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ORIGINAL ARTICLE

Beneficial effects of oral tilactase on patients with hypolactasia

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ABSTRACT

Background A lactose-free diet is commonly prescribed to subjects with hypolactasia. We tested the effectiveness of a single ingestion of tilactase (a β-D-galactosidase from *Aspergillus oryzae*) in adults with hypolactasia, previously assessed by lactose H_2-breath test.

Materials and methods After measurement of orocecal transit time (OCTT, by lactulose H_2-breath test) and lactose H_2-breath testing plus placebo, a total of 134 subjects were positive to hypolactasia and underwent lactose H_2-breath testing plus either low (6750 U) or standard (11 250 U) doses of tilactase. The appearance of gastrointestinal symptoms during the tests was monitored.

Results OCTT was longer in malabsorbers (subjects without bloating, abdominal pain and/or diarrhoea, $n = 25$) than in intolerants (bloating, abdominal pain and/or diarrhoea, $n = 109$, $P < 0.02$). Malabsorbers had longer time to H_2 peak ($P < 0.03$), lower H_2 peak levels ($P < 0.002$) and smaller integrated H_2 excretion levels ($P < 0.005$) than intolerants. After tilactase ingestion, integrated H_2 levels were decreased by 75% (low dose) and 87% (standard dose) in malabsorbers, and by 74% (low dose) and 88% (standard dose) in intolerants. In the latter group, total symptom score were decreased by 76% (low dose) and by 88% (standard dose) ($P < 0.0001$).

Conclusion A single oral administration of tilactase is highly effective in decreasing symptoms and hydrogen excretion of hypolactasia assessed by lactose H_2-breath test. If confirmed by long-term observations, ingestion of tilactase might be a better option than exclusion diets in intolerant subjects with hypolactasia.

Keywords Breath test, lactase, milk.

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Introduction

Primary hypolactasia is a genetically determined deficiency of the small intestinal brush border enzyme lactase [1–3]. The prevalence of hypolactasia is low in northern Europe (about 2% in Scandinavia), but it can approach 100% in South-East Asian populations [4,5]. About 50 million Americans malabsorb lactose [6] and in Italy the prevalence might be as high as 40–70% [7–10]. The prevalence of lactose malabsorption increases with age [11]. Although adults with hypolactasia can usually eat limited amounts of lactose-containing food without considerable symptoms ('malabsorbers'), about 50% of patients experience symptoms ('intolerants') [12] including bloating, abdominal pain, diarrhoea [4,13,14], with a considerable impact on the quality of life.

Furthermore, since exclusion diet for dairy products and milk is frequently recommended in subjects with lactose intolerance [15], early diagnosis and treatment of hypolactasia might prevent secondary osteopenia and osteoporosis, a risk for bone fractures, induced by a reduced oral intake of calcium and phosphorus [16–18]. Despite jejunal biopsy still being considered the standard for diagnosis of hypolactasia, the H_2-breath test is a valid clinical opportunity [13,19–22]. Indeed, the H_2-breath test has optimal correlation with positive genotyping [2,3,23,24] and helps in subjects with negative genotyping but likely to have a secondary cause of hypolactasia [23].

An exclusion diet is frequently recommended to subjects suspected for lactose intolerance, often without an established diagnosis [15]. Alternative therapeutic approaches may include loperamide (longer contact of intestinal enzyme with substrate) [25,26], yoghurt and β-galactosidases producing probiotics [17,27]. Most promising is the use of synthetic lactase analogues.

Previous studies were conducted, in small, not controlled settings, adding soluble enzymes to milk some hours before its consumption, thus obtaining a pre-incubated milk [27–31]. This therapeutic option is impractical due to the necessity of adding the enzyme some hours before meal consumption.

β-Galactosidase obtained from *Kluyveromyces lactis* improved symptoms in intolerants when added to milk [8]. Recently, it has

become commercially available tilactase, a β-D-galactosidase from *Aspergillus oryzae* (Tilactase) as chewable tablets to be taken before a lactose-containing meal. This procedure makes pre-incubation in milk unnecessary and intolerant subjects have the possibility to eat not only milk, which is the limitation for other forms of lactases, but also dairy products.

In this study, we tested the effectiveness of orally administered tilactase on gastrointestinal symptoms and breath H_2 excretion in a large group of adults with hypolactasia. Since the efficacy of different doses of tilactase has not been properly tested in a clinical setting, we compared low and standard doses of tilactase.

Materials and methods

Subjects and setting

Enrolled were 382 consecutive outpatient individuals referred to the Department of Internal Medicine at the University Medical School of Bari. Individuals had persisting symptoms (longer than 6 months) suggestive of hypolactasia, small intestinal bacterial overgrowth (SIBO) and/or functional gastrointestinal disorders (i.e. irritable bowel, functional dyspepsia). Subjects with alarm symptoms (e.g. anaemia, weight loss, chronic pain, ineffective therapies) or older than 50 years underwent further investigations (endoscopy plus standard intestinal biopsies when required, X-ray of the large intestine by barium enema, blood tests) [32]. Exclusion criteria were: lack of compliance, history of liver, renal, lung, heart, metabolic, or neurological diseases, treatments with laxatives, antibiotics, prokinetics, or any other drug known to influence colonic flora or gastrointestinal motility in the month preceding the study. All of the subjects gave their written informed consent to the study protocol, which was approved by the Human Subjects Committees at the University Medical School in Bari.

Experimental protocol

The experimental protocol is summarized in Fig. 1. Excluded from the study were 207 patients (mainly because of organic gastrointestinal diseases). Subjects suspected for hypolactasia ($n = 175$, no evidence of organic diseases), underwent the lactulose H_2-breath test to confirm that they were H_2 producer and to exclude the presence of SIBO, a potential confounding factor [33]. Thus, 10 patients were excluded because of no H_2 production ($n = 3$) or SIBO ($n = 7$). If H_2 production was confirmed and SIBO was excluded, the orocecal transit time (OCTT) was calculated. The other 165 subjects underwent the first lactose H_2-breath test with the administration of placebo. This test resulted positive for 134 patients. The remaining 31 lactose breath test-negative subjects underwent a fructose H_2-breath test and had a final diagnosis of either fructose malabsorption or irritable bowel syndrome.

The study comprised the effect of tilactase on H_2-breath levels and symptom severity in patients randomly distributed in the following subgroups: (i) a single low dose (three tablets for a total of 6750 U) in 72 subjects (17 malabsorbers and 55 intolerants); and (ii) a single standard dose (five tablets for a total of 11 250 U) in 43 subjects (6 malabsorbers and 37 intolerants). Both tests were performed in another 19 subjects (2 malabsorbers and 17 intolerants). Thus, a total of 91 subjects (19 malabsorbers and 72 intolerants) received the low-dose of tilactase, while a total of 62 subjects (8 malabsorbers and 54 intolerants) received the standard-dose of tilactase.

Either placebo or tilactase were administered immediately before milk ingestion. Tilactase contained β-D-galactosidase from *A. oryzae* (Lacdigest®, Italchimici, Pomezia, Italy). Each tablet contains 2250 U. According to the definition of lactase unit, one enzymatic unit is defined as the amount of the enzyme that can release 1 μmole of ONP (O-nitrophenol) at the conditions set by Extract Chemie (EC) or by Food Chemical Codex (FCC). The equivalence is defined: 1 FCC unit = 1·46 EC unit. Lacdigest enzymatic activity is indicated as EC units. As underscored by the manufacturer, one tablet is capable of hydrolysing 5 g of lactose, which is the amount contained in 100 mL of cow's milk. Thus, three tablets (equal to 6750 U) should be sufficient to hydrolyse about 66% of the lactose contained in 500 mL of cow's milk, while five tablets should be sufficient to hydrolyse 100% of the total lactose content.

The primary end-points of the study were to test the effect of low and standard doses of oral tilactase on H_2-breath levels and in preventing symptoms in patients with hypolactasia. The secondary end-point was to test the safety and tolerability of oral tilactase substitution in the clinical setting.

Breath testing

The day before each breath test subjects underwent a 12-h carbohydrate-free diet [34,35], followed by a 12-h fasting period. Before starting the test, subjects underwent a through mouth wash with 50 mL of a 1% chlorexidine solution to prevent oro-pharingeal fermentation of sugars by local microflora. Smoking and physical exercise were not allowed 2 h before and during breath samplings [36–38], as well as eating. Subjects collected alveolar air, avoiding deep inspiration or hyperventilation before exhalation. Breath samples following the ingestion of each substrate were analysed at baseline in duplicate and at each time point by a portable, previously validated device (LactoFAN®, Lans Medical BV, the Netherlands). The accuracy of the detector was ±2 p.p.m., with a resolution of 1 p.p.m. and a range of 0 to 500 p.p.m. Each breath test was performed at least 72 h apart to avoid the effect of colonic acidification [8].

