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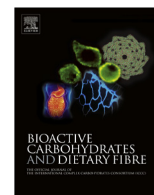

Anti-obesity effects of enzymatically-digested alginate oligomer in mice model fed a high-fat-diet

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ABSTRACT

Alginate is an acidic linear polysaccharide isolated from brown seaweed, and is widely used in food, supplement, and medical industries. In this study, the anti-obesity effects of dietary acid-hydrolyzed (A-AO) and enzymatic-digested (E-AO) alginate oligomers were investigated in male mice fed a high-fat diet. E-AO showed stronger anti-obesity effects than A-AO, as judged by the reduction in body and adipose tissues weights. Further studies demonstrated that the anti-obesity effects of E-AO were superior to those of original alginate polymer (AP). E-AO also showed anti-obesity effects in female obese mice, in which E-AO suppressed the plasma leptin level. Dietary AP and E-AO suppressed the increase in serum triglyceride (TG) levels induced in mice by oral administration of corn oil. AP and E-AO inhibited pancreatic lipase. *In vitro* analysis showed that E-AO inhibited lipid accumulation in differentiated 3T3-L1 adipocytes, whereas AP had no effect, suggesting that a direct effect of E-AO on adipocytes, in addition to the suppression of gastrointestinal lipid absorption, is partly responsible for the superior anti-obesity effects of E-AO.
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1. Introduction

Obesity, a metabolic disorder characterized by an excessive accumulation of fat in adipose tissues, has become an important public health problem in developed nations. Obesity is closely associated with lifestyle-related diseases such as hyperlipidemia, hypertension, type 2 diabetes mellitus, and certain cancers (Eckel et al., 2004; Field et al., 2001; Matsuzawa, 2006; You, Yang, Lyles, Gong, & Nicklas, 2005). Therefore, strategies to prevent or reduce obesity are of great importance and are urgently required worldwide. In addition to general recommendations with regard to proper diet and exercise, several different approaches to treat obesity have been proposed, such as the use of functional food supplements that have anti-obesity effects (Han, Kimura, & Okuda, 2005).

Edible seaweeds such as *Laminaria japonica* and *Undaria pinnatifida* have been traditionally cultivated and consumed in Asian countries such as Japan, Korea, and China. These brown algae have attracted attention as healthy foods because they contain beneficial constituents with various bioactivities that might help prevent lifestyle-related diseases, including obesity (Miyata, Koyama, Kamitani, Toda, & Yazawa, 2009). Brown algae contain a fat-soluble alkaloid, fucoxanthin, and related compounds. Anti-obesity effects of fucoxanthin in *in vitro* and *in vivo* studies have been reported (Maeda, Hosokawa, Sashima, Murakami-Funayama, & Miyashita, 2009). In addition to alkaloids, brown algae are rich sources of polysaccharides such as alginate and fucoidan. The values of such polysaccharides in the food, agriculture, and medicinal industries are well documented (Ajithkumar, Andersson, Siika-aho, Tenkanen, & Åman, 2006; Surenjav, Zhang, Xu, Zhang, & Zeng, 2006). Various beneficial effects of seaweed-derived polysaccharides have been reported, such as antioxidant effects, protection again infectious and inflammatory diseases, and modification of immunological responses (Han et al., 2009; Hida, Miura, Adachi, & Ohno, 2005; Qi et al., 2005; Sun et al., 2009; Ueno et al., 2012).

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We have focused on the bioactivities of alginate and enzymatically depolymerized alginate oligomers (Iwamoto et al., 2005; Kurachi et al., 2005). Alginate, an acidic linear polysaccharide, is usually manufactured from the large brown seaweeds *Macrocystis pyrifera* and *Ascophyllum nodosum* at an industrial level. It is used in a wide range of commercial applications, including thickening agents and dispersion stabilizers. The polysaccharide comprises two uronic acids, α-L-guluronate (G) and β-D-mannuronate (M). The molecular size, M/G ratio, and conformational structure appear to affect the physicochemical properties and bioactivities of alginates (Asada et al., 1997; Jeong et al., 2006; Kimura, Watanabe, & Okuda, 1996; Logeart, Prigent-Richard, Boisson-Vidal, Chaubet, Durand, Jozefonvicz, & Letourneur, 1997; Logeart, Prigent-Richard, Jozefonvicz, & Letourneur, 1997; Otterlei et al., 1991). Alginate oligomers (AOs), prepared through the enzymatic degradation of alginate polymers (APs), have several biological activities. For instance, intraperitoneal administration of AO induces the production of multiple cytokines in mouse serum, oral administration of AO suppresses IgE production in mice, and ingested AO suppresses the rise in blood pressure in a spontaneous hypertensive rat model and decreases blood pressure in clinical studies (Chaki et al., 2002; Hiura, Chaki, & Ogawa, 2001; Uno, Hattori, & Yoshida, 2006; Yamamoto, Kurachi, Yamaguchi, & Oda, 2007). Furthermore, AO promotes the growth of bifidobacteria, whereas AP has no such effect (Akiyama et al., 1992). We have shown that the ability of AO to induce the secretion of tumor necrosis factor (TNF)-α from RAW264.7 cells is greater than that of the original APs (Kurachi et al., 2005). Because AO has a fairly low viscosity in aqueous solutions even at a high concentration and does not form a gel in the presence of calcium, AO is more suitable for applications in *in vivo* systems (Kurachi et al., 2005). Recent studies have suggested that a part of orally administered AO transfers to the circulation after gastrointestinal absorption (Chaki, Kajimoto, Ogawa, Baba, & Hiura, 2007; Nishikawa, Yokose, Yamamoto, Yamaguchi, & Oda, 2008). To clarify the usefulness of AO in an *in vivo* system, in this study we investigated the anti-obesity effects of AO and AP in an obese mouse model induced by a high-fat diet.

