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Enzymatically synthesized glycogen reduces lipid accumulation in diet-induced obese rats

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ABSTRACT

Based on a recent study indicating that enzymatically synthesized glycogen (ESG) possesses a dietary, fiber-like action, we hypothesized that ESG can reduce the risk of obesity. In this study, the antiobesity effects of ESG were investigated in a model of diet-induced obesity. Male Sprague-Dawley rats were divided into 4 groups and fed a normal or high-fat diet, with or without 20% ESG, for 4 weeks. Body weight, food intake, lipid deposition in the white adipose tissues and liver, fecal lipid excretion, and plasma lipid profiles were measured. At week 3, the body fat mass was measured using an x-ray computed tomography system, which showed that ESG significantly suppressed the high-fat diet–induced lipid accumulation. Similar results were observed in the weight of the adipose tissue after the experiment. Moreover, ESG significantly suppressed the lipid accumulation in the liver but increased fecal lipid excretion. The plasma concentrations of triacylglycerol and nonesterified fatty acid were lowered after a high-fat diet, whereas the total bile acid concentration was increased by ESG. However, the hepatic messenger RNA (mRNA) levels of enzymes related to lipid metabolism were not affected by ESG. Conversely, the mRNA levels of long-chain acyl-CoA dehydrogenase and medium-chain acyl-CoA dehydrogenase were up-regulated by ESG in the muscle. These results suggest that the combined effects of increased fecal lipid excretion, increased mRNA levels of enzymes that oxidize fatty acids in the muscle, and increased total bile acid concentration in the plasma mediate the inhibitory effect of ESG on lipid accumulation.

Abbreviations: ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; cDNA, complementary DNA; CHL, cholesterol; CPT, carnitine palmitoyltransferase; CT, computed tomography; ESG, enzymatically synthesized glycogen; FAS, fatty acid synthase; HF, high fat; LCAD, long-chain acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; mRNA, messenger RNA; NEFA, nonesterified fatty acid; PL, phospholipid; PPAR, peroxisome proliferator–activated receptor; SCFAs, short-chain fatty acids; TBA, total bile acids; TG, triacylglycerol; UCP, uncoupling protein; VLCAD, very-long-chain acyl-CoA dehydrogenase.

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1. Introduction

Glycogen is a highly branched (1 → 4) and (1 → 6) linked α-D-glucan with a huge molecular weight that ranges from 10^6 to 10^9 d and is commonly existent in animals and microorganisms as a storage form of glucose. In mammals, glycogen is present mainly in the liver and muscle, comprising about 5% and 1% of the wet weights of these tissues, respectively. It is used to maintain the blood glucose level and as an energy source for muscular contraction [1]. Besides the liver and muscle, several other tissues such as skin, cartilage, fat pads, kidney, and leukocytesm contain small amounts of glycogen [2]. Glycogen is naturally synthesized from uridine diphosphate-glucose through the action of glycogenin (EC 2.4.1.186), glycogen synthase (EC 2.4.1.11), and branching enzyme (EC 2.4.1.18) [1].

Recently, our group developed an in vitro method for synthesizing glycogen from starch by using isoamylase (EC 3.2.1.68), branching enzyme, and amylomaltase (EC 2.4.1.25), which we call *enzymatically synthesized glycogen* (ESG) [3]. The physicochemical properties such as the number-averaged chain length, the internal chain length, the exterior chain length, the intrinsic viscosity, and the transmission electron microscopic images of ESG were equivalent to those of natural sources of glycogen [4]. Its physiological functions, mainly its immunomodulatory activity, have also been investigated [5–7]. The evaluation of ESG by the Association of Official Analytical Chemists method 2002-02 revealed that it contains about 20% dietary fiber, and a single oral administration test in rats determined that the glycemic index of ESG is about 80 [8,9]. The undigested portion of ESG was shown to reach the cecum and be converted to short-chain fatty acids (SCFAs) by the microbiota [9], thus suggesting that the digestion-resistant portion of ESG works as a soluble and fermentable fiber. Several studies have shown that dietary fiber possesses beneficial effects on lipid metabolism [10,11]. Enzymatically synthesized glycogen decreased the weight of epididymal fat tissue and plasma triacylglycerol (TG) levels and increased the high-density lipoprotein/total cholesterol (CHL) ratio in rats fed a normal diet [9]. However, no detailed exploration on the action of ESG on lipid metabolism has been conducted based on the current literature.

