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Effect of partially hydrolyzed oat β-glucan on the weight gain and lipid profile of mice

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ABSTRACT

Oat β-glucan hydrolysates with different molecular weights were prepared and their physicochemical, hypocholesterolemic, and weight-reducing characteristics were evaluated. The enzymatic hydrolysis by cellulase caused a decrease in the molecular weight of oat β-glucan (1450–370 × 10^3 g/mol), which also affected swelling power and bile acid/fat binding capacities. In addition, mice were fed high-fat diet supplemented with β-glucans with three different molecular weights (1450, 730, 370 × 10^3 g/mol). The diets treated with β-glucans significantly reduced the body weight of the mice. However, the molecular weight of β-glucans did not appear to significantly affect the serum lipid profile.

1. Introduction

In recent years, obesity has been considered as one of the main causes of cardiovascular disease, diabetes, and cancer (Brown & Siahpush, 2007) and an increase in the prevalence of obesity and overweight has been widely reported. Over the past 20 years, obesity rates doubled to 30% of the adult populations in the United States where the economic costs of obesity were estimated to be more than $117 billion in 2002 (Brisbon, Plumb, Brawer, & Paxman, 2005). Likewise, there is also a rapid rise of obesity in Europe (Buschemeyer & Freedland, 2007).

Obesity occurs when energy input exceeds energy expenditure. That is, the predominant obesity-causing factor is the dietary energy imbalance that may allow adipocytes to pathologically grow (Hwang et al., 2005). While pharmaceutical treatments for obesity have been extensively researched, only two drugs, orlistat and sibutramine, were approved for long-term use in significantly obese patients by Food and Drug Administration (FDA). However, attention should be paid to their adverse effects including gastrointestinal discomforts, flatulence, and diarrhea (Heck, Yanovski, & Calis, 2000). In addition to the prescription drugs, nutritional supplements for weight loss are popular in the over-the-counter market. Although such treatments are widely used, none have been proved to be safe and effective (Pitter & Ernst, 2004). Hence, it is necessary to find more effective and safe treatments through the inhibition of digestion and absorption of dietary fat which is a target for obesity treatment.

A number of studies have documented that dietary fibers provide a variety of human health benefits such as improvement of pancreatic and bowel functions (Wrick et al., 1983). In particular, oat β-glucan out of various dietary fibers has been considered as a valuable bioactive component for functional food applications (Wood et al., 1994). It is well known that oat β-glucan reduces postprandial blood glucose (Wood et al., 1994) and blood cholesterol levels (Rimm et al., 1996). These beneficial effects have encouraged the food industry to develop new functional foods containing oat β-glucan. However, the potential food applications of β-glucan are still limited to cereal-based baked products because of its unique rheological properties such as high viscosity. Furthermore, the physiological activities of β-glucan may be viscosity-dependent (Wood et al., 1994).

Since the molecular weight of a polymer is an important factor to change viscosity, several methods were used to prepare for β-glucan samples with different molecular weights such as acidic hydrolysis (Doublier & Wood, 1995; Johansson et al., 2006), enzymatic hydrolysis (Tosh, Wood, Wang, & Weisz, 2004), and physical treatments (Wang, Wood, & Cui, 2002). However, more detailed correlations between the molecular weight of β-glucan and its biological properties have been hardly found in the literature. Even, the efficacy of weight loss of β-glucan as a function of molecular weight has not been reported yet to our best knowledge.

In this study, oat β-glucans with different molecular weights were enzymatically prepared and their effects on weight loss and lipid profile were tested in an animal model system.

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2. Materials and methods

2.1. Materials

Oat bran concentrate (OBC) containing 43% β-glucan on a dry basis was purchased from Nature® Advanced Oat Technologies (Lot No. 050610-1-08, Missoula, MT, USA). Cellulase isolated from *Trichoderma reesei* and dextran standards of different molecular weights were obtained from Novozymes A/S (Celluclast® 1.5L, Bagsvaerd, Denmark) and from Sigma–Aldrich Chemical Co. (St Louis, MO, USA), respectively. All other chemicals were of the highest commercial grade.

2.2. Preparation of partially hydrolyzed oat β-glucans by enzymatic hydrolysis

Oat bran concentrate was suspended in distilled water (6.25% (w/v), pH 4.8, 50 °C) and the cellulase (840–8400 EGU/g) was added, followed by incubation at 50 °C for 5 h. The incubated samples were then heated to 100 °C for 30 min to inactivate the enzyme. After an equal volume of ethanol was added, the precipitate was collected by centrifugation (9700 *g*, 10 min) and dried at 40 °C (Johansson et al., 2006).