Lactulose H_2-breath test was first performed to detect significant H_2 excretion, to calculate OCTT and to exclude SIBO. The test consisted of two baseline measurements followed by the ingestion of 10 g lactulose in 100 mL tap water. Breath samples were collected every 10 min up to a maximum of 360 min, hydrogen concentrations were analysed and OCTT established as previously

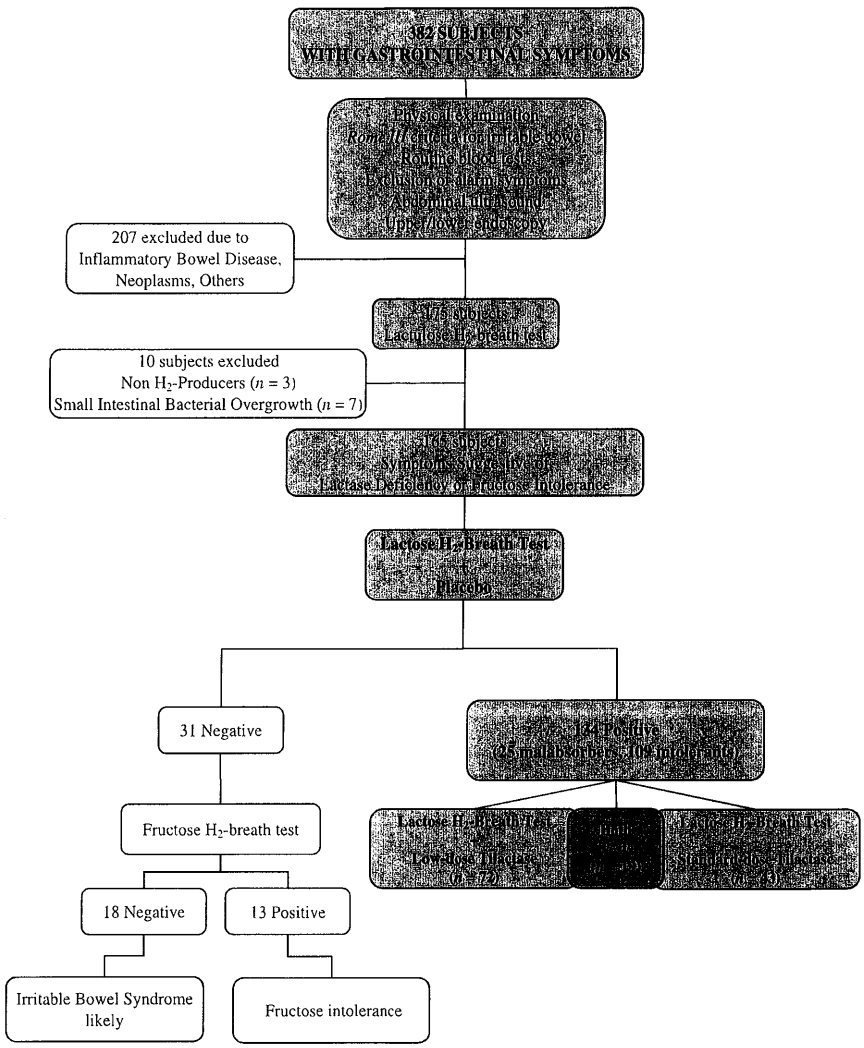

Figure 1 Flow chart used for patient screening.

described [39–41]. The presence of SIBO was defined according to recent criteria [42].

Lactose H_2-breath test was performed by administering 500 mL of cow's whole milk (25 g of lactose). Breath hydrogen test measures lactose non-absorption. It is simple to perform, non-invasive and has a sensitivity and specificity that are superior to the absorption test [43]. Air samples were collected every 30 min from 0 to 240 min and at 360 min. The test was considered positive (lactose malabsorption) if H_2 concentrations were at least 20 p.p.m. above baseline [8,13,34]. H_2 peak levels (p.p.m.), time to H_2 peak (min), and area under the curve of H_2 levels during the observation time (AUC, H_2 p.p.m. × 360 min) were calculated [44,45].

Symptoms

During each lactose breath test, subjects were recorded for the presence and severity of bloating, abdominal pain, and bowel movements by a previously validated visual analogue scale (VAS) [46,47], at the same time points of lactose breath test. VAS score-time related curves for each symptom were constructed at the end of each test. The AUC of each symptom was also calculated [44,45].

A semiquantitative analysis of bowel movements was performed (0 = no evacuations; 1000 = one evacuation; 2000 = two or three evacuations; 3000 = more than three evacuations). A total symptom score was constructed in each patient as the sum of the integrated VAS (AUCs × 360 min) of bloating and abdominal pain plus the score for bowel movements.

Data analysis

Data are given as mean ± standard error of the mean (SEM). Time-related changes of continuous variables were analysed using analysis of variance (ANOVA) repeated measures followed by post-hoc tests for multiple comparisons. Differences of two groups

were evaluated by the Mann–Whitney *U*-test or by Student's *t*-test, where appropriate. Correlations were assessed by calculating Spearman's r_s. The chi-squared test was used to compare categorical variables and proportions. A two-tailed probability (*P*) value of less than 0·05 was considered statistically significant [48,49]. Calculations were performed with the NCSS2007 software (Hintze J. Kaysville, UT, USA, http://www.ncss.com).

Results

Among 165 H_2-producer subjects, 134 (81·2%) had a positive lactose breath test according to the established criteria [8,13,34] (91 females and 43 males, mean age 37 ± 1 (SEM) years, range 14–81). Twenty-five subjects (19%) were lactose malabsorbers (mean age 36 ± 4 years, 10 males, 15 females) and 109 (81%) were lactose intolerants (38 ± 1 years, 33 males, 76 females). Groups did not differ for age or gender.

Lactulose breath test for OCTT

In the 134 subjects with hypolactasia, OCTT was 120·6 ± 4·1 min, with slightly longer values in females than males (125·3 ± 5·3 min and 110·7 ± 6·3 min, respectively, P = NS). OCTT was shorter in subjects with intolerance than in those with malabsorption (116·0 ± 4·1 min vs. 140·8 ± 12·3 min, $P < 0·02$). Both malabsorbers and intolerant subjects exhibited an OCTT significantly longer than that observed in a large historical control group of 142 healthy subjects matched for sex and age (99·5 ± 1·6 min, $P < 0·0001$, ANOVA) [39,40].

Lactose breath test

Subjects with lactose malabsorption had longer time to reach the cut-off value of 20 p.p.m. (179 ± 16 min vs. 135 ± 6 min, $P < 0·03$), longer time to reach the H_2 peak (228 ± 14 min vs. 200 ± 5 min, $P < 0·03$), lower H_2 peak levels (45·7 ± 4·5 p.p.m. vs. 72·4 ± 3·8 p.p.m., $P < 0·002$), and smaller integrated H_2 excretion levels (AUC: 8202 ± 970 vs. 13836 ± 725 p.p.m. × 360 min, $P < 0·005$) than subjects with lactose intolerance.

Effect of tilactase on breath hydrogen excretion following lactose breath test

Baseline levels of excreted H_2 during lactose/placebo and lactose/tilactase breath test were similar (1·51 ± 0·2 p.p.m. and 1·32 ± 0·2 p.p.m., respectively, P = NS). Neither the low nor the standard doses of tilactase ingestion caused side effects, as confirmed by a careful follow up during the test and up to 24 h after the lactose breath test (phone interview).

In the whole study group of 134 subjects the low dose of tilactase produced a consistent decrease (75%) of integrated H_2 levels (12 785 ± 644 p.p.m. × 360 min) compared to placebo (3154 ± 331 p.p.m. × 360 min, $P < 0·0000002$). The standard dose produced a further decrement (–12%) of integrated H_2 levels (1636 ± 229 p.p.m. × 360 min, $P < 0·0000002$ vs. placebo).

The net effect of different doses of tilactase was evident in both malabsorbers and intolerants (Fig. 2). Either doses of tilactase were followed by a significant decrement of time-dependent changes of H_2 excretion and AUCs.

Integrated H_2 levels decreased by 75% (low dose) and by 87% (standard dose) in lactose malabsorbers and by 74% (low dose) and 88% (standard dose) in subjects with lactose intolerance (P = NS, low vs. standard dose in both subgroups).

A similar trend existed for the intolerant subgroup of 17 subjects undergoing lactose breath test with both doses (low dose tilactase 2180 ± 441 p.p.m. vs. high dose tilactase 1862 ± 488 p.p.m. × 360 min; P = NS).

Effect of tilactase on symptoms in intolerant subjects studied by the lactose breath test

With placebo, the total symptom score in intolerant subjects was 13 301 ± 953, while integrated AUCs for abdominal bloating and abdominal pain were 7400 ± 639 and 5470 ± 953 mm × 360 min, respectively. Tilactase ingestion (both doses) resulted in a significant decrement in symptom score (Fig. 3), with total symptom score decreasing by 76% (to 3193 ± 879) with the low dose and by 88% (to 1318 ± 396) with the standard dose of tilactase ($P < 0·0001$ vs. placebo). Twenty-six subjects (24%) had one (n = 9 subjects) or more (n = 17 subjects) bowel movements with the lactose/placebo breath test. This symptom persisted in one subject after low dose and one subject after standard dose tilactase ($P < 0·05$).

