Oral xylose isomerase decreases breath hydrogen excretion and improves gastrointestinal symptoms in fructose malabsorption – a double-blind, placebo-controlled study


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SUMMARY

Background
Incomplete resorption of fructose results in increased colonic hydrogen production and is a frequent cause of abdominal symptoms. The only treatment available is diet.

Aim
To study whether orally administered xylose isomerase (XI), an enzyme that catalyses the reversible isomerisation of glucose and fructose, can decrease breath hydrogen excretion in patients with fructose malabsorption.

Methods
Patients received 25 g fructose in 100 mL water together with either placebo or XI capsules. Primary endpoint was the reduction in breath hydrogen excretion, as assessed by the area under the breath hydrogen curve over 4 h (AUC). A secondary endpoint was the reduction in abdominal pain, bloating and nausea assessed on a visual analogue scale (VAS, range: 0–10). A P value <0.05 was considered statistically significant.

Results
Sixty-five patients in whom fructose malabsorption had been diagnosed by positive breath hydrogen test within the previous year, were included in the study [15 males, 50 females; mean age 43.3 (s.d. = 14.4), range: 21–73 years]. The median AUC was 885 ppm/240 min in the XI group compared to 2071 ppm/240 min in the placebo group (P = 0.00). Median scores for abdominal pain (0.7 vs. 1.3) and nausea (0.2 vs. 0.6), but not for bloating (P = 0.053), were significantly improved after XI (P = 0.009 and P = 0.005) as compared with placebo.

Conclusions
Oral administration of xylose isomerase significantly decreased breath hydrogen excretion after ingestion of a watery fructose solution. Nausea and abdominal pain were significantly improved by xylose isomerase.
INTRODUCTION

Fructose is present in modern diets either as a constituent of the disaccharide sucrose or as the monosaccharide. Average daily intake varies from 11 to 54 g. \(^1\) Fructose is used as a sweetener or as high fructose corn syrup in soda, fruit juices or candy, and it is naturally present in fruits like apples, peaches, pears and oranges. \(^2\) From 1970 to 1997, daily per capita intake of fructose increased from below 1 g to over 70 g while consumption of sucrose, a disaccharide in which fructose is linked to glucose, decreased about 34%. \(^3\)

The absorptive capacity for fructose that is not accompanied by glucose is small. \(^4\) The normal absorptive capacity for fructose depends on other nutrients as well, and still is poorly understood. It has been suggested that healthy subjects have the capacity to absorb up to 25 g of fructose, whereas many have incomplete absorption and intolerance with intake of 50 g of fructose. \(^5\) Ingestion of food that contains fructose in excess of glucose may result in symptoms such as abdominal bloating or diarrhoea and also may provoke symptoms in patients with irritable bowel syndrome (IBS). \(^6\)–\(^8\) It has been suggested that as little as 3 g of fructose may induce symptoms in functional bowel disorders. Gender may influence fructose malabsorption. \(^9\)

Incompletely absorbed fructose reaches the colon, where bacterial carbohydrate metabolism results in production of short chain fatty acids and gases like hydrogen. This may cause symptoms like abdominal pain, bloating, discomfort and diarrhoea. \(^10\)–\(^12\) These symptoms are not specific for fructose malabsorption but also occur with other incompletely absorbed carbohydrates, like dietary fibres, sorbitol, lactose in case of lactase deficiency or carbohydrate malabsorption in severe restriction of pancreatic digestive or intestinal absorptive capacity. \(^11\)–\(^13\)–\(^15\) Recently the term FODMAP, describing fermentable oligo-, di- and monosaccharides and polyols has been used to characterise short chain carbohydrates which potentially may trigger abdominal symptoms and for which dietary restriction has been suggested to be efficacious. \(^16\)–\(^17\) Continued ingestion of high amounts of fructose has been suggested to result in a variety of other impairments. Since the year 1975 it has been discussed that enhanced dietary fructose could induce obesity, accelerated ageing, insulin resistance and non-alcoholic fatty liver disease. \(^18\)–\(^22\) In addition, some types of depression could be related to long time consumption of high fructose diet. \(^23\)

Hydrogen produced in the colon is absorbed and excreted in breath. This can be used for a diagnostic breath hydrogen test. The sensitivity of this breath hydrogen test, however, is influenced by the prevalence of hydrogen non-excretion, which has been reported to be up to 18%. \(^24\) Incomplete fructose absorption usually is identified by a positive result on a breath hydrogen test after ingestion of 25–50 g of fructose.

