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Amelioration of obesity in high-fat diet-fed mice by chestnut starch modified by amylosucrase from *Deinococcus geothermalis*

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ABSTRACT

The ameliorative effect of enzymatically modified chestnut starch on high-fat diet (HFD)-induced obesity in a mouse model and the underlying mechanism were investigated. Chestnut starch was enzymatically modified by amylosucrase from *Deinococcus geothermalis* (DGAS). *In vitro* analyses including Englyst's assay and determination of the starch digestive pattern to investigate starch digestibility revealed that chestnut starch naturally contained higher slowly digestible starch and resistant starch than corn starch. Modification of chestnut native starch by DGAS increased the proportion of resistant starch, rendering it unavailable for catalysis by small-intestinal mucosal α-glucosidase. The amylose ratio and branch-chain of amylopectin in chestnut starch were increased by DGAS. In an *in vivo* study, HFD (45% kcal from fat)-induced obese C57BL/6 model mice were orally administered DGAS-modified chestnut starch at the dose of 1500 mg/kg b·w for 10 weeks. Supplementation of DGAS-modified chestnut starch to obese mice significantly reduced features of obesity as compared with HFD- or native chestnut starch-fed mice. Food intake and the gut hormones PYY and GLP-1 were not significantly changed. However, we found that feeding DGAS-modified chestnut starch led to a significant increase in empty cecum weight, indicating short-chain fatty acid production in the cecum. Additionally, DGAS-modified chestnut starch induced the short-chain fatty acid receptor GPR43-mediated suppression of insulin signaling. Changes in all these factors were resistant starch content-dependent. In conclusion, DGAS modification of chestnut starch increases non-digestible resistant starch and this ameliorates diet-induced obesity via GPR43-mediated suppression of insulin signaling, thereby presumably reducing fat accumulation in white adipose tissue.

1. Introduction

Starch not only is a carbohydrate energy source but also provides various physiological benefits depending on its digestibility. Starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) based on the *in vitro* Englyst assay for nutritional characteristics (Englyst, Kingman, & Cummings, 1992). While RDS is rapidly converted to glucose molecules by enzyme digestion, SDS is completely, albeit much more slowly, digested by small-intestinal amylases (Englyst et al., 1992; Lehmann & Robin, 2007). In contrast, RS fiber is resistant to hydrolysis by small-intestinal α-amylase and thus reaches the large intestine where it is fermented by gut bacteria (Keenan et al., 2015). RS is categorized into four types (RS1–4). Briefly, RS1 is mostly entrapped in a non-digestible matrix, RS2 is non-gelatinized starch from raw foods, such as raw potato starch, RS3 is a retrograded starch formed during cooling of gelatinized starch, and RS4 is a starch with novel chemical bonds formed by chemical modification with the addition of ester and ether groups (Keenan et al., 2015; Sajilata, Singhal, & Kulkarni, 2006). Starch, particularly RS, acts as a prebiotic in the gut as it serves as a substrate for fermentation by gut bacteria, yielding microbial metabolites such as short-chain fatty acids (SCFAs) (Bird, Conlon, Christophersen, & Topping, 2010; Zaman & Sarbini, 2016). In line with its prebiotic effect, RS reportedly provides physiological

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benefits, such as anti-obesity effect (Zhou et al., 2009), anti-colonic cancer effect (Topping & Clifton, 2001), hypoglycemic effect, which aids in preventing the onset of diabetes (Hedemann, Hermansen, Pedersen, & Bach Knudsen, 2017; Zhou et al., 2008), and hypocholesterolemic effect (Han et al., 2003).

RS has been extensively studied, and in particular, animal models for studying anti-obesity effects of high-amylose maize RS type 2 (HAMRS2) have been well established. The Keenan lab reported the anti-obesity effect of RS-containing diet and suggested two possible mechanisms: (1) gut–brain axis control of satiety by gut-secreting hormone peptide tyrosine tyrosine (PYY) and glucagon-like protein 1 (GLP-1) (De Silva & Bloom, 2012; Zhou et al., 2015a,b) and (2) prebiotic functions of SCFAs produced via RS fermentation (Keenan et al., 2006). However, despite accumulating evidence of the fat-reducing effect of HAMRS2 in various animal models (Charrier et al., 2013; Keenan et al., 2006; Shen, Keenan, Raggio, Williams, & Martin, 2011; Zhou et al., 2009), the underlying mechanisms in fat tissues remain unclear.

Amylosucrase (EC 2.4.1.4, ASase) is a glucosyltransferase that synthesizes α-1,4 glucan (an amylose-like polymer) from sucrose as a sole substrate (Seo et al., 2008; Zhang et al., 2016). ASase not only synthesizes amylose (AM) but also elongates the side chains of amylopectin (AP) in gelatinized starch by transferring glucose from sucrose to the non-reducing end of AP. The increase in branch-chain length of AP renders the crystalline structure of the starch more stable and less accessible to digestive enzymes. Therefore, the RS and SDS contents of ASase-treated starch are higher than those of normal starch. (Kim, Kim, & Yoo, 2015; Kim, Kim, Choi, Park, & Moon, 2016; Kim, Kim, Moon, & Choi, 2014; Kim et al., 2013; Ryu et al., 2010; Shin, Choi, Park, & Moon, 2010). However, most studies have focused on the physicochemical properties of ASase-treated starch, and research on biological effects on, e.g., obesity, diabetes, and immunity, is lacking. Moreover, most studies have used ASase from *Neisseria polysaccharea* (NPAS), which has lower thermal stability and activity than ASase from *Deinococcus geothermalis* (DGAS) (Seo et al., 2008). Moreover, DGAS exhibits different transglucosylation properties than NPAS (Jung et al., 2009; Seo et al., 2016). RS formation by DGAS can expand the source of RS, which is currently limited to HAMRS2 and NPAS-treated starches.