Correlations between symptoms and different markers of H_2 production in intolerant subjects undergoing lactose/placebo breath test are depicted in Fig. 4. There were positive and statistically significant correlations ($0·0001 < P < 0·01$) between integrated AUC for bloating and total symptom score with H_2 peaks and integrated H_2. By contrast, AUC for bloating and total symptom score decreased at increasing values of time to H_2 peak. As expected, correlations disappeared completely with tilactase, due to the remarkable decrease of symptoms (data not shown).

Discussion

In a large group of adults with hypolactasia, the present placebo-controlled study clearly shows that a novel formulation of tilactase is highly effective also at low dose in decreasing breath H_2 excretion (an objective marker of lactose malabsorption) following the ingestion of lactose-containing milk. Furthermore, tilactase ingestion effectively decreases specific symptoms in lactose-intolerant subjects without causing side effects.

Despite the widespread diffusion of hypolactasia [4–10], clear guidelines on how to manage lactose malabsorption in adults are still unclear [15,50]. Hardly acceptable and potentially dangerous deprivation diet [16,17] is currently suggested to

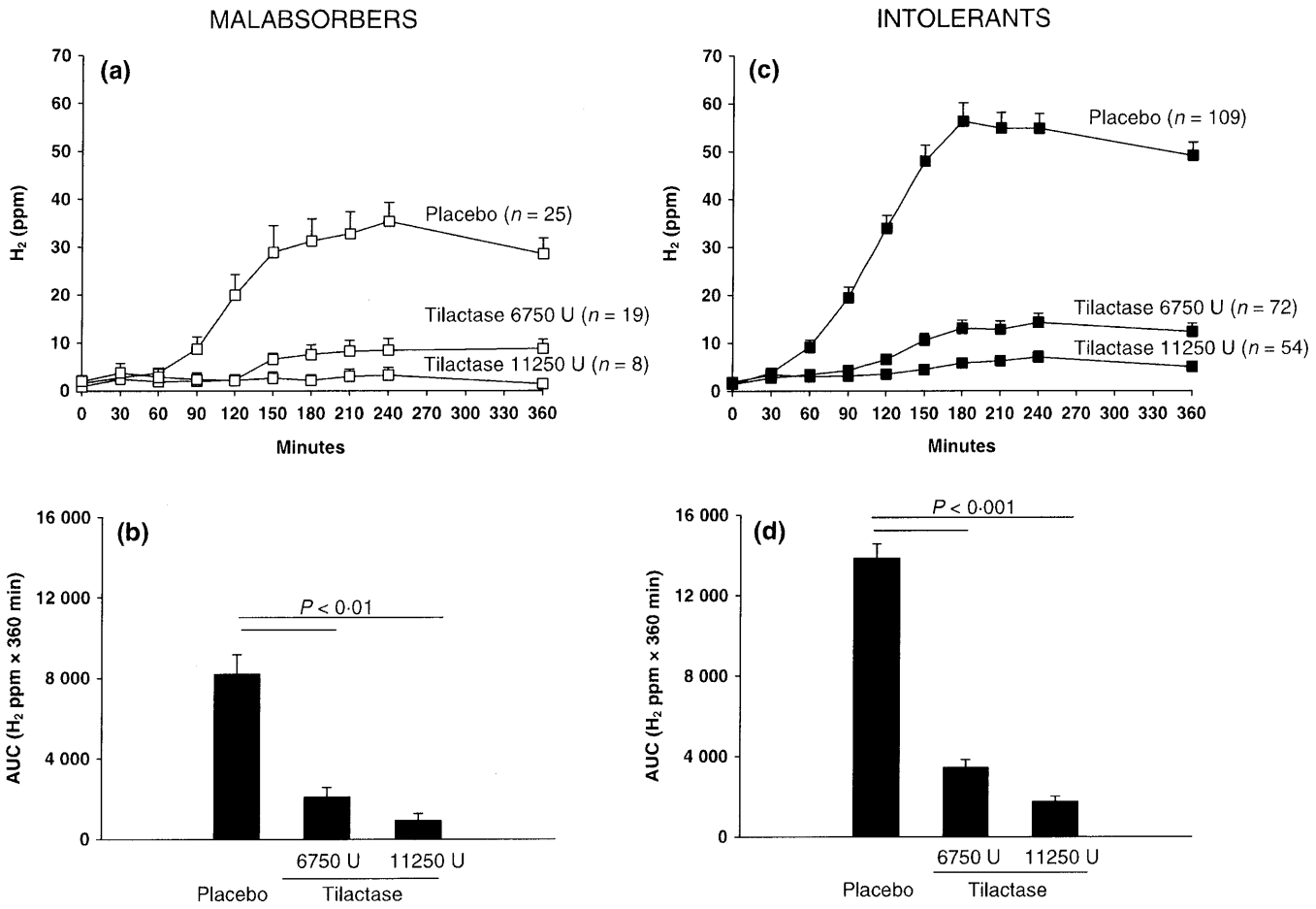

Figure 2 Effect of tilactase on expired H_2 in subjects with hypolactasia according to lactose malabsorption (no symptoms) or intolerance (symptoms). Graphs are shown as time-dependent changes of H_2 in breath (a,c) and as integrated area under curve (AUC) (b,d) following the ingestion of 500 mL of cow's whole milk in subjects treated with placebo and low dose (6750 U) or standard dose (11 250 U) of tilactase. Symbols and bars indicate means while vertical lines indicate standard error of the mean. Malabsorbers: (a) significant ($P < 0{\cdot}01$) decrease of H_2 levels between 90 and 360 min with low-dose tilactase and between 180 and 360 min after standard-dose tilactase. (b) Significant decrease of integrated AUC with the low and the standard doses of tilactase, compared with placebo. A trend existed also between either doses of tilactase. Intolerants: (c) a significant ($P < 0{\cdot}01$) decrease of H_2 levels existed between 60 and 360 min in subjects treated with the low and the standard doses of tilactase. (d) Significant decrease of integrated AUC with low and standard doses of tilactase, compared with placebo. A trend existed also between either doses of tilactase.

patients [15] since large and conclusive placebo-controlled studies on supplemental therapies with oral enzymes in adults are lacking.

In previous studies, milk was pre-hydrolysed by β-galactosidase obtained by *K. lactis* [8] and the effect on maximum H_2 concentration and cumulative H_2 excretion was better than that from *Aspergillus niger* [17,51]. β-D-galactosidase from *A. oryzae* was also effective in children when added to milk [52]. Variable results, however, might depend not only on different enzyme preparations but also on timing in adding the enzymes to the milk (e.g. mealtime or hours before the meal) as well as on the different doses employed [17,19,51,53].

Oral β-D-galactosidase from the fungus *A. oryzae* (tilactase) has been successfully tested in early small studies [54,55]. The recent introduction on the Italian market of tilactase allowed us to perform the first experimental study in a large clinical setting.

The lactose H_2-breath test is currently considered clinically useful in the diagnosis of lactase non-persistence (i.e. malabsorption). According to previous criteria we found a

