ISRCTN17344943 https://doi.org/10.1186/ISRCTN17344943

Evaluation of the gastrointestinal effects of breads containing in situ produced arabinoxylan-oligosaccharides in healthy volunteers

Submission date 26/04/2011	**Recruitment status** No longer recruiting	[_] Prospectively registered [_] Protocol [_] Statistical analysis plan [_] Results [_] Individual participant data [_] Record updated in last year
Registration date 06/06/2011	**Overall study status** Completed	
Last Edited 06/06/2011	**Condition category** Digestive System	

Plain English summary of protocol
Not provided at time of registration

Contact information

Type(s)
Scientific

Contact name
Prof Kristin Verbeke

Contact details
Translational Research Center for Gastrointestinal Disorders (Targid) and Leuven Food Science and Nutrition Research Centre (LFoRCe)
University Hospital Gasthuisberg
K.U. Leuven
Herestraat 49, O&N1
Box 701
Leuven
Belgium
3000

Additional identifiers

Protocol serial number
ML4790

Study information

Scientific Title
Evaluation of the gastrointestinal effects of breads containing in situ produced arabinoxylan-oligosaccharides in healthy volunteers: a monocentric, randomised, double-blind, controlled cross-over study

Study objectives
The present study focuses on the potential of in situ produced 'prebiotic' AXOS in improving markers associated with good health and wellbeing in healthy volunteers. Prebiotics are compounds that cannot be utilised by enzymes of the upper gastrointestinal tract of humans but that are fermented selectively by some types of intestinal bacteria that reside in the large intestine, thereby exerting a beneficial health effect on their host. Ingestion of prebiotics causes a shift in the composition of the intestinal bacterial population, typically characterised by a relative increase in certain beneficial bacteria such as Bifidobacterium species. This shift in the intestinal microbiota and the associated fermentation activities, typically involving increased production of short chain fatty acids (SCFA), are associated with improved overall health, reduced gut infections, better absorption of minerals and suppression of colon cancer initiation.

The study product of this study was bread made by addition of endoxylanase enzymes to a flour mixture derived from wheat and rye, resulting in the generation of arabinoxylan-oligosaccharides (AXOS) during the bread making process.

The primary objectives were to assess whether intake of the AXOS-enriched bread alters the levels of microbiota in the faeces, in particular faecal bifidobacteria and whether it has an effect on the levels of short-chain fatty acids (SCFA) in the faeces, a hallmark of beneficial prebiotic effects.

The study also aimed at investigating the effect of consumption of AXOS-enriched bread on phenol and p-cresol content in urine, markers of colonic protein fermentation. In addition, the study was also set up to investigate, through the use of questionnaires, whether consumption of AXOS-enriched bread affects stool frequency, stool consistency and gastrointestinal symptoms.

Wheat flour bread without in situ produced AXOS (during run in and wash out periods) and fibre enriched bread without in situ produced AXOS were used as references.

Ethics approval required
Old ethics approval format

Ethics approval(s)
The initial version of the protocol of clinical trial ML4790 and the informed consent sheet form were approved by the Ethics Committee of the University Hospital UZ Leuven on February 12, 2008. An amendment of this protocol was submitted to the Ethics Committee and was approved on February 21, 2008.

Primary study design
Interventional

Study design
Monocentric randomised double-blind controlled cross-over study

Study type(s)
Screening

Health condition(s) or problem(s) studied
Gastrointestinal effects of arabinoxylan-oligosaccharides

Interventions
The subjects were randomised in two groups, Group 1 and Group 2, differing in the treatment sequence. The study ran over 12 weeks, divided over four consecutive periods of three weeks each.

During the first period (run-in period) and the third intake period (wash-out period) all subjects of Group 1 and Group 2 consumed bread made with refined wheat endosperm flour without added enzymes (W-).

During the second and the fourth intake period Group 1 subjects consumed wheat/rye bread without in situ produced AXOS (WR-) and a wheat/rye bread with in situ produced AXOS (WR+), respectively, and vice versa for Group 2 subjects.

