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The effect of dietary fibre preparations from potato starch on the growth and activity of bacterial strains belonging to the phyla *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*

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ABSTRACT

In the present study, it was ascertained whether dietary fibre preparations derived from potato starch with prebiotic properties would promote the growth of *Bacteroidetes* strains while also inhibiting *Firmicutes* strains at the same time. The fibre preparations were added to faecal samples from obese children and incubated for 24 hours at 37 °C under anaerobic conditions. It was found that the studied preparations stimulate the growth of bacterial strains belonging to the phyla *Bacteroidetes* and *Actinobacteria*, while reducing the quantity of strains belonging to the phylum *Firmicutes* – especially *Clostridium* (enzyme-resistant citric acid-modified dextrin D1 $p = 0.02$, enzyme-resistant tartaric acid-modified dextrin D2 $p < 0.01$). The concentration of SCFA in the stool of obese children after a period of incubation with the fibre preparations was higher in comparison to stool not containing these preparations D1 ($p = 0.03$) and D2 ($p = 0.04$). Dietary fibre preparations obtained from potato starch may be used prophylactically to prevent obesity.

1. Introduction

Over the past several years, the incidence of obesity has considerably increased. While the main cause of obesity is oversupply of calories vs. insufficient energy expenditure, this condition is determined by many factors. It is important to elucidate the role of the various factors affecting obesity from the point of view of developing treatments to counter it. Studies conducted by Backhed et al., Gordon et al., and De Filippo et al. indicate that human obesity is likely linked to the composition and relative abundance of gut microbiota. Backhed, Manchester, Semenkovich, and Gordon (2007) determined the proportions of *Bacteroidetes* and *Firmicutes* in normal-weight and

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overweight mice and found that the relative abundance of *Bacteroidetes* was much lower (20%) in overweight mice than in leaner mice (40%; Backhed et al., 2004; Backhed et al., 2007). Fleissner et al. (2010) reported that feeding mice a diet rich in animal fats and low in dietary fibre led to a reduction in the population of *Bacteroidetes* strains and an increase in *Firmicutes* strains. It has been found that an increase in *Bacteroidetes* in gut microbiota and the related changes in the gut metagenome are correlated with weight loss in overweight and obese individuals, alleviation of metabolic disorders, increased sensitivity to insulin in adults with metabolic syndrome and/or type 2 diabetes, reduced inflammation in patients with inflammatory bowel disease, and a higher proportion of regulatory T cells in peripheral blood.

The types and relative proportions of microorganisms determine the kind of metabolites produced, which is of great importance for the host, as the metabolites may be either harmful or beneficial. These metabolites include short-chain fatty acids (SCFAs), generated as a result of the fermentation of undigested polysaccharides by certain types of bacteria in the colon (Archer, Johnson, Devereux, & Baxter, 2004; Cani, Dewever, & Delzenne, 2004; Delzenne, Cani, Daubioul, & Neyrinck, 2005; Tarini & Wolever, 2010). SCFAs fulfil a number of advantageous functions. Butyric acid stimulates the growth of intestinal epithelium, provides nourishment to intestinal cells, and enhances their maturation and appropriate differentiation. Propionic acid helps the growth of hepatocytes, while acetic acid improves the development of peripheral tissues. SCFAs regulate the metabolism of glucose and lipids, stimulate the proliferation and differentiation of enterocytes, and lower the pH of intestinal contents, thus improving the absorption of mineral compounds by making them more soluble (Blaut & Clavel, 2007; Lin et al., 2012). It has been shown that while SCFAs constitute a source of energy, they actually counteract obesity by reducing fat accumulation in adipose tissue, accelerating energy expenditure, and increasing the production of satiety hormones (Gao et al., 2009; Keenan et al., 2006; Kimura et al., 2013). The influence of butyric acid on energy homeostasis may be attributable to stimulation of leptin synthesis in adipocytes, induction of GLP-1 secretion by intestinal L cells, and increased oxidation of fatty acids (Gao et al., 2009; Nicholson et al., 2012). Research into gut microbiota metabolites has shown that propionic acid may provide an additional source of energy for the human host, as it is used in the processes of glucose and lipid synthesis (Bates, Akerlund, Mittge, & Guillemin, 2007; Cani et al., 2008). The composition and activity of gut microbiota should be taken into consideration in evaluating the risk factors for obesity and metabolic syndrome. Importantly, they may be regulated through diet. A diet containing high quantities of dietary fibre and prebiotics leads to beneficial relative proportions of the phyla *Bacteroidetes* and *Firmicutes* within the gut microflora, and to a beneficial increase in the relative abundance of *Prevotella* vs. *Bacteroides* within *Bacteroidetes* (Abrams, Griffin, Hawthorne, & Ellis, 2007).