There is a lack of information on the prevalence of incomplete absorption of fructose in the healthy population, but self selection of persons coming to the test may play an important role. In studies on small numbers of healthy subjects between 11% and 50% had a positive breath hydrogen test after 25 g of fructose, and between 38% and 81% of subjects had a positive breath hydrogen test after 50 g of fructose. In normal subjects who ingested 25 g of fructose, incomplete absorption documented by a positive hydrogen breath test had only a marginal effect on symptoms, whereas after 50 g of fructose there was a more pronounced effect on symptoms in previously asymptomatic normal subjects with documented incomplete absorption. \(^1\)

The same review also summarised results of hydrogen breath testing in patients with functional gut disorders. \(^1\) In these patients, the prevalence of a positive hydrogen breath test after 25 g of fructose was between 39% and 80%, increasing with the concentration of the fructose solution used. \(^25\)

When studies in patients or normal subjects using 25 g of fructose were compared, patients with functional gut disorders and a positive hydrogen breath test in general had a higher likelihood of symptoms as compared with previously asymptomatic normal subjects with a positive breath hydrogen test. However, after 50 g of fructose, the proportion of patients with a positive breath hydrogen tests and the proportion of patients who had symptoms related to incomplete fructose absorption was similar to normal subjects. \(^1\) These observations suggest that patients with functional gastrointestinal disorders may have a greater likelihood to be referred to a fructose hydrogen breath test, and that they may be more sensitive to lower doses of malabsorbed fructose.

Xylose isomerase (XI, EC 5.3.1.5, CAS 9023-82-9) is an enzyme that catalyses the reversible isomerisation of glucose and fructose. \(^26\) It has been used for the industrial conversion of glucose to fructose in the manufacture of high fructose corn syrup. \(^27\) XI is available from industrial production and has been proven not to provoke allergic reactions in man. Furthermore, GRAS (Generally Recognized As Safe) status of the enzyme has been affirmed by the FDA.
We hypothesised that orally administered XI is able to catalyse the conversion of poorly absorbable fructose into well-absorbable glucose in the human intestine in vivo and thereby reduce breath hydrogen excretion after ingestion of fructose in patients with incomplete fructose absorption. To assess this hypothesis, we performed a double-blind randomised crossover study in patients with previously established fructose malabsorption. Breath hydrogen excretion was assessed in patients who received either XI or placebo with an oral watery fructose load. A secondary endpoint was the assessment of the effect of XI on symptoms using a visual analogue scale (VAS).

PATIENTS AND METHODS

Study design
This randomised, double-blind, placebo-controlled, prospective, crossover interventional study was conducted in the Department of Environmental Dermatology and Venereology of the Medical University of Graz, Austria, from June 2009 until May 2010 in accordance with the principles of Good Clinical Practice (GCP) corresponding to the ICH guidelines (International Conference on Harmonisation of drugs and medical devices).

The protocol was registered at ClinicalTrials.gov (NCT00916487) and at EudraCT (EUDRACT2008-005861-80) and approved by the Ethics Committee of the Medical University of Graz (EK 20-024 ex 08/09).

Inclusion and exclusion criteria
The study was performed in patients who were referred for a fructose hydrogen breath test for the evaluation of abdominal symptoms, and in whom fructose malabsorption was confirmed within the preceding 12 months. Tests were performed in the departments of gastroenterology or dermatology of our university. Fructose malabsorption was confirmed if there was an increase in end expiratory breath hydrogen concentration of at least 20 ppm over baseline after 25 or 30 g of fructose; 30 g was used at the department of gastroenterology and 25 g was used at the department of dermatology. Exclusion criteria were pregnancy, breast feeding, diabetes mellitus and gastrointestinal surgery, endoscopy or antibiotic treatment within the preceding 4 weeks. Before enrolment, every patient signed a declaration of consent after having been provided with thorough verbal and written information.

Endpoints
Primary endpoint was the assessment of breath H₂ excretion after fructose intake by calculating the area under the breath hydrogen curve (AUC) over a 4 h observation period. AUC after fructose plus XI was compared with AUC after fructose plus placebo. Secondary endpoint was the reduction of the symptoms abdominal pain, bloating and nausea using a visual analogue score.