Stool and urine collection
From the morning of day 19 till the morning of day 21 of each intake period, urine was collected by each subject. To each container (5 liter volume) for urine collection dispensed to the subjects by members of the investigational team, 1.0 g neomycine was added to prevent bacterial growth. Subjects delivered the collected urine at the clinic to members of the investigational staff, who measured the volume of the urine fractions. 10 ml of the urine was immediately transferred to the central laboratory facility of the University Hospitals (U.Z. Leuven) for analysis of urea and creatinine content. A 20 ml aliquot was stored at -20°C until further analyses. On the evening of day 20 or the morning of day 21, one stool was collected by each subject in a plastic recipient (round box with lid, 500 ml volume) dispensed to the subjects by members of the investigational team. The recipients with stool were stored immediately after collection at home at 4°C until delivery at the clinic. All stool samples were delivered to the clinic within 20 h of stool production. Upon delivery, 2.5 g of each stool sample was immediately fixed by members of the investigational staff in paraformaldehyde for microbial analysis by fluorescent in situ hybridisation (FISH) and other aliquots of 2.0 g were stored at -20°C until further analyses of short chain fatty acids.

Analytical procedures
Microbiological analysis of faeces (delivered at the end of each of the 4 periods):
Stool samples were analysed to evaluate the influence of the consumption of different bread types on the composition of the faecal microbiota. Fluorescent in situ hybridisation (FISH) was used to count the number of different bacterial groups in paraformaldehyde fixed stool samples. For total bacterial cell counts, 4-6-diamidino-2-phenylindole (DAPI) was used. The probes used for group-specific FISH analysis were Bif164 for the Bifidobacterium sp. group, Lac158 for the Lactobacillus sp. group, Ec1531 for the Enterobacteriaceae and an equimolar mixture of Chis150 and Clit135 for the Clostridium histolyticum/lituseburense group. Rod-shaped bacteria fluorescing with the Lac158 probe were scored as Lactobacillus rods. Filters were mounted on clean microscope slides with Vectashield (Vector Laboratories, Burlingame, Calif.). Digital images of the slides, viewed with a Leica (Wetzlar, Germany) DMRXA epifluorescence microscope, were taken with a Kodak Megaplus 1.4 charge-coupled device camera. These images were analysed and fluorescent cells were counted by using Quantimet HR600 image analysis software (Leica). Depending on the amount of fluorescent cells, 10 to 30 microscopic fields were counted.

Short chain fatty acids in faeces (delivered at the end of each of the 4 periods):
To vials containing faecal samples (2 g) the following was added: 0.5 ml 9.2 M sulfuric acid, 0.4 ml of 0.75 % (v/v) 2-methylhexanoic acid (internal standard), 0.4 g NaCl and 2 ml diethyl ether. After shaking the vials for 2 minutes, the vials were centrifuged (3 min at 3000 x g) and the diethyl ether phase transferred to glass vials. The diethyl ether phase containing the organic acids was analysed on a gas-liquid chromatograph equipped with a EC-1000 Econo-Cap column (Alltech, Deerfield, IL, USA; dimensions: 25 m x 0.53 mm, film thickness 1.2 µm; acid-modified polyethylene glycol as liquid phase) and a flame ionisation detector. Nitrogen was used as a carrier gas at a flow rate of 20 ml per minute and the column temperature and injector temperature were set at 130 and 195°C, respectively. Concentrations of SCFAs were calculated based on standards with known concentrations of the different acids. The concentration of total SCFA was calculated as the sum of the concentrations of acetic, propionic and butyric acid.

