in addition to various other components such as polysaccharides. Sung et al. [38] reported that PGA produced using B. subtilis subsp. chungkookjang as the biocatalyst led to the synthesis of high molecular weight γ-PGA (over 2,000 kDa) with effective purification of the polymer [38].

A recent study reported that high molecular weight γ-PGA of over 2,000 kDa (hm γ-PGA) enhanced immune function [21]. In addition, chungkookjang or natto powder was found to improve lipid metabolism in diabetic rats [38]. However, although hm γ-PGA is thought to play an independent physiological role, little research has been done on this topic.

In the present study, we explored the effects of hm γ-PGA produced using B. subtilis subsp. chungkookjang on lipid metabolism, adiposity, and hepatic lipogenic enzyme expression in rats fed a fat-rich diet. A pilot study was performed to examine the weight-loss and lipid-lowering effects of hm γ-PGA in humans.

**Materials and Methods**

**Preparation and Molecular Weight Determination of Poly-γ-PGA Glutamic Acid**

High molecular weight γ-PGA (hm γ-PGA) derived from Bacillus subtilis subsp. chungkookjang was prepared at a pilot-scale plant (BioLeaders Corporation, Daejeon, Korea) as described previously [38]. Samples of γ-PGA with molecular masses of 1, 10, 50, 500, and 2,000 kDa were prepared by gel permeation chromatography (GPC) using polyacrylamide (1–9,000 kDa) as a standard material. γ-PGA solution was diluted with 0.1 M NaNO₃ and injected into a GPC system equipped with a ViscoGel GMPW column (column length × 30 cm; Viscotek, Houston, TX, USA) at 40°C and a flow rate of 0.8 ml/min. γ-PGA was detected with a Viscotek LR25 laser refractometer and polyacrylamide was used as the standard material for molecular weight determination. Viscosity was determined by a viscometer with 5 ml of buffer. The solution was then vortexed and the homogenate was immediately centrifuged at 12,000 × g for 15 min at 4°C. The supernatant was assayed for G6PD activity.

**Serological Analysis**

Serum levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) were measured using an automatic blood chemical analyzer (Prime E automatic photometer; Asan Pharmaceutical Co., Hwaseong, Korea). Serum leptin was measured with a rat enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden) following the manufacturer’s instructions.

**Tissue G6PD Activity**

Glucose-6-phosphate dehydrogenase (G6PD) activity was determined according to Bergmeyer’s method with slight modification [4]. Approximately 250 mg of liver tissue was homogenized at room temperature with 5 ml of buffer. The solution was then vortexed and the homogenate was immediately centrifuged at 12,000 × g for 15 min at 4°C. The supernatant was assayed for G6PD activity. Total protein in the tissues was assayed by the Bradford method using bovine serum albumin (BSA) as a standard [41]. G6PD activity was expressed as μmol NADPH release/mg protein.

**Quantification of Triglyceride and Cholesterol Content**

Lipids in the liver tissue and feces were extracted using the Bligh and Dyer method [5]. Total lipids were extracted from frozen livers with chloroform-methanol [2:1 (v/v)]. The lipid extract was dried, dissolved in 2-propanol, and used for total lipid and cholesterol analysis. Total lipid and cholesterol content was measured using a commercially available kit (Asan Pharmaceutical Co).

**mRNA Expression of G6PD Enzyme in the Liver**

G6PD mRNA expression was assayed using real-time RT-PCR. Total RNA was isolated with TRI reagent (Sigma, St. Louis, MO, USA). cDNA was reverse transcribed from 1 μg of total RNA and oligo(dT) 12-18 primer using SuperScript II RNAse H-reverse transcriptase (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions. Real-time quantitative (RTQ) PCR was performed using an iQ5 real-time PCR detection system (Bio-Rad, Hercules, CA, USA) using iQ SYBR Green Supermix. We designed primers using