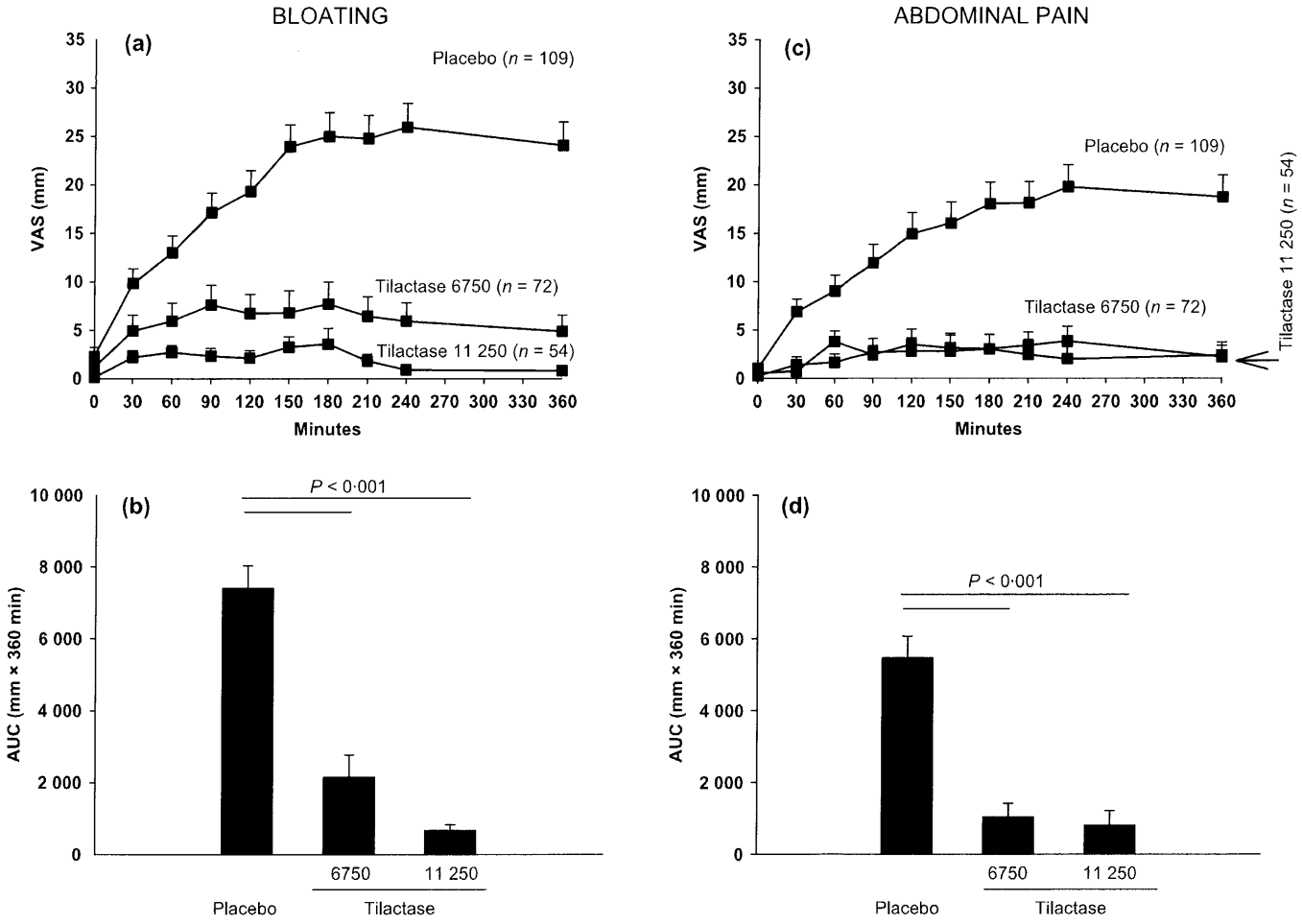

Figure 3 Effect of tilactase on gastrointestinal symptoms in subjects with lactose malabsorption (no symptoms) or intolerance (symptoms). Graphs are shown as time-dependent changes of VAS for bloating and abdominal pain (a,c) and as integrated area under curve (AUC) (b,d) following the ingestion of 500 mL of unskimmed cow's milk in subjects treated with placebo and low dose or standard dose of tilactase. Symbols and bars indicate means while vertical lines indicate standard error of the mean. Bloating: (a) significant ($P < 0{\cdot}01$) decrease of H_2 levels between 30 and 360 min. with low and standard dose tilactase. (b) Significant decrease of integrated AUC with low and standard doses of tilactase, compared with placebo. A trend existed also between either doses of tilactase. Abdominal pain: (c) a significant ($P < 0{\cdot}05$) decrease of H_2 levels between 30 and 360 min in subjects treated with low and standard doses of tilactase. (d) Significant decrease of integrated AUC with low and standard doses of tilactase, compared with placebo.

prevalence of lactose breath test-positive subjects of 77·3% [8,13,34]. New criteria (uncommon in clinical practice) involving a single time-point measurement of H_2 p.p.m. (6th hour, > 6 p.p.m.) or a cumulative value (at the 5th, 6th, and 7th hours, > 15 H_2 p.p.m.) have been also proposed to increase sensitivity [34]. However, we found no gain in our series with the first option, i.e. only one additional 'negative' subject would have become positive to lactose breath test (prevalence 78·1%). Both figures are close to those reported by Di Stefano *et al.* using 20 g of lactose (400 mL milk) instead of 25 g of lactose (500 mL milk) [34]. Thus, our regimen seems appropriate in sensitivity and specificity, at least in this series. With the second option, most subjects would have refused a 7-h breath test, and this was the reason why this criterion was not chosen in the present study.

Since the status of intolerance remains unchanged in most individuals [56], performing two sequential lactose breath tests – the second with tilactase and invariably blind to the

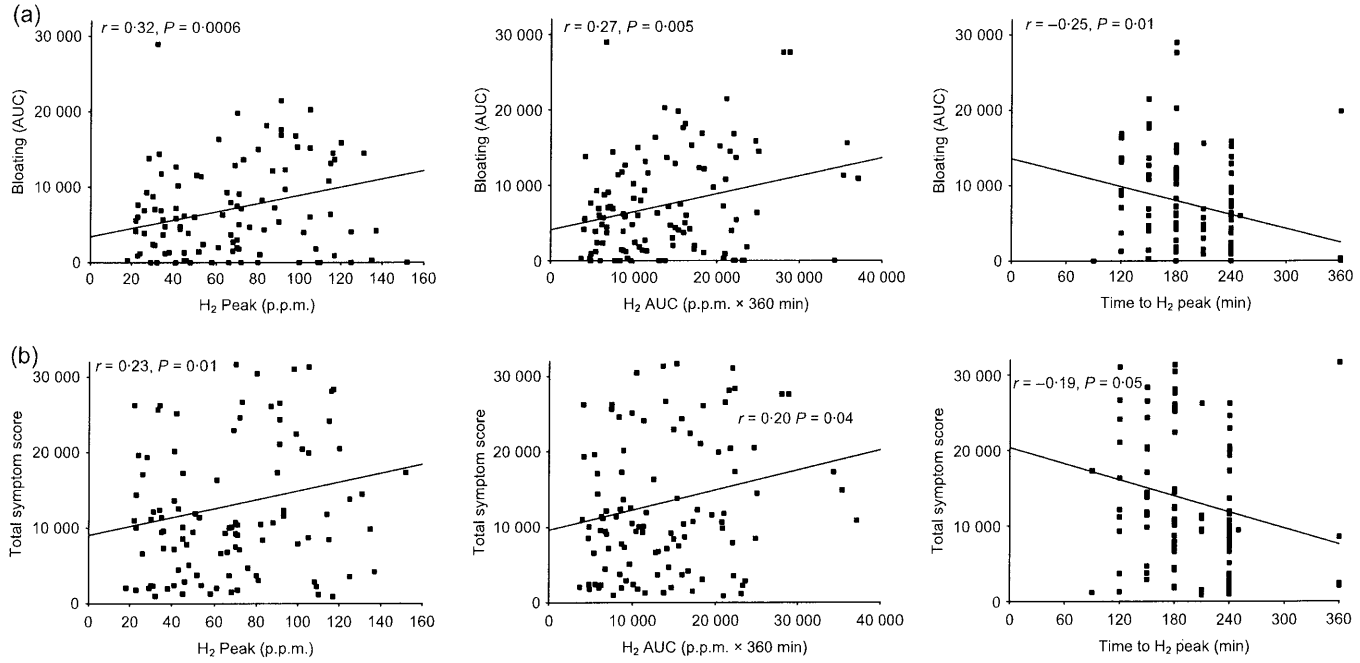

Figure 4 Correlations between symptoms and gastrointestinal symptoms and markers of H_2 excretion in intolerant subjects (n = 109). (a) Integrated score for bloating positively correlated with H_2 peak and integrated H_2 excretion and negatively correlated with time to reach maximum H_2 peak; (b) Total symptom score (including also bowel movements, see Methods) was positively correlated with H_2 peak and integrated H_2 excretion and negatively correlated with time to reach maximum H_2 peak.

subject tested – is likely to reflect the true change in symptom score upon enzyme ingestion.

Gastrointestinal symptoms potentially due to hypolactasia can be misleading and mimic the symptoms of other conditions (i.e. irritable bowel syndrome [57], intestinal neoplasms, SIBO, coeliac disease and inflammatory bowel diseases). Thus, a definitive diagnosis is essential before attributing symptoms to lactose intolerance. An *a priori* chronic milk-exclusion diets in the absence of a diagnosis, in fact, may have a negative impact on everyday life.

The diagnosis might become even easier if one considers that affordable, portable, compact, easy-to-use equipment have recently become available [56,58].

The prevalence of hypolactasia can be as high as 40–70% in southern Italy [7–10], and the presence of symptoms that affect quality of life has been reported in about 50% of this population [4,13], although the prevalence can be highly variable due to geographical and genetic differences and differences in diagnostic approach [59–61].

In this study, more than 80% of subjects developed symptoms following ingestion of 500 mL of milk, even if less than one-third had bowel movements, possibly the most cumbersome symptom. This trend has also been described in a Chinese population with genetically determined hypolactasia [58]. Oral tilactase might be valuable in a geographical area with high prevalence of hypolactasia, and possibly more practical than pre-hydrolysed milk whose taste might be altered with exogenous liquid lactase [27].