Studies on rats suggest that prebiotics used as dietary supplements beneficially reduce the development of obesity and the severity of non-alcoholic fatty liver disease (NAFLD) as a result of gut microflora modification; they also decrease the amount of fatty tissue due to lower *de novo* synthesis of fatty acids (Cani, Neyrinck, Maton, & Delzenne, 2005; Delzenne et al., 2005; Vilsbøll, Krarup, Madsbad, & Holst, 2003). Thus, the consumption of prebiotic substances may be one of the factors preventing overweight and obesity in children as well as limiting the secondary metabolic conditions of obesity.

Unfortunately, commonly-used prebiotics as fructooligosaccharides, xylooligosaccharides, isomaltooligosaccharides, and transgalactooligosaccharides often cause gastric problems that frequently outweigh their main advantage – their low energy content (Crittenden & Playne, 2002; Gibson, 1999, 2004; Roberfroid, 1998; Sako, Matsumoto, & Tanaka, 1999; Swennen, Courtin, & Delcour, 2006). These prebiotics are also poorly tolerated by the human body. Therefore, researchers look for compounds that would be better tolerated and could be consumed in large quantities. Promising substances in this respect include starch products, such as dietary fibre preparations containing resistant dextrins, branched dextrins, resistant maltodextrins (Hamaker & Tuncil, 2014; Monsivais, Carter, Christiansen, Perrigue, & Drewnowski, 2011), and soluble corn fibre (Ye, Arumugam, Haugabrooks, Williamson, & Hendrich, 2015). The objective of the present study was to determine whether prebiotic dietary fibre preparations from potato starch would stimulate the growth of *Bacteroidetes* strains while inhibiting *Firmicutes* strains. Moreover, the composition of SCFA and BCFA in the stool of obese children was analysed.

2. Materials and methods

2.1. *Preparation of dietary fibre*

Enzyme-resistant citric acid-modified dextrin (dietary fibre preparation D1) was prepared according to the method of Kapusniak, Jochym, Barczynska, Slizewska, and Libudzisz (2008), P.392894 (Kapusniak, Barczynska, Jochym, Slizewska, & Libudzisz, 2015a). Thus, potato starch was sprayed with a hydrochloric acid solution (0.5%, w/w) to obtain a final HCl concentration of 0.1% on a dry starch basis (dsb). A citric acid solution (0.5%, w/v) was then added to obtain a final organic acid concentration of 0.1% dsb. The thoroughly mixed sample was dried at 110 °C to obtain a final moisture content below 5%. The dried sample (10 g) was placed in an anti-pressure bottle (SIMAX), capped, and heated at 130 °C for 3 hours in an Economy Laboratory Furnace (ELF) model 11/6 (Carbolite, Hope, England).

Enzyme-resistant tartaric acid-modified dextrin (dietary fibre preparation D2) was prepared following the method of Kapusniak et al. (2008), P.392895 (Kapusniak, Barczynska, Jochym, Slizewska, & Libudzisz, 2015b). Thus, potato starch was sprayed with a hydrochloric acid solution (0.5%, w/w) to obtain a final HCl concentration of 0.1% on a dry starch basis (dsb). A tartaric acid solution (20%, w/v) was then added to obtain a final organic acid concentration of 40% dsb. The sample was mixed well, dried at 60 °C overnight, and then dried at 110 °C for 3 hours to obtain a final moisture content below 5%. The dried sample (10 g) was placed in an anti-pressure bottle (Simax), capped, and heated at 130 °C for 2 hours in an Economy Laboratory Furnace (ELF) model 11/6 (Carbolite, Hope, England). Both products were cooled in a desiccator and milled into powder with a particle size of <1 mm. Dietary fibre preparations were then washed with ethanol (80%, v/v) to remove excess tartaric acid and low molecular weight material formed during