p-Cresol and phenol in urine (delivered after 48 h collection at the end of each of the four periods):
Total p-cresol and phenol content in urinary samples was assayed by Gas chromatography-mass spectroscopy (GC-MS). The pH of 950 µL urine was adjusted to 1.0 with 50 µL concentrated sulphuric acid (Merck KaA, Darmstadt, Germany). After addition of 50µL 2,6-dimethylphenol solution (20mg/100ml; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) as internal standard, the solution was heated for 30min at 90°C to deproteinise and hydrolyse conjugated p-cresol and phenol. After cooling down, p-cresol and phenol were extracted in 1.0 ml ethyl acetate (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The acetyl acetate layer was dried over anhydrous sodium sulfate and finally 0.5 µL was analysed on a GC-MS (Trace GC-MS, Thermo-Finnigan, San José, CA, USA). The analytical column was a 30 m x 0.25 mm internal diameter, 0.50 µm RxiTM-5-MS (Restek, Bellefonte, PA, USA). Helium GC grade was used as a carrier gas with a constant flow of 1.3 ml/min. The oven was programmed from 55°C (isothermal for 5min), and increased by 10°C/min to 160°C and by 20°C/min to 280°C. Mass spectrometric detection was performed in electron impact full scan mode from m/z 59 to m/z 590 at 2 scans/s.

Recording of stool parameters and gastrointestinal symptoms:
Gastrointestinal symptoms were monitored daily during the last week of every 3-week intake period. The volunteers were asked to daily grade the following symptoms: abdominal cramps, flatulence and bloating. The severity of the symptoms was graded on a 4-step scale ranging from no (0), minimal (1), mild (2), moderate (3) to severe (4) symptoms as described by van Munster et al. (1994). During the last week of every 3-week intake period, defecation frequency as well as consistency of the stool according to the Bristol Stool Form Scale were recorded daily through appropriate questionnaires. The average stool frequency was calculated as the number of stools divided by the number of days of diary recording, the average stool consistency as the sum of Bristol Stool Form Scales divided by the number of stools and the composite parameter of stool frequency and consistency (also called Bristol composite measure) as the sum of Bristol Stool Form Scales divided by the number of days of diary recording.

Intervention Type
Other

Phase
Not Applicable

Primary outcome(s)
Total short chain fatty acid concentrations in faeces were significantly higher upon consumption of WR+ (wheat/rye bread with in situ produced AXOS) compared to intake of W- (wheat flour

bread without in situ produced AXOS). Butyrate levels were 1.7 times higher after consumption of WR+ than in the run-in or wash-out period (W- consuming periods). Consumption of WR+ tended to increase selectively the faecal concentration of bifidobacteria (P = 0.06) relative to intake of W-.

Key secondary outcome(s)
1. Urinary phenol and p-cresol excretion were significantly lower after WR+ intake compared to WR- (wheat/rye bread without in situ produced AXOS)
2. Average stool frequency and the composite stool frequency/consistency parameter were increased after intake of WR+ compared to WR-. No adverse effects on gastrointestinal symptoms were reported during WR+ intake.

Completion date
11/06/2008

Eligibility

Key inclusion criteria
1. Age between 18 and 46 years
2. Good general health (determined by self-assessment)
3. Regular eating habit (3 meals per day during at least 5 days per week)

Participant type(s)
Patient

Healthy volunteers allowed
No

Age group
Adult

Lower age limit
18 Years

Sex
All

Key exclusion criteria
1. History of abdominal surgery
2. Intake of antibiotics less than two months before the start of the study
3. Medical treatments influencing gut transit less than 2 weeks before the start of the study

Date of first enrolment
20/03/2008

Date of final enrolment
11/06/2008

Locations

Countries of recruitment
Belgium

Study participating centre
Translational Research Center for Gastrointestinal Disorders (Targid) and Leuven Food Science and Nutrition Research Centre (LFoRCe)
Leuven
Belgium
3000

Sponsor information

Organisation
Puracor NV (Belgium)

Funder(s)

Funder type
Industry

Funder Name
Puracor NV (Belgium)

Results and Publications

Individual participant data (IPD) sharing plan

IPD sharing plan summary
Not provided at time of registration