Optimal dosage of tilactase should be tailored according to the overall amount of ingested lactose. Although either dose of oral tilactase was highly effective in both malabsorbers and intolerants challenged to 25 g of lactose, our findings point to a role for lactase enzymes in symptomatic subjects.

The finding in lactose malabsorption (i.e. only elevated H_2-breath excretion) does not support, in our opinion, the use of tilactase unless symptoms suggestive of intolerance will appear.

Apparently, the problem of decreased efficacy of oral tilactase due to potential gastric inactivation of the enzyme was not encountered in our study [27]. Thus, a standard tilactase dose may eventually be used in subjects with high H_2 production after ineffective low doses, when large quantities of lactose-containing products are eaten or when symptoms persist.

We found that OCTT was shorter in intolerant than in malabsorber subjects. This finding, together with the longer time to reach H_2 peak concentrations in malabsorbers, is in accordance with a previous study performed in a sample of a Chinese population with genetically determined hypolactasia, in

which both lactose digestion index and amount of digested lactose were calculated [58]. Others have reported a similar behaviour in asymptomatic malabsorbers [62–64]. In intolerant subjects, lactose might trigger a faster transit time due to a direct effect of lactose on factors regulating intestinal motility (i.e. osmoreceptor in the upper intestine and/or hormone release) [56,65]. It is likely that subjects with moderate hypolactasia digest lactose better because they have more intestinal lactases, but also because of longer exposure time of the disaccharide in the intestinal lumen. In addition, the presence of individual variability in intestinal motility patterns cannot be excluded [66]. Thus, the onset of symptoms in lactose-intolerant subjects might originate from several factors, including the amount of lactose ingested, the degree of hypolactasia, the speed of small intestinal transit and lactose processing by colonic microflora, gender, age and pregnancy [56,58,67]. Indeed, adding living bacteria in fresh yoghurt is associated to delayed OCTT and fewer symptoms in patients with lactose maldigestion due to improved bacterial lactose digestion [62].

It has been previously suggested that levels of breath H_2 excretion might reflect the degree of lactose digestion and influence the occurrence of symptoms following lactose ingestion [62,68]. In this respect, an additional finding of the present paper is the evidence of a strong association between H_2-breath levels and the intensity of gastrointestinal symptoms following lactose ingestion in intolerant patients. In fact, in our series, a significant positive correlation was found between the integrated H_2-breath levels and the total symptom score. Another study using genotyping of the lactase-phlorizin hydrolase –13 910 polymorphism showed that the CC genotype of the DNA variant –13 910 T/C upstream of the LCT gene is associated with lactase non-persistence [2]. This genetic condition is strongly correlated to a positive H_2-breath test [3], although in breath test-positive individuals with a negative genetic test a secondary causes of lactase deficiency should be investigated [23]. No correlation was found between severity of symptoms and the level of breath H_2 excretion by Di Stefano *et al.* [34]. Despite Vonk *et al.* found 'no difference in breath H_2 excretion between groups of tolerants and intolerants', H_2-breath levels tended to be higher in intolerants (see Table 1 of their manuscript [58]). Either different scores employed for symptoms (quantitative vs. semiquantitative) or differences in populations might account for such discrepant results. In accord with our findings, however, Vonk *et al.* [58] found that subjects with lactose malabsorption had longer time to reach the H_2 peak after lactose ingestion, implying a slow rate of colonic delivery of lactose. In this respect, we found that by adding tilactase, a significant decrement in H_2 excretion was evident in both malabsorbers and intolerant patients. In this latter group, we observed a virtually complete disappearance of symptoms, and this favours the relationship between H_2 levels and degree of lactose maldigestion or intolerance.

Several therapeutic approaches have been previously proposed for the treatment of symptomatic hypolactasia [9,25–27]. Although these approaches are helpful, they do not allow for dairy products ingestion, which are important constituents of the Mediterranean diet. Moreover, one should question about these approaches for practical use in regularly daily life of patients.

In this respect, our findings suggest a practical therapeutic approach to milk intolerance, which is also likely to prevent complications of reduced calcium intake, vitamin deficiency and to ensure a better quality of life [16,17,69].

In conclusion, our results find a strong association between H_2-breath levels and intensity of gastrointestinal symptoms following lactose ingestion in lactose-intolerant patients. Oral β-D-galactosidase from *A. oryzae* is highly effective in decreasing symptoms of hypolactasia and hydrogen excretion of lactose maldigestion in adult with hypolactasia, in the absence of side effects. Thus, oral tilactase is a valid therapeutic strategy in a subgroup of lactose-intolerant subjects.

Conflict of interest

Piero Portincasa has received speaker's fees and research funds from Italchimici. All other authors: none declared.

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Oral Tilactase Enzymes (β-D-galactosidase) from *Aspergillus oryzae* Effectively Decrease Breath Hydrogen Excretion and Gastrointestinal Symptoms in Lactose Malabsorption And Intolerance. Clinical Relevance In Southern Italy.

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Abbreviations: AUC, area under the curve; IBS, irritable bowel syndrome; LBT, lactose breath test; OCTT , orocecal transit time; ppm, parts per million; SIBO, small intestinal bacterial overgrowth

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STUDY HIGHLIGHTS

What Is Current Knowledge

- Lactose malabsorption is frequent in southern Europe and Italy
- Lactose intolerance generates symptoms which can mimic those of other gastrointestinal diseases
- Milk- and dairy products-free diets are commonly prescribed even without reaching a final diagnosis of lactose intolerance by lactose H_2 breath test.
- Calcium metabolism and bone mass can be negatively affected by exclusion diets in intolerant subjects.
- Oral lactase substitutes have been recently marketed in Italy and might be a valid therapeutic approach in lactose intolerant subjects.

What Is New Here

•This study is the first and the largest one to test the efficacy of two different regimens of oral tilactase enzymes (β-D-galactosidase obtained from fungus *Aspergillus oryzae*) in lactose malabsorbers and intolerants.
• The effect of tilactase enzymes was evaluated by using objective (H_2 breath excretion) and subjective tools (self-reported visual analogue scales of symptoms)
• Both low and standard doses of tilactase enzymes effectively reduced H_2 breath excretion and symptoms without side effects.
• Oral tilactase enzymes are effective and likely to have a positive impact on the quality of life and to hasten the effect on bone mass following long-term lactose-deprivation diets in lactose intolerant subjects.

ABSTRACT (word count: 239)

OBJECTIVE: Hypolactasia is frequent in southern Italy where consumption of milk and fresh dairy products is very popular. A strict lactose-free diet is commonly prescribed in "symptomatic" subjects, often without a final diagnosis of lactose intolerance. The effect of oral tilactase enzymes on gastrointestinal symptoms and breath hydrogen excretion were tested in adults with hypolactasia.

METHODS: Screening 137 subjects with "lactose intolerance" resulted in 106 subjects undergoing orocecal transit time (OCTT, lactulose H_2-breath test) and (single blind fashion), lactose H_2-breath test with placebo or β-D-galactosidase from *Aspergillus oryzae* (Lacdigest) with low (6750 u) or standard (11250 u) dose, to monitor the effect on the 6-hours H_2 breath excretion and gastrointestinal symptoms.

RESULTS: OCTT was longer in malabsorbers (n=20) than intolerant (n=86) subjects (151.0±13.1 min vs. 114.6±4.5 min, $P<0.002$). Malabsorbers had longer time to reach the H_2 peak, lower H_2 peak levels, and smaller integrated H_2 excretion levels ($0.001<P<0.02$) than intolerant subjects. With tilactase enzymes, integrated H_2 levels were decreased by 73% (low dose) and 85% (standard dose) in malabsorbers, by 75% (low dose) and 86% (standard dose) in intolerants. In the latter group total symptom score decreased by 77% (low dose) and by 99% (standard dose) ($P<0.0001$).

CONCLUSIONS: Administration of oral tilactase enzymes administration is safe and highly effective in decreasing the subjective and objective effects of lactose malabsorbition and intolerance in adults with hypolactasia. Oral substitution with low or standard dose lactase enzymes should be considered instead of exclusion diets or different enzymatic strategies.

INTRODUCTION

Primary lactose malabsorption (hypolactasia) is a genetically determined deficiency of the small intestinal brush border enzyme lactase (a glycoside hydrolase of the β-galactosidase family of enzymes,) which results into deficient cleavage of the disaccharide lactose into the two absorbable monosaccharides, glucose and galactose (1-3).
While the frequency of hypolactasia is low in northern Europe (about 2% in Scandinavia), it can approach 100% in Southeast Asian populations (4, 5). About 50 million Americans malabsorb lactose (6) and in Italy the prevalence might be as high as 40-70% (7-10). High concentrations of lactase are physiologically present in first months of life (11), but most adults have decreased lactase activity after weaning while others, especially in populations where fresh milk forms a significant part of adult diet (for example in northern Europe), have persistent lactase activity probably due to phenotypic selection. The prevalence of lactose malabsorption increases with age, and moreover, the prevalence of intolerance symptoms in malabsorbers decreases (12). Accordingly, daily calcium intake was similar among the adults and elderly studied. Although adults with lactase deficiency can usually eat limited

amounts of fresh milk or lactose-containing food without experiencing symptoms (the so called "malabsorbers"), it is estimated that about 50% of patients worldwide may experience gastrointestinal symptoms (the so called "intolerants") (13) including bloating, abdominal pain and/or diarrhoea (4, 14, 15). Symptoms may have a considerable impact on the quality of life but, most important, may also mimic highly prevalent gastrointestinal diseases, either functional or organic.