Verum and placebo
The XI capsule (258.2 mg) (Fructosin; Sciotec, Tulln, Austria) contains 43.12 mg of this enzyme showing an activity of approximately 1.6 µkat per caps. Further ingredients, amongst others are microcrystalline cellulose (70.43 mg), gelatine (47.24 mg), maltose (25.13 mg), shellac (22.04 mg) and hydroxypropylcellulose (20.4 mg). Placebo capsules are composed of microcrystalline cellulose (200 mg) and gelatine (47.24 mg). To ensure reproducible activity of the enzyme a crystallisation process that yields highly active and stable crystals of XI was developed (patent application EP2161034). These crystals were then incorporated into small microparticles with a diameter of approximately 1 mm. This size ensures that pellets are emptied during the digestive phase of stomach emptying. Pellets had an enteric coating with a thin film of shellac to protect the enzyme from acidic denaturation during stomach transit. The pellets were packed into gelatine capsules which dissolve quickly in the stomach releasing the pellets.

Preparation, randomisation protocol and use of test kits
The producer of XI capsules prepared numbered test kits containing (i) fructose for two tests (25 g each in two 100 mL plastic bottles), and (ii) three capsules of XI and three capsules of placebo which were randomly assigned to sealed bags marked with ‘X’ and ‘Y’ respectively. Test kits were sent by courier to the test centre. XI and placebo capsules looked and tasted identical. This procedure guaranteed blinding of researchers and participants. Subjects were randomised to groups A and B according to a scheme defined before starting inclusion and randomly assigned to a predefined test kit number. Subjects of group A had to start with capsules contained in the sealed bag ‘X’, and subjects of group B started with capsules contained in bag ‘Y’. For the second test, the capsules in the remaining bag (Y or X, respectively) had to be taken. Deblinding of test kits was done in a joint meeting of investigators (P. K., T. S.) and representatives of the producer of capsules.

Therefore, randomisation was performed on several levels. XI and placebo capsules were randomly assigned to sealed bags marked with ‘X’ or ‘Y’ for each test kit before
test kit preparation. Test kits were numbered consecutively. Subjects were randomly assigned to a numbered test kit and, independently, to group A and B. Assignment to groups A and B determined the order of use of bags ‘X’ and ‘Y’ for the first or second test respectively. All randomisation steps were performed according to lists provided by a statistical institute (Data collection, Statistics, Risk assessment; Austrian Agency for Health and Food Safety) before starting package preparation and oral provocations using the random sample SAS (9.2; SAS Inc., Cary, NC, USA).

Test procedure
Two tests were performed at an interval between 4 days and 21 days. One day prior to each visit, subjects were asked to consume a lactose- and fructose-restricted diet. All the subjects fasted overnight and were not allowed to smoke 12 h before the test. The fasting end expiratory breath H\textsubscript{2} concentration (termed 0 min) was measured using a Gastro+ Gastrolyser (Bedfont Scientific Ltd, Rochester, England) which has a sealed electrochemical sensor specific for H\textsubscript{2}. Immediately thereafter subjects had to take three capsules ‘X’ or ‘Y’ and then to ingest 25 g of fructose freshly dissolved in 100 mL of mineral water taken from one single production batch which was added to the plastic bottles which contained the fructose. Subjects were advised to drink the solution within 5 min. Thereafter, end expiratory breath H\textsubscript{2} concentration was recorded every 30 min for the first 2 h, then every 60 min for the next 2 h (for a total of 240 min) by a study physician (M. A.-M.). The second test was performed identically with the remaining capsules (‘Y’ or ‘X’).

Analysis of breath H\textsubscript{2} concentrations
After plotting the breath H\textsubscript{2} concentrations (in ppm) vs. time for the 4 h observation period the area under the curve (AUC) was calculated for each subject for both tests and expressed as ppm/240 min. Fasting breath H\textsubscript{2} curve (AUC) was calculated for each subject for both time for the 4 h observation period the area under the curve exceeding this baseline of 5 ppm, the AUC was calculated using a Gastro+ Gastrolyser (Bedfont Scientific Ltd, Rochester, England) which has a sealed electrochemical sensor specific for H\textsubscript{2}. Immediately thereafter subjects had to take three capsules ‘X’ or ‘Y’ and then to ingest 25 g of fructose freshly dissolved in 100 mL of mineral water taken from one single production batch which was added to the plastic bottles which contained the fructose. Subjects were advised to drink the solution within 5 min. Thereafter, end expiratory breath H\textsubscript{2} concentration was recorded every 30 min for the first 2 h, then every 60 min for the next 2 h (for a total of 240 min) by a study physician (M. A.-M.). The second test was performed identically with the remaining capsules (‘Y’ or ‘X’).