2.3. Physicochemical properties

2.3.1. Molecular weight

Gel permeation chromatography (GPC) was carried out at room temperature with a prep-HPLC system (LC-900, Japan Analytical Instrument, Tokyo, Japan) with a JAIGEL-W254–255 column and a JAI RI-50 refractive index. The flow rate of mobile phase (deionized water) was 3.5 ml/min and the injection volume (0.5%, w/v) was 1.0 ml. The molecular weight of the samples was determined by dextran standards with known M_w values.

2.3.2. Viscosity

Apparent viscosity was measured with a rotational Rheostress RS1 rheometer (Thermo Haake, Karlsruhe, Germany) using a plate-and-plate type geometry (35 mm). The β-glucan samples were suspended in distilled water (30 mg/ml) and heated at 80 °C for 3 h with agitation. After cooling down at room temperature, the resulting suspensions were loaded on a rheometer and the steady shear rate sweep was carried out over shear rates from 20 to 200/s at 25 °C.

2.3.3. Swelling power

Swelling power was determined by the modified method of Sasaki and Matsuki (1998). The mixture of 0.3 g of sample and 10 ml of deionized water was placed in a shaking water bath at 70 °C for 10 min and then transferred into a boiling water bath. After boiling for 10 min, the tubes were cooled with tap water for 5 min and centrifuged at 1700 *g* for 4 min. Swelling power was expressed as the ratio of wet sediment weight to dry sample weight.

2.4. Bile acid and fat binding capacities

2.4.1. Bile acid binding

In vitro bile acid binding capacity was measured according to the previous procedures with modification (Body, Eastwood, & Maclean, 1966). The samples were added to 0.01 M sodium phosphate buffer (pH 7.0) containing 200 μM cholic acid at a concentration of 2.5 mg/ml, stirred at 37 °C for 2 h, and then filtered (0.2 μm syringe filter, Waters Co., USA). The resulting solutions (0.2 ml) were treated with 70% sulfuric acid (1 ml) for 5 min and 25% furfural (0.2 ml) was then added. After 1 h, the absorbance was measured at 510 nm.

2.4.2. Fat binding

In vitro fat binding capacity was examined according to the method reported by Lin and Humbert (1974). β-Glucan samples (0.2 g) were dispersed in olive oil (10 ml) and the mixtures were placed at ambient conditions for 1 h with agitation on a vortex mixer every 15 min. After centrifugation at 1600 *g* for 20 min, the supernatant was decanted and the residue was weighed. The fat absorption was obtained from the amount of olive oil bound to 1 g of dry sample.

2.5. Animal experiment

2.5.1. Animals and diets

Four-week-old C57BL/6J male mice were purchased from Central Lab. Animal Inc. (Seoul, Korea). After a week's acclimatization period on standard rodent chow (Samyang, Seoul, Korea), the animals were stratified by weight and divided into one of five groups ($n = 10$ per group). They were fed a normal diet (ND, AIN-76A purified rodent diet with 65% corn starch #111753 (Dyets Inc., Bethlehem, PA, USA)), a high-fat diet (HFD, 40% beef tallow modified AIN-76A purified rodent diet #101556 (Dyets Inc., Bethlehem, PA, USA)), and three experimental diets (BG, high-fat diet containing β-glucan having the composition of 44% starch, 13% protein, and 3.97 kcal/g energy) for 6 weeks (Table 1). Each of the experimental groups was fed high-fat diet containing β-glucans with three different molecular weights (1,450,000 g/mol, 730,000 g/mol, 370,000 g/mol) which were designated as BG1450, BG730, and BG370, respectively. The mice were housed in cages with wire-mesh bottoms in conditioned rooms (23 °C; relative humidity 55%) and food and water were provided ad libitum for the 6-week period. Food intakes were measured twice weekly and body weights were recorded once a week. The care and treatment of mice were approved by the Hanyang University Lab Animal Care Committee, which were in accordance with the Korean Guide for the Care and Use of Laboratory Animals.

2.5.2. Serum collection

The mice were anesthetized with dry ice and dissected 24 h after food was removed at the end of the experimental term. Blood collected from the heart was placed at room temperature for 2 h and then centrifuged at 1500 *g* for 30 min at 4 °C to obtain serum. The separated serum was then snap frozen in liquid nitrogen and stored at −70 °C for further analysis.

Table 1
Compositions of the experimental diets (g/kg diet).

Ingredients	Groups [a]		
	ND	HFD	BG
Casein	200	200	169
DL-Methionine	3	3	3
Corn starch	650	150	150
Sucrose	0	150	65
Cellulose	50	50	50
Corn oil	50	0	0
Beef tallow	0	400	400
Mineral Mix S10022G	35	35	35
Vitamin Mix V10037	10	10	10
Choline bitartrate	2	2	2
Oat bran concentrate	0	0	200
Total energy (kcal)	3592	5542	5543

[a] ND, Normal diet; HFD, high-fat diet; BG, high-fat diet containing β-glucan with different molecular weights.