Precise and objective diagnosis of lactose intolerance, therefore, might prevent osteopenia and osteoporosis (16, 17). The diagnosis of lactase deficiency is commonly achieved by measuring increase of breath H_2 levels following administration of lactose-containing solution or simply whole milk: this test is currently the clinical standard (14, 18-20), although genotyping might soon become available (2, 3).

An exclusion diet for dairy products and milk is frequently recommended in subjects in which lactose intolerance. In some cases a lactose-free diet is even initiated when the diagnosis of lactose intolerance is uncertain or is only based on clinical suspicion. This diet, however, may result in a reduced oral intake of calcium and phosphorus. This has been shown in lactose intolerant subjects who have inadequate bone mass peak and increased risk of osteoporosis (21). Other therapeutical approaches for lactose intolerance may include administration of loperamide (to allow longer contact of intestinal enzyme with substrate) (22, 23), yoghurt and β-galactosidases producing probiotics (17, 24). Most promising and practical is the use of synthetic lactase analogues; β-galactosidase obtained from *Kluyveromyces lactis* improved symptoms in intolerants when added to milk before ingestion (8). Recently, oral enzymes (tilactase) have become available in the Italian market to be taken as chewable tablets (c.t.) before a lactose-containing meal. In principle, the use of oral tilactase enzymes has few advantages: it allows to eat a series of dairy products, for example, and it does not need preincubation. Especially, using oral enzymes can overcome several practical problems that have negative impact on the overall compliance of patients with lactose intolerance.

Therefore in this study was sought to test the effect of orally administered tilactase enzymes on gastrointestinal symptoms and breath H_2 excretion in a large group of adult subjects with hypolactasia. Since the efficacy of different doses of tilactase enzymes have not been properly tested in a clinical setting, a low and standard dose of tilactase enzymes was compared in this study. We show here that oral tilactase enzymes are highly effective in reducing symptoms and H_2 breath excretion in subjects with lactose malabsorption and intolerance.

METHODS

Subjects and setting

The study included 312 consecutive individuals referred to the outpatient clinic of the Department of Internal Medicine at the University Medical School in the regional hospital of Bari, the largest city with a population of 350,000 in Apulia, southern Italy. Screening of study participants is is shown in the flow chart of **Figure 1**. Individuals were referred mainly by their physicians because of persisting symptoms (i.e. more than 6 months) located either in the upper and/or lower abdomen suggestive of lactose intolerance, small intestinal bacterial overgrowth (SIBO) and/or functional gastrointestinal disorders (i.e. irritable bowel, functional dyspepsia). Subjects with alarm symptoms (e.g. anaemia, weight loss, chronic pain, ineffective therapies) or older than 50 years underwent further investigations including upper a/o lower endoscopy and blood tests in order to rule out inflammatory bowel diseases, celiac disease (25), intestinal neoplasms, colonic diverticula, and thyroid diseases. If needed, patients underwent Rx barium enema for characterization of colonic diverticula and severe dolicocolon, as a potential cause of lower intestinal symptoms. All of the subjects gave their written informed consent to be included in the study. Excluded from the study were non-compliant subjects or those with an history of liver, renal, lung, heart, metabolic, or neurological disease and those taking laxatives, antibiotics, prokinetics, or any other drug known to influence colonic flora or gastrointestinal motility in the month

preceding the study.

Experimental protocol

The protocol was part of the routine workup dedicated to the diagnosis of gastrointestinal diseases (organic, functional) in patients seen for chronic gastrointestinal symptoms. According to the screening flow chart of **Figure 1**, 167 patients were excluded mainly because of organic gastrointestinal diseases, while 145 patients without evidence of organic diseases and with symptoms highly suggestive of lactose intolerance underwent the lactulose H_2-BT, to confirm that the subject was H_2-producer and to exclude the presence of small intestinal bacterial overgrowth (SIBO). When both conditions were present, the orocecal transit time (OCTT) was calculated. Thus, 8 patients were excluded because of no H_2 production (n=2) or SIBO (n=6). The other 137 subjects underwent the first lactose H_2-breath test (LBT) preceded by administration of placebo. The placebo tablets had exactly the same appearance and taste of those containing tilactase but did not contain enzymes or lactose. This test resulted positive in the study group for 106 patients (70 Females and 36 Males, mean age 36±1 (SEM) years, age range 14-74 years). The remaining 31 LBT-negative subjects underwent a fructose H_2-BT and a final diagnosis of either fructose malabsorption or IBS was made. The study comprised the effect of tilactase enzymes on H_2 breath levels and symptom severity in the following groups: A) low dose (3 c.t., for a total of 6750 u) in 88 subjects (17 malabsorbers, 71 intolerants); B) standard dose (5 c.t., for a total of 11250 u) in 19 subjects (2 malabsorbers and 17 intolerants) who had previously undergone test A) plus another 18 subjects who gave consent to undergo only one test (standard tilactase dose). Thus, a total number of 37 subjects (5 malabsorbers and 32 intolerants) joined test B). Either placebo or tilactase enzymes were administered immediately before milk ingestion, as recommended by the manufacturer instructions. Tilactase enzymes consisted of a drug recently marketed in Italy, containing β-D-galactosidase from *Aspergillus oryzae (Lacdigest®*, Italchimici, Pomezia, Italy). Each c.t. contains 2250 u. According to the definition of lactase unit, one enzymatic unit is defined as the amount of the enzyme that can release 1 μmole of ONP (O-nitrophenol) at the conditions set by Extract Chemie (EC) or by Food Chemical Codex (FCC). The equivalence being: 1 FCC unit = 1.46 EC unit. Lacdigest enzymatic activity is indicated as EC units. As indicated by the manufacturer, one c.t. is capable of hydrolysing 5 g of lactose which is the amount contained in 100 ml of cow's milk. Thus, 3 c.t. (equal to 6750 u) should be sufficient to hydrolyze about 66% of the lactose contained in 500 ml of cow's milk, while 5 c.t. should be sufficient to hydrolyze 100% of the total lactose content. The low dose was tested because prior pilot experiments showed that most of the symptoms could be effectively reduced by this cost-saving regimen. Thus, the primary end-points of the study were to test the effect of low and standard doses of oral tilactase enzymes on H_2 breath levels and in preventing three major gastrointestinal symptoms (abdominal bloating, pain, and bowel movements) in subjects with lactose malabsorption without symptoms (malabsorbers) or with symptoms (intolerant). The secondary end-point was to test the safety, and tolerability of oral tilactase enzymes substitution in our clinical setting.

Breath testing

Before each breath test subjects were instructed to undergo a 12-hours carbohydrate-free diet; only meat, fish and olive oil were allowed at lunch and dinner the day before the test, to avoid prolonged intestinal H_2 production due to persistence of non-absorbable or slowly fermentable material in the colon (26, 27). Dinner was followed by a 12-hour fasting period until 8.00AM of the following day. In the clinic, subjects underwent a through mouth wash with 50 mL of a 1% chlorexidine solution (*Plak out®*, Polifarma Benessere, Roma, Italy) to prevent potential oro-pharingeal fermentation of sugars by local microflora. Smoking, eating, and physical exercise were not allowed 2 h before and during breath samplings (28-30). Subjects were instructed to collect alveolar air avoiding deep inspiration or hyperventilation before exhalation. Breath samples following the ingestion of each substrate were analyzed at baseline in duplicate and at each time point. For this, a portable, previously validated

device was used (*LactoFAN®*, Lans Medical BV, The Netherlands, kindly). The manifold was equipped with a microfuel cell for detection of H_2 as part per million (ppm). According to the manufacturer, the accuracy of the detector was ±2 ppm, with a resolution of 1 ppm and a range of 0 to 500 ppm. Each breath test was performed at least 72 h apart to avoid the effect of colonic acidification (8).
Lactulose H_2 breath test was performed first to detect significant H_2 excretion, to calculate OCTT and to exclude SIBO. The test consisted of two baseline measurements followed by the ingestion of 10 g lactulose; for this, 15 ml of *Laevolac®* suspension (Roche, Italy) diluted in 100 ml of tap water were administered. Breath samples were collected thereafter every 10 minutes up to a maximum of 360 min. Breath hydrogen concentrations were analyzed as previously described (31, 32). The first rise of 10 ppm above baseline values followed by a positive trend of another two consecutive measurements was taken as OCTT and expressed in minutes. The presence of SIBO was defined according to recently published criteria (33).
Lactose H_2 breath test was performed by administering 500 ml of cow's unskimmed milk containing 25 g of lactose. Air samples were collected at 0, 30, 60, 90, 120, 150, 180, 210, 240 and 360 min. The test was considered positive for lactose malabsorption in the presence of H_2 concentrations of at least 20 ppm above baseline, according to a commonly used criterion (8, 14, 26). H_2 peak levels in ppm, time to H_2 peak in min., and area under the curve of H_2 levels during the observation time (AUC expressed as H_2 ppm x 360 min) were calculated by the trapezoidal rule (34, 35).