Symptom assessment
The symptoms bloating, nausea and abdominal pain were assessed individually before ingestion of the test solutions and at each time of breath hydrogen measurement by visual analogue scale covering a range of 0 (none) to 10 (most severe).

Statistics
Nonparametric methods were used for the inference statistical evaluation.

The regular distribution analysis was performed according to Kolmogorov-Smirnov and to Shapiro-Wilk. The Wilcoxon signed-rank test was used to compare the two related samples XI and placebo. A P value <0.05 was considered statistically significant.

The same statistical tests (Kolmogorov-Smirnov and Wilcoxon) were used for the VAS symptom scores for bloating, abdominal pain and nausea.

Data were analysed with SAS 9.2 and SPSS version 19 (SPSS Inc., Chicago, IL, USA).

RESULTS
Sixty-five patients (15 men and 50 women; mean age 43.3 (s.d. = 14.4), range: 21–73 years) participated in the study. No subject dropped out from the trial. One of 65 tested subjects did not complete the symptom questionnaire. In the current study, peak increases in end expiratory hydrogen concentrations after fructose did not exceed 20 ppm in 19 patients after placebo; after ingestion of XI, four of these patients had an increase in breath hydrogen concentration after fructose exceeding 20 ppm. On the other hand, XI decreased peak hydrogen excretion after fructose to less than 20 ppm in 16 patients who had an increase exceeding 20 ppm after placebo.

The median AUC after 25 g of fructose was 885 ppm/240 min (25th percentile 60, 75th percentile 4009) in the XI group, and 2071 ppm/240 min (25th percentile 487, 75th percentile 5898) in the placebo group (Table 1, Figure 1). This difference in AUC was statistically significant (Z-value = −3.585, two-sided asymptotic significance; \( P = 0.00 \)) in favour to the XI group.

There were marked inter-individual differences in scores for all three symptoms, covering the whole range of the VAS scale from 0 to 10. In general, bloating was scored to be the most bothersome symptom although its median score was only 0.8 (range: 0–8.2) in the placebo group and 0.2 (range: 0–10) in the XI group. This difference failed to reach statistical significance (\( Z = −1.935, \ P = 0.053 \)). Median scores for abdominal pain and nausea were significantly lower after XI as compared with placebo (\( Z = −2.626, \ P = 0.009 \) and \( Z = −2.81, \ P = 0.005 \) respectively) (Table 2, Figure 2).

DISCUSSION
Xylose isomerase (EC 5.3.1.5, CAS 9023-82-9), also referred to as glucose isomerase, is an enzyme that catalyses the chemical reaction \( \text{d-xylose} \rightleftharpoons \text{d-xylulose} \) and
fructose $\leftrightarrow$ glucose. It has been used since 1957 industrially to convert glucose to fructose in the manufacture of high fructose corn syrup and is one of the highest tonnage industrial enzymes. Glucose isomerase enzyme preparations are used in the production of high fructose corn syrup described in § 184.1866 of the FDA GRAS Notice Inventory. They are derived from recognised species of precisely classified nonpathogenic and nontoxicogenic microorganisms, including *Streptomycyes rubiginosus*, *Actinoplanes missouriensis*, *Streptomycyes olivaceus*, *Streptomycyes olivochromogenes* and *Bacillus coagulans*, that have been grown in a pure culture fermentation that produces no antibiotics.

As XI is also able to convert poorly absorbable fructose to well-absorbable glucose *in vitro*, we hypothesised that this enzyme could be used for conversion of excess fructose to glucose in the human small intestine *in vivo*.

To test this hypothesis, capsules containing enteric coated micropellets of XI were manufactured. This capsule design has been used in the past for protecting drugs from the acidic environment of the stomach, allowing gastric emptying of micropellets with a size between 2 mm and 3 mm through the pylorus with food and quick release of the enzymes in the neutral environment of the duodenum. Ingested watery solutions of poorly absorbable carbohydrates start reaching the cecum after an oro-cecal transit time of $60 \pm 7$ min [mean $\pm$ standard deviation (s.d.)] in symptomatic and $96 \pm 16$ min (mean $\pm$ s.d.) in asymptomatic persons; during this time the enzyme presumably would catalyse the isomerisation of fructose to glucose, which is actively absorbed by the intestinal mucosa via the SGLT-1 transporter. We hypothesised that due to active absorption of glucose, the equilibrium of the isomerisation process would be pushed towards glucose. As a consequence, less fructose would enter the colon and be metabolised by colonic bacteria, resulting in less hydrogen production, and possibly also reduced symptoms related to incompletely absorbed fructose.