In preliminary studies, starches of chestnut, yam, and rice subjected to DGAS-modification have been examined for their obesity-preventive effects (unpublished). DGAS-modified chestnut starch showed the most significant anti-obesity effect *in vivo*. Therefore, in this study, we investigated whether modification of chestnut starch by DGAS increases the RS content and we confirmed that DGAS-modified chestnut starch prevents obesity. Furthermore, we aimed to elucidate the mechanism, particularly in view of preventing fat accumulation by prebiotic activity.

2. Materials and methods

2.1. Materials

Chestnut starch was prepared according to the alkaline method reported by Lee et al. (2015). All other chemicals used in this study were of analytical reagent grade and were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). DGAS was expressed in and purified from recombinant *Escherichia coli* harboring pHCDGAS according to Seo et al. (2016).

2.2. Preparation of DGAS-modified chestnut starch

DGAS treatment of pre-gelatinized chestnut starch was conducted according to the method of Kim et al. (2015) with some modifications. Starch samples (60 g, dry weight basis or d.b.) as an acceptor were suspended in 50 mM Tris-HCl buffer (pH 8.0; 2500 mL) containing 300 mM sucrose as a donor. The starch dispersion was autoclaved at 121 °C for 20 min and then cooled to reaction temperature (40 °C). This substrate dispersion was transferred into a water-jacketed vessel (5000 mL) with one port lid, and then, DGAS (0.15 U/mL; 30 U in total) was added. The reaction was conducted at 40 °C for 48 h under constant stirring (300 rpm) using an overhead stirrer (MS3030D; Misung Scientific, Yangju, Korea) equipped with a cross-stirring rod. At 48 h, an equal volume (2000 mL) of ethanol was added to the reaction mixture to inactivate DGAS and precipitate the glucan polymer, and the reaction mixture was cooled to 4 °C for 24 h. DGAS-treated starch was recovered by centrifugation (11,000 × *g*, 4 °C, 20 min). The starch pellet was washed five times with double-distilled water, freeze-dried, ground, and passed through a 100-mesh sieve. The starch sample was stored in a desiccator at room temperature for further analysis.

2.3. Structural properties of DGAS-modified chestnut starch

To determine the iodine-binding index (IBI) of starch, starch sample (100 mg) was added to 10 mL of urea-dimethyl sulfoxide solution, and the mixture was stirred for 15 min in boiling water. Then, it was dried in an oven at 100 °C for 45 min. One milliliter of sample was mixed with 95 mL of distilled water, after which 2 mL of I_2-KI solution and 100 mL of distilled water were added to the mixture. After 15 min of reaction, the optical density at 660 nm was measured to determine iodine staining for calculating the IBI.

Branch-chain distribution of AP in the starch was determined by high-performance anion-exchange chromatography (HPAEC) after debranching treatment. Starch sample (10 mg, d.b.) was suspended in 90% aqueous dimethyl sulfoxide (1 mL), and heated in a boiling water bath for 1 h. Then, the starch solution was mixed with absolute ethanol (6 mL) and centrifuged at 4500 × *g* for 15 min, after which the supernatant was removed. The precipitate was dissolved in 1 mL of preheated 10 mM sodium acetate buffer (pH 3.5) and cooled to 40 °C. The starch solution was mixed with isoamylase (280 U/mg on oyster glycogen; Megazyme, Bray, Ireland), incubated at 40 °C for 24 h, and heated in a boiling water bath for 10 min to inactivate isoamylase. The debranched starch solution was passed through a 0.45-μm disposable syringe filter (Whatman, Kent, UK) and injected into an HPAEC system to examine AP branch-chain distribution of chestnut starches (Kim et al., 2013). The HPAEC system (ICS5000 series; Dionex, Sunnyvale, CA, USA) with a pulse amperometric detector (PAD; Dionex) was equipped with a CarboPac PA-200 column (3 × 250 mm; Dionex). Separation was achieved using a linear gradient from 150 mM NaOH to 600 mM sodium acetate (in 150 mM NaOH) at a flow rate of 0.5 mL/min.

X-ray diffraction patterns of starches were observed using diffraction angles between (2θ) 3.5° and 40° at a scanning speed of 6°/min using an X-ray diffractometer (M18XHF; Mac Science, Yokohama, Japan) with the following conditions: target, Cu-Kα; voltage, 40 kV; and current, 40 mA.

2.4. In vitro Englyst assay and digestion pattern analysis using small-intestinal mucosal α-glucosidase

The amounts of RDS, SDS, and RS in starches and feeds of different sources were determined by the Englyst assay (Englyst et al., 1992). The sample (1 g, starch or feed) was mixed with water (4 mL) in a 50-mL conical tube and prewarmed in a water bath at 37 °C for 15 min. Hydrolysis of prewarmed sample solution was initiated by adding an enzyme solution (2 mL) containing amyloglucosidase from *Aspergillus niger*, α-amylase from porcine pancreas, and invertase from Baker's yeast (all from Sigma-Aldrich

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