Symptoms
During each LBT subjects were instructed to record the presence and severity of bloating, abdominal pain (cramps), and bowel movements. For this purpose, a visual analogue scale (VAS) was used which consisted of a 100 mm-horizontal line (extreme left= symptom completely absent; right end=max intensity of the symptom). After appropriate instructions, subjects had to mark the severity of symptoms along its length at each time point (36, 37). First measurement was taken in the fasting patient before the ingestion of milk (baseline, time 0) and then at 30, 60, 90, 120, 150, 180, 210, 240 and 360 min thereafter. As during this time period no other food was allowed, it was assumed that symptoms were likely to be related to lactose maldigestion and intolerance. VAS score-time related curves for each symptom were constructed. The area under curve of each symptom was also calculated by the trapezoidal rule (34, 35). An arbitrary score to weight for clinical relevance was chosen for semiquantitative analysis of bowel movements,: 0= no evacuations, 1000=one evacuation, 2000= two or three evacuations, 3000= more than three evacuations. For each patient, a total symptom score was constructed as sum of the integrated VAS (AUCs x 360 min) of bloating and abdominal pain plus the score for bowel movements.

Data analysis
Data are given as the mean ± standard error of the mean (SE). Time-related changes of continuous variables were analysed using *ANOVA* repeated measures followed by *post-hoc* tests for multiple comparisons. Differences of two groups were evaluated by the Mann-Whitney *U*-test or by Student's t test, where appropriate. Correlations were assessed by calculating Spearman's r_s. The chi-square test was used to compare categorical variables and proportions. A two-tailed probability (P) value of less than 0.05 was considered statistically significant (38, 39). Calculations were performed with the *NCSS 2007* statistical software (Kaysville, UT, USA) (35).

RESULTS
When considering the initial group of 137 H_2-producer subjects, 106 (77.3%) had a positive LBT according to the established criteria (8, 14, 26). Twenty sybjects (19%) were lactose malabsorbers, i.e. no complaint recorded during the LBT observation time (8 Males, 12 Females, mean age 33±3 years) and 86 (81%) were lactose intolerants, i.e. presence of abdominal symptoms during the LBT

observation time (37±2 years, 28 M, 58F). Groups did not differ for age and gender.

Lactulose breath test

In the 106 subjects with lactase deficiency, OCTT by lactulose breath test was 121.5±4.6 min and tended to be slightly longer in females than males (125±6 min vs. 116±6 min, P=NS). OCTT was significantly longer in subjects with lactose malabsorption than intolerance (151.0±13.1 min vs. 114.6±4.5 min, P<0.002). When data were compared with those obtained from an historical and extended control group of 142 healthy subjects matched for sex and age (31, 32), both malabsorbers and intolerant subjects exhibited a longer OCTT than the control healthy group (99.5±1.6 min, ANOVA, P<0.0001).

Lactose breath test

Subjects with lactose malabsorption had longer time to reach the cut-off value of 20 ppm (192±20 min vs. 135±7 min, P<0.01), longer time to reach the H_2 peak (233±17 min vs. 199±6.0 min, P<0.02), lower H_2 peak levels (44.6±5.3 ppm vs. 73.1±4.5 ppm, P<0.001), and smaller integrated H_2 excretion levels (AUC: 8087±1201 vs. 13912±861 ppm x 360 min, P<0.01) than subjects with lactose intolerance.

Effect of tilactase on breath hydrogen excretion following lactose breath test

Baseline levels of excreted H_2 during LBT obtained with placebo and tilactase enzymes were similar (1.42±0.2 ppm and 1.31±0.2 ppm, respectively, P=NS). Neither the low or standard dose of tilactase ingestion caused side effects, as confirmed by a careful follow up during the test and thereafter up to 24 h after the LBT (phone interview). In the whole study group of 106 subjects the low dose of tilactase produced a consistent decrease (75%) of integrated H_2 levels (from 12813±765 to 3219±340 ppm x 360 min in the placebo and tilactase group, respectively, P<0.0001). The standard dose of tilactase produced a further decrement of integrated H_2 levels (1797±947 ppm x 360 min, P<0.001 *vs* LBT-placebo). The net effect of different doses of tilactase was evident in both malabsorbers and intolerants, as shown in **Figure 2**. Either doses of tilactase were followed by a significant decrement of time-dependent changes of H_2 excretion and AUCs. It shoud be noted that integrated H_2 levels decreased by 73% (low dose) and by 85% (standard dose) in lactose malabsorbers and by 75% (low dose) and 86% (standard dose) in lactose intolerant subjects. The further riduction with standard dose, however, did not achieve statistical significance. In the intolerant subgroup of 17 subjects undergoing the LBT with both doses of enzymes, a similar trend was showned (low dose tilactase 2180±441 *vs* high dose tilactase 1862±488 ppm x 360 min; P=0.097).

Effect of tilactase on symptoms in intolerant subjects studied by the lactose breath test

As previously underscored, lactose intolerant subjects were those developing abdominal symptoms like bloating, pain/cramps, bowel movements following ingestion of milk-containing lactose. With placebo, the total symptom score was 13744±1079 while the integrated AUCs for abdominal bloating and abdominal pain were 8003±750 and 5241±654 mm x 360 min, respectively. Tilactase ingestion both at low and standard doses resulted in a significant decrement of both symptoms (**Figure 3**). The total symptom score decreased by 77% (to 3284±984) with the low dose and by 99% (to 1027±1455) with the standard dose of tilactase (P<0.0001 vs. placebo). Twenty-three subjects (27%) had one (n=7 subjects) or more (n=16 subjects) bowel movements with the LBT-placebo test. This symptom persisted in one subject after low dose and 1 subject after standard dose tilactase.

The correlations between symptoms and different markers of H_2 production in intolerant subjects undergoing LBT-placebo are depicted in **Figure 4**. There were positive and statistically significant correlations (0.0002<P<0.01) between integrated AUC for bloating and total symptom score with H_2 peaks and integrated H_2, which was absent in the case of abdominal pain. By contrast, AUC for bloating and total symptom score decreased at increasing values of time to H_2 peak. As expected all

correlation was completely lost after ingestion of tilactase enzymes, due to major decrease of symptoms (data not shown).

DISCUSSION

We show here that in a large group of subjects with adult hypolactasia, a novel formulation of oral tilactase enzymes recently marketed in Italy is highly effective at low and standard doses in decreasing breath H_2 excretion (an objective marker of lactose malabsorption) following the ingestion of lactose-containing milk. Most important, tilactase ingestion effectively decreases specific symptoms in lactose intolerant subjects without causing side effects. Oral β-D-galactosidase from the fungus *Aspergillus oryzae* has been successfully tested in prior small studies (40, 41); to our knowledge this is the first report on the use of oral tilactase enzymes in a large clinical setting in a geographical area with high prevalence of lactose intolerance. Previous studies have tested different enzymes in different settings. Milk was prehydrolysed by *β*-galactosidase obtained by *K. Lactis* (8) and the effect on maximum H_2 concentration and cumulative H_2 excretion was better than that from *Aspergillus niger* (17, 42). β-D-galactosidase from *Aspergillus oryzae* was also effective in children when added to the milk (43). Variable results, however, might depend on different enzyme preparations but also on timing in adding the enzymes to the milk (e.g. mealtime or hours before the meal) as well as by the different doses employed (17, 18, 42, 44).

The lactose H_2 breath test is currently considered as the clinical standard for the diagnosis of lactase non-persistence, i.e. malabsorption. We have classified subjects according to previously proposed criteria (8, 14, 26) and found that the prevalence of LBT-positive subjects was 77.3%. New criteria involving a single time-point measurement of H_2 ppm at the 6^{th} hour (greater than 6 ppm) or a cumulative value of more than 15 H_2 ppm obtained at the 5^{th}, 6^{th}, and 7^{th} hours have been also proposed to increase sensitivity, although not used in clinical practice (26). However, we found no gain in our series with the first option, i.e. only one additional "negative" subject would have become positive to LBT (prevalence 78.1%). Both values are close to that reported by Di Stefano et al. (26) who used 20 g lactose (400 mL milk) instead of 25 g lactose (500 mL milk) used in the present study. Thus, our regimen seems appropriate in sensitivity and specificity, at least in this series. With the second option, most subjects would have refused a 7-hours breath test, and this was the reason why this criterion was not applied in the present study.