To prove this hypothesis, we studied the effect of XI on breath hydrogen excretion after ingestion of fructose. Our study group consisted of patients who had presented for the evaluation of abdominal symptoms and in whom fructose malabsorption was established within the previous 12 months by the use of a fructose hydrogen breath test. It has been demonstrated in the past, that this group may include patients with functional gastrointestinal disorders who are more likely to develop symptoms at lower doses of ingested fructose, possibly representing visceral hypersensitivity which has been demonstrated in patients with IBS.

Although we had originally planned to repeat the fructose hydrogen breath test immediately before start of the study, this repeat procedure was declined by the ethics committee of our university, to spare patients of the symptoms associated with an oral fructose load. Unexpectedly, it turned out that in the course of the study in 29% of patients the increase in end expiratory hydrogen concentration was less than 20 ppm. This
suggests a considerable intraindividual variability of breath hydrogen excretion, which needs future attention in trials using breath hydrogen test. In this context, it has to be kept in mind, that the cut off value of 20 ppm which is commonly used clinically to establish malabsorption is only one point on the hydrogen excretion curve, plotted as hydrogen excretion over time. Considerably more information on colonic gas production and excretion can be gained from cumulative flatus collections or AUC. In the current study, AUC was used to assess colonic hydrogen accumulation over the 240 min observation period.

In those patients who did not exceed the cut off level of 20 ppm, there was still a demonstrable increase in the breath hydrogen over the baseline, indicating excess hydrogen production due to fructose malabsorption. The dose of 25 g fructose in our study is on the lower end of the dose range which has been used by different authors. At this dose, patients with functional gastrointestinal disorders are more likely to develop symptoms than normal subjects. This is relevant for the potential clinical application of our study results, because if a drug treatment would be considered in fructose malabsorption, this should be limited to patients who develop symptoms, regardless whether or not symptoms may be aggravated by an underlying visceral hypersensitivity. Since pharmacological treatment options for functional gastrointestinal disorders are very limited detection of small amounts of fructose being malabsorbed has a clinical relevance, directing attention at excluding even small amounts of fructose from the diet.

In this study, oral supplementation of XI revealed a statistically significant decrease in breath hydrogen excretion over a 4 h period after ingestion of fructose, as compared with placebo. Decrease in the area under the breath hydrogen curve after XI suggests that XI catalysed the isomerisation of fructose which resulted in a lower amount of fructose reaching the colon. It is reasonable to end the measurement period at 4 h since breath hydrogen excretion after ingestion of poorly absorbable carbohydrates reaches its peak before that time. In a previous study on lactose malabsorption, hydrogen peak occurred at 160 ± 16 min (mean ± s.d.), regardless whether patients were symptomatic or not, and in a study with cumulative measurement of anal hydrogen excretion, cumulative hydrogen excretion curves flattened after 210–240 min in the majority of subjects.

A surprising finding in our study was, that, although patients had presented in the preceding year for evaluation

![Table 2](image_url)
of abdominal symptoms, indicating that they were bothered by their symptoms enough to warrant diagnostic evaluation at a tertiary care centre, only few patients had high scores on the VAS assessment of symptom severity during this study. This may indicate a large intra-individual variability in the severity of symptom scores presumably associated with incomplete fructose absorption. Although median symptom scores therefore were quite low in our study, scores for nausea and abdominal pain were significantly improved by XI. There was no significant effect on bloating after administration of XI; however, it has been shown in the past that reproducibility of symptoms associated with large bowel dilatation is higher with better characterised symptom of pain as compared with the less specific symptom of bloating.38

In summary, our proof of concept study has demonstrated that in patients with incomplete fructose absorption, oral administration of xylose isomerase can reduce breath hydrogen excretion in vivo and reduce symptoms associated with fructose malabsorption.

Future studies will have to demonstrate whether XI is effective with carbohydrate mixtures which are present in fructose containing food, and to assess long-term effects and safety and to identify patients who most benefit from the treatment. The presence of fructose malabsorption in patients with functional gut symptoms may represent not only an opportunity for dietary manipulation, as stated by Barrett et al.,17 but for additional application of XI to assist in the amelioration of such symptoms.

Isomerisation of fructose by XI may also be useful to avoid high fructose uptake into the body. Further studies are required to confirm this hypothesis.

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