Obesity is now considered both a disease and a social problem, not only in developed countries but also in developing countries [12]. Although many studies have been performed on the management and prevention of obesity [13–15], the problem has not diminished. *Obesity* is generally defined as an excessive storage of fat in the viscera and subcutaneous adipose tissue, induced by an imbalance between energy intake and expenditure [12]. We hypothesized that dietary supplementation with ESG reduces the risk of obesity. Research that determines the antiobesity effect of specific food components factor will contribute to a better the understanding and reduction of the human obesity problem. To test our hypothesis, we studied the effects of ESG on fat deposition in the adipose tissue and livers of diet-induced obese rats and their plasma lipid profiles. We also examined lipid excretion in the feces and messenger RNA (mRNA) expression levels of enzymes related to lipid metabolism to understand the underlying molecular mechanism.

2. Methods and materials

2.1. Materials

Enzymatically synthesized glycogen was synthesized in our laboratory, as described previously [3]. The animal diets were purchased from Research Diets, Inc (New Brunswick, NJ, USA). All other chemicals were analytical grade and obtained from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Animal experiments

The animal experiments performed in this study were approved by the Institutional Animal Care and Use Committee (permission no. 21-07-01) and performed according to the Kobe University Animal Experimentation Regulations. Three-week-old male Sprague-Dawley rats were purchased from Japan SLC, Inc (Shizuoka, Japan), and housed in a temperature-controlled (25°C ± 3°C) room at 60% ± 5% humidity under a 12-hour light/dark cycle. After 1 week of acclimatization, 30 rats were randomly divided into 4 groups: 8 rats in the control (C) group, 7 rats in the control-ESG20% (C20%) group, 8 rats in the high-fat (HF) group, and 7 rats in the HF-ESG20% (HF20%) group. For 4 weeks, each group was fed the relevant diet, as listed in Table 1.

Food intake and body weight were measured once a week. After 3 weeks, rats were anesthetized with isoflurane by inhalation and scanned using an experimental animal x-ray computed tomography (CT) system (the Latheta LCT-100 [Hitachi Aloka Medical, LTD, Tokyo, Japan]). Contiguous slice images were obtained at 1-mm intervals from the diaphragm to the base of the tail, and quantitative assessments were performed using the Latheta software (version 2.10; ALOKA, Hitachi Aloka Medical, LTD, Tokyo, Japan). The total fat mass, which consisted of visceral and subcutaneous fat, was evaluated, and its ratio to the total body mass was calculated.

Table 1 – Ingredient composition and caloric content of the experimental diets fed to the rats

	Diet group			
	C	C20%	HF	HF20%
Casein (g/1000 g)	190	190	258	258
L-Cystine (g/1000 g)	2.8	2.8	3.9	3.9
Corn starch (g/1000 g)	299	133	0	0
Maltodextrin (g/1000 g)	33	33	162	0
Sucrose (g/1000 g)	332	332	89	89
ESG (g/1000 g)	0	200	0	200
Soybean oil (g/1000 g)	24	24	32	32
Lard (g/1000 g)	19	19	317	317
Cellulose (g/1000 g)	47	13	65	26
Mineral mix S10026 (g/1000 g)	9.5	9.5	13	13
Dicalcium phosphate (g/1000 g)	12	12	17	17
Calcium carbonate (g/1000 g)	5.2	5.2	7.1	7.1
Potassium citrate (g/1000 g)	16	16	21	21
Vitamin mix V10001 (g/1000 g)	9.5	9.5	13	13
Choline bitartrate (g/1000 g)	1.9	1.9	2.6	2.6
Calorie (kcal/1000 g)	3845	3846	5242	5261

All diets were purchased from Research Diets, Inc.