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Original article

Gut microbiota mediated benefits of barley kernel products on metabolism, gut hormones, and inflammatory markers as affected by co-ingestion of commercially available probiotics: a randomized controlled study in healthy subjects

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SUMMARY

Background and aims: Barley kernel based products have been shown to induce benefits on blood glucose regulation, cardio-metabolic risk markers and appetite regulating hormones in a time perspective of 11–16 h after intake. The mechanisms have been assigned to gut fermentation of indigestible carbohydrates. The purpose of the present study was to evaluate if the modulatory effects of barley on markers of metabolic- and appetite regulation are affected by a dietary background including a mixture of commercially available probiotics.

Methods: Barley kernel bread was included in the normal diet of 21 healthy subjects in two 4-day intervention periods; with (BB-pro) or without (BB) dietary supplement with a combination of probiotics (*Bifidobacterium animalis* DN-173 010, *Lactobacillus reuteri* DSM 17938, and *Lactobacillus plantarum* 299v). A white wheat flour based bread was included as a reference product (WWB-ref) in a separate 4-day bread intervention period. A cross-over design was applied concerning BB- and WWB-ref; the BB-pro intervention was last in the test sequence. The BB-pro intervention was preceded by 10 days priming with probiotics. The 4 day BB- and WWB-ref intervention periods included dietary supplementation with placebo, and the interventions were preceded with 10 days priming with the placebo. The day after each intervention period, blood samples were collected at fasting and postprandially after a standardized breakfast (0–210 min) for determination of markers of glucose metabolism (blood glucose, serum (s-) insulin), inflammation (s-IL-6, s-IL-18, s-CRP, PAI-1), and concentrations of gut derived hormones involved in satiety and glucose homeostasis (plasma (p-) PYY, p-GLP-1) and intestinal barrier integrity (p-GLP-2). Breath hydrogen was determined as a marker of colonic fermentation.

Results: Four days intervention with BB, in comparison to WWB-ref, lowered blood glucose response after a subsequent standardized breakfast (0–210 min, $P < 0.05$). BB and BB-pro interventions increased p-GLP-1 (0–120 min, $P < 0.05$) and breath H_2 (0–210 min, $P < 0.05$). BB-pro intervention, in comparison to BB and WWB-ref, increased levels of s-PAI-1 ($P < 0.05$), and p-GLP-2 (0–210 min, $P < 0.05$) after the standardized breakfast.

Conclusions: With the exception of increased p-GLP-2 and an unexpected increase in s-PAI-1 concentrations, co-ingestion of a mixture of probiotics did not affect the metabolic outcome of BB; neither positively nor importantly negatively.

The study was registered at: ClinicalTrials.gov, register number NCT01718418 (www.clinicaltrials.gov/ct2/show/NCT01718418).

Abbreviations: AUC, area under the curve; iAUC, incremental area under the curve; BB, barley kernel based bread; BB-pro, barley kernel based bread consumed with probiotics; DF, dietary fiber; MetS, metabolic syndrome; NSP, non-starch polysaccharides; RS, resistant starch; WG, whole grain; WWB, white wheat flour based bread; WWB-ref, white wheat flour based reference bread.

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1. Introduction

Epidemiological studies show that whole grain (WG) and dietary fiber (DF) intake is associated with lower prevalence of metabolic syndrome (MetS) related diseases, such as obesity and type 2 diabetes [1,2]. Today there is a growing insight regarding the role of gut microbiota in modulating host metabolism and systemic low-grade inflammation. The metabolic benefits of WG and DF appear, at least partly, to be associated with colonic fermentation of indigestible carbohydrate substrates [3,4]. Both prebiotic- (inulin and oligofructose extracts) and to some extent also probiotic (strains of *Lactobacillus* and *Bifidobacterium* spp.) approaches have been demonstrated to improve components of the MetS in human- and animal studies [5]. However, most substantial benefits on MetS components, e.g. glucose- and weight regulation, have been observed with prebiotic substrates [5]. In addition to oligofructose- and inulin extracts, other DF which not in strict meaning are referred to as prebiotics have shown benefits on metabolic variables through mechanisms related to colonic fermentation. Consequently, in rodent models, feeding resistant starch (RS) from high amylose maize resulted in improved glucose regulation and an increased release of gut hormones important in e.g. glucose- and appetite regulation (GLP-1 and PYY) [6], and reduced abdominal fat [7]. In addition there are reports of improved insulin sensitivity also in healthy subjects following intake high amylose maize RS [8]. Examples of other potential sources of prebiotic substrates are barley kernel based foods, which e.g. have shown the capability to facilitate glucose regulation, increase gut derived hormones (GLP-1) and lower markers of inflammation in healthy subjects through a gut microbiota mediated mechanism [9–12]. Foods containing probiotics are frequent on the market, and it could be argued that co-ingestion of probiotic strains could affect health outcome emanating from gut fermentation of indigestible carbohydrate substrates. The purpose of the present study was to investigate if metabolic benefits previously observed in healthy humans after ingestion of barley kernel based products are affected by co-ingestion of a mixture of commercial probiotics. For this purpose, in a crossover study design, 21 healthy young adults were provided barley kernel based bread for 4 days without (BB) or with (BB-pro) dietary supplementation with probiotics (strains of *Lactobacillus* and *Bifidobacterium* spp.). A white wheat flour based bread was included as a reference product (WWB-ref) in a separate 4 day bread intervention period. The BB-pro intervention was preceded by 10 days of priming with the probiotics. The BB- and WWB-ref interventions included dietary supplementation with placebo, and the interventions were preceded with 10 days of priming with the placebo. The day after each intervention period, blood samples were collected at fasting and postprandial a standardized breakfast for determination of markers of glucose metabolism (blood glucose, serum (s-) insulin), inflammation (s-IL-6, s-IL-18, s-CRP, s-PAI-1), and concentrations of gut derived hormones involved in satiety and glucose homeostasis (plasma (p-) PYY, p-GLP-1) and intestinal barrier integrity (p-GLP-2).