2. Materials and methods

2.1. Preparation of alginate oligomers (AO)

APs (IL-6M and ULV-L3) were obtained from Kimika Co. Tokyo, Japan. IL-6M and ULV-L3 are trade name of alginate products of the company, which are prepared from *Durvillaea* sp. and *Lessonia nigrescens*, respectively. Approximate M/G ratio of IL-6M and ULV-L3 are estimated to be ~2.2 and 1.0–1.3, respectively. E-AO was prepared by digesting AP with bacterial alginate lyase (Nagase ChemteX Co. Osaka, Japan) as described previously (Nishikawa et al., 2008). Thus, E-AO has an unsaturated terminal structure with a double bond. A-AO, which does not have the unsaturated terminal structure with a double bond, was also prepared. In brief, 1% AP in 200 mL of acidic solution (pH 4.0) with HCl was incubated at 121 °C for 80 min. The sample solution was cooled to room temperature, neutralized with NaOH, and lyophilized using a vacuum freeze dryer (FD-1; Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The results of gel-filtration chromatography suggested that the mean degree of polymerization of E-AO and A-AO was 4.

2.2. Animals and cell line

Specific pathogen-free male and female ddY mice (4 weeks old, 18–20 g) were obtained from Texam (Nagasaki, Japan). The mice were housed at a constant room temperature of 24 °C with a 12-h light/dark cycle photoperiod and free access to standard laboratory food (CE-2; CLEA, Tokyo, Japan) as a normal diet (N diet) and water *ad libitum*. High-fat diet 32 (CLEA, Tokyo, Japan) was used in obesity experiments. All mice were treated according to the guidelines of the Japanese Association for Laboratory Animal Science and the Guidelines for Animal Experiments of Nagasaki University, Japan. The preadipogenic mouse 3T3-L1 fibroblast cell line was obtained from the Institute of Development, Aging, and Cancer of Tohoku University. The cells were maintained at 37 °C in Dulbecco's modified Eagle medium (DMEM) containing 1 mg/mL glucose, 10% fetal calf serum (FCS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (growth medium) in a humidified atmosphere with 5% CO_2 and 95% air.

2.3. Measurement of body and adipose tissue weights in mice fed a high-fat diet

After a week acclimation, the mice were randomly divided into four diet groups: normal diet, high-fat diet, high-fat diet supplemented with 10% (w/w) AO, and high-fat diet supplemented with 10% AP. Mean consumptions of these diets were estimated to be 4 g/mouse/day, and mean doses of AP and AO were estimated to be 10–20 g/kg body weight/day. The composition (w/w) of the normal diet was approximately 5% fat, 25% protein, 55% carbohydrate, and 6% mineral mixture. The composition of the high-fat diet was 32% fat, 26% protein, 32% carbohydrate, and 4% mineral mixture. During the 14 days of the diet, the body weights of the mice were measured daily. At the end of the experiment, the mice were sacrificed under anesthesia. Epididymal and retroperitoneal adipose tissues from male mice and periovular and retroperitoneal adipose tissues from female mice were immediately removed and weighed.

2.4. Multiplex bead array assay

A multiplex bead array assay (Merck-Millipore, MA, USA) was used for the quantitative measurement of multiple obesity-related hormones such as GLP-1, PYY, insulin, and leptin according to the manufacturer's recommended procedure. In brief, the premixed standards were reconstituted in 0.25 mL of distilled water. The standard stock was serially diluted in PBS to generate eight points for the standard curve. The assay was performed in a 96-well filtration plate supplied with the assay kit. Premixed beads (25 μL) coated with target capture antibodies were transferred to each well of the filter plate and washed three times with multiplex bead array assay wash buffer. Premixed standards or serum samples (20 μL) were added to each well containing washed beads. The plate was shaken for 30 s and then incubated at room temperature for 30 min with low-speed shaking. After incubation and washing, premixed detection antibodies (50 μL) were added to each well. After 30 min incubation, streptavidin-phycoerythrin (50 μL) was added to each well and incubated for 30 min. The incubation was terminated after shaking for 10 min at room temperature. The beads were washed three times and resuspended in 100 μL of multiplex bead array assay buffer. The beads were read on a multiplex suspension array system, and the data were analyzed using multiplex bead array assay software.

2.5. Measurement of serum TG levels in mice after oral administration of a lipid emulsion

Male ddY mice (6 weeks old) were divided into four groups (n=5). After 18 h fast, the test groups were orally administered a lipid emulsion (20 mL/kg body weight) containing 50% corn oil and 1% egg lecithin with or without test compounds (AO or E-AP at a dose of 10 mg/kg body weight) using a procedure reported

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