2. Materials and methods

The study was registered at: ClinicalTrials.gov, register number NCT01718418 (www.clinicaltrials.gov/ct2/show/NCT01718418).

2.1. Ethical statement

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Regional Ethical Review Board in Lund, Sweden (Reference 2010/457). Written informed consent was obtained from all test subjects.

2.2. Test subjects

The test subjects were volunteer students recruited from Lund University, Sweden. Twenty-three subjects were enrolled. After start of the study two subjects dropped out without given any reason, resulting in 8 women and 13 men with the age of (mean ± s.d) 23.9 ± 0.7 years and with body mass indices (BMI) (mean ± s.d) of 22.6 ± 0.4 kg/m^2 completed the study. The inclusion criteria were age between 20 and 35 years, BMI within 19–25 kg/m^2, fasting blood glucose ≤6.1 mmol/L, non-nicotine user (smoker or snuffer) and overall healthy with no known metabolic disturbances or food allergies, including lactose- or gluten intolerance. In order to reduce the possible influence of hormonal variations (estrogen, progesterone), women without hormonal-based contraceptives were not included. If needed, the subjects were encouraged to use painkillers without anti-inflammatory effects. Intake of antibiotics or probiotics was not allowed within two weeks before or during the study.

2.3. Interventions

The study was divided in three intervention periods where test subjects were provided barley kernel based bread with (BB-pro) or without (BB) dietary supplementation with probiotics (strains of *Lactobacillus* and *Bifidobacterium* spp., see below). Each bread intervention period lasted for 4 days. A white wheat flour based bread was included as a reference product (WWB-ref) in a separate 4 day bread intervention period. The BB-pro intervention was preceded with 10 days priming with the probiotics. The 4 day BB- and WWB-ref interventions included dietary supplementation with a placebo, and the interventions were preceded with 10 days priming with the placebo (the placebo is described below). Thus, each intervention period lasted in total 14 days; 10 days with probiotic or placebo +4 days with bread together with probiotics or placebo. The test products were included in the test subjects' normal diet. The BB was kindly produced by Credin A/S (Juelsminde, Denmark) and contained barley kernels (75% of the dry matter (DM)) and whole-grain barley flour (10% of the DM) and a small proportion of white wheat flour (15% of the DM). The WWB consisted of a commercial bread (Dollar Storfranska, Lockarp, Malmö, Sweden). The bread portions were selected to provide 75 g available starch per day. During the first three days with bread, the daily portions were divided into equal sizes to be ingested in the morning and the evening. On day four with bread, i.e. the day prior to an experimental day, the morning portion provided 25 g available starch and a late evening meal 50 g available starch. The reason for the consumption approach the day prior to the experimental day was to have the opportunity to compare results emanating from this study with previously studies addressing similar research questions and where subjects were provided test evening meals in a similar manner, e.g. Priebe, M. G., et al., 2010, AJCN, and Johansson, E. V., et al., 2013, Nutr J, 2013 [9,10].

The selection of probiotic products were based on being commercially available and commonly consumed, and the dosage of the probiotic bacteria were according to the manufacturers' instructions. All the products were blinded as much as possible to the test subject.

2.3.1. Probiotic mixture included in the BB-pro intervention

- Probiotic yoghurt (200 mL/day): Activia®, *Bifidobacterium animalis* DN-173 010, Danone AB, Solna, Sweden ($20 \cdot 10^9$ CFU/day).