This single-blind study was designed to avoid the cumbersome and potentially harmful consequences of two sequential LBTs (a first diagnostic and a second with placebo). A randomized double blind placebo-controlled design, albeit meaning a more rigorous approach, would have exposed intolerant subjects (the majority in this and other studies (45)) to a second placebo LBT and to unnecessary symptoms, since the intolerance status has been reported to be stable over time (45). In our experience, this approach would have negatively affected the overall compliance of the subjects, as shown by a prior pilot study. Instead, we were able to complete the study and to randomize the following tests with low or standard dose of tilactase enzymes in the subgroup willing to undergo both regimens. The information provided by this study we believe is reliable as we matched the objective measurement of H_2 breath excretion with the subjective assessment of symptoms, graded on a self-reported visual analogue scale which was fully explained before starting the test and could not be influenced by external observers. Others studies have employed semiquantitative scores (8, 26, 46). Since the status of intolerance remains unchanged in most individuals (45), performing two sequential LBTs –the second with tilactase enzymes and invariably blind to the subject tested- is likely to reflect the true change in symptom score upon enzyme ingestion.

The results of this study can have practical implications for a number of reasons.

First, gastrointestinal symptoms reported by a number of potential lactose intolerant subjects can be misleading and mimic those found in other conditions, i.e. irritable bowel syndrome (47), intestinal neoplasms, SIBO, celiac disease, and IBD. Thus, we believe that it is essential to achieve a definitive

diagnosis before attributing symptoms to to lactose intolerance, and this aspect should be discussed with the patient and his general practitioners. Starting the *a priori* chronic milk-exclusion diets in the absence of a diagnosis, in fact, may have a negative impact on the everyday life, can be cumbersome and might decrease the overall patient's compliance. The diagnosis might become even easier if one considers that affordable, portable, compact, easy-to-use equipments have recently become available for screening numerous patients by designing the so-called "field tests" (45, 46).

Second, the therapeutic approach with oral tilactase enzymes can be even more valuable in a particular geographical setting. The deficiency of the intestinal enzyme lactase can be as high as 40-70% in southern Italy (7-10). The presence of gastrointestinal symptoms, which appear to affect the quality of life, has been reported in about 50% of subjects with hypolactasia (4, 14), although the prevalence can be highly variable (48-50). In this study the majority of subjects (more than 80%) developed symptoms following ingestion of 500 mL milk, even if less than one-third had bowel movements, possibly the most cumbersome symptom. This trend has also been described in a Chinese population with genetically determined lactase deficiency (46). Apulia, however, is an Italian region at the heart of the Mediterranean area where daily consumption of solid dairy products (including the hand-made fresh cheese "provolina", buffalo "mozzarella", "scamorza" plus a full variety of cottage cheeses) is very popular and represents a major source of daily dietary calcium intake. Thus, oral tilactase enzymes might be valuable in a geographical area with high-prevalence of hypolactasia, and possibly more practical than prehydrolysed milk which taste might be altered with exogenous liquid lactase (24).

Third, optimal dosage of tilactase enzymes should be tailored according to the overall amount of ingested lactose. Although either doses of oral tilactase enzymes were highly effective in both malabsorbers and intolerants challenged with 25 g lactose, our findings point to a role for lactase enzymes in symptomatic (intolerant) subjects. In this subset of subjects, in fact, it is essential to achieve the best compliance and to prevent the risk of osteoporosis due to illogical exclusion diets (21). The simple finding of lactose malabsorption (i.e. only elevated H_2 breath excretion) does not support, in our opinion, the use of tilactase enzymes, unless symptoms suggestive of intolerance will appear following ingestion of greater amounts of lactose-containing foods or due to subsequent changes of luminal intestinal function resulting in a condition of (secondary) hypolactasia. Furthermore, the ingestion of tilactase enzymes at low dose (able to hydrolyze about 66% of the total amount of lactose contained in 500 ml of milk) induced in our series a 75% decrease of integrated H_2 breath levels in the whole group of patients. The ingestion of the standard dose of tilactase, by contrast, (able to hydrolyze about 100% of the lactose contained in 500 ml of milk) induced only an additional 10% decrease of the integrated H_2 breath levels, as compared with placebo. Of note, symptoms decreased by more that 75% and were virtually abolished with the low and standard dose, respectively. Apparently, the problem of decreased efficacy of oral tilactase due to potential gastric inactivation of the enzyme was not encountered in our study (51). Thus, our results indicate that there is not a true additional benefit in ingesting a standard tilactase dose; this approach may eventually be employed in subjects with high H_2 production after failure of low doses, or when large quantities of lactose-containing products are likely to be eaten, or when symptoms persist.

We found that OCTT was delayed in malabsorbers, compared with intolerants. This finding, and the longer time to reach H_2 peak concentrations in malabsorbers, is in accord with a previous study (46) performed in a sample of a Chinese population with genetically determined lactase deficiency tested with 25 g lactose used in a different test (^{13}C-lactose/^{3}H-glucose). Others have reported a similar behavior in asymptomatic malabsorbers (52-54). Likely, subjects with moderate hypolactasia better digest lactose because they have more intestinal lactases, but also because of longer exposure time of the disaccharide in the intestinal lumen. Indeed, this was also the conclusion in the study of Vonk *et al.* (46) who calculated both lactose digestion index and amount of lactose digested. By contrast, intolerant subjects appear to have a faster OCTT *per se*; moreover, lactose might trigger a faster transit time due to a direct effect of lactose on factors regulating intestinal motility (45), i.e. osmoreptor in the upper

intestine (55) and/or hormone release (45). Thus, the onset of symptoms in lactose intolerant subjects might originate from several factors, including the amount of lactose ingested, the degree of hypolactasia, the speed of small intestinal transit and lactose processing by colonic microflora (45, 46). Indeed, adding living bacteria in fresh yoghurt is associated to delayed OCTT and fewer symptoms in patients with lactose maldigestion due to improved bacterial lactose digestion (56).

Levels of breath H_2 excretion might reflect the degree of lactose digestion and influence the occurrence of symptoms following lactose ingestion (52, 57). In our series a significant positive correlation was found between the integrated H_2 breath levels and the total symptom score. This was also confirmed by Bodlaj *et al.* (2) employing genotyping of the lactase-phlorizin hydrolase -13910 polymorphism and showing that the CC genotype of the DNA variant -13910 T/C upstream of the LCT gene is associated with lactase non-persistence. This genetic condition is strongly correlated to a positive H_2 breath test (3). By contrast, no correlation was found between severity of symptoms and the level of breath H_2 excretion in a previous study (26). Although Vonk *et al.* (46) found "no difference in breath H_2 excretion between groups tolerant and intolerants", a trend appears towards higher H_2 breath levels in intolerant subjects (see Table 1 of the manuscript). Either different scores employed for symptoms (quantitative vs. semiquantitative) or differences in populations might account for such discrepant results. In accord with our findings, however, Vonk *et al.* (46) found that subjects with lactose malabsorption had longer time to reach the H_2 peak after lactose ingestion implying a slow rate of colonic delivery of lactose. In this respect, we found that by adding tilactase enzymes, a significant decrement in H_2 excretion was evident in both malabsorbers and intolerant patients. In this latter group we observed a virtually complete disappearance of symptoms, and this favours the relationship between H_2 levels and degree of lactose maldigestion or intolerance.

Several therapeutic approaches have been proposed to treat symptomatic hypolactasia (9). Loperamide was used to prolong OCTT and improve lactose handling (22, 23), but it was not clinically fully effective. This approach is in favour of the finding that asymptomatic malabsorbers had indeed a longer OCTT. Other therapeutic approaches considered the administration of milk with low lactose concentrations, yoghurt, β-galactosidases-producing probiotics (24), and β-D-galactosidase-containing solutions to be added to the milk (pre-hydrolyzed). Although all the above mentioned approaches are helpful, they do not allow for dairy products ingestion, which are important constituents of the Mediterranean diet. Moreover, one should question about their practical use on a day-to-day basis.

In this respect, our findings point to a practical therapeutic approach to milk intolerance likely to prevent complications of reduced calcium intake (16, 58), vitamin deficiency (17) and to ensure a better quality of life.

In conclusion, our results point to the existence of a strong association between H_2 breath levels and intensity of gastrointestinal symptoms following lactose ingestion in lactose intolerant patients. Oral β-D-galactosidase from *Aspergillus oryzae* is highly effective in decreasing the subjective and objective effects of lactose maldigestion in adult with hypolactasia without causing side effects. Oral tilactase enzymes represent a valid therapeutic strategy in a subgroup of symptomatic lactose-intolerant subjects.

LEGEND TO FIGURES

Figure 1

Patients screening flow chart

Legend: SIBO, small intestinal bacterial overgrowth.

Figure 2

Effect of tilactase enzymes in subjects with lactose malabsorption or intolerance. Graphs are shown as time-dependent changes of H2 in breath (A, C) and as integrated area under curve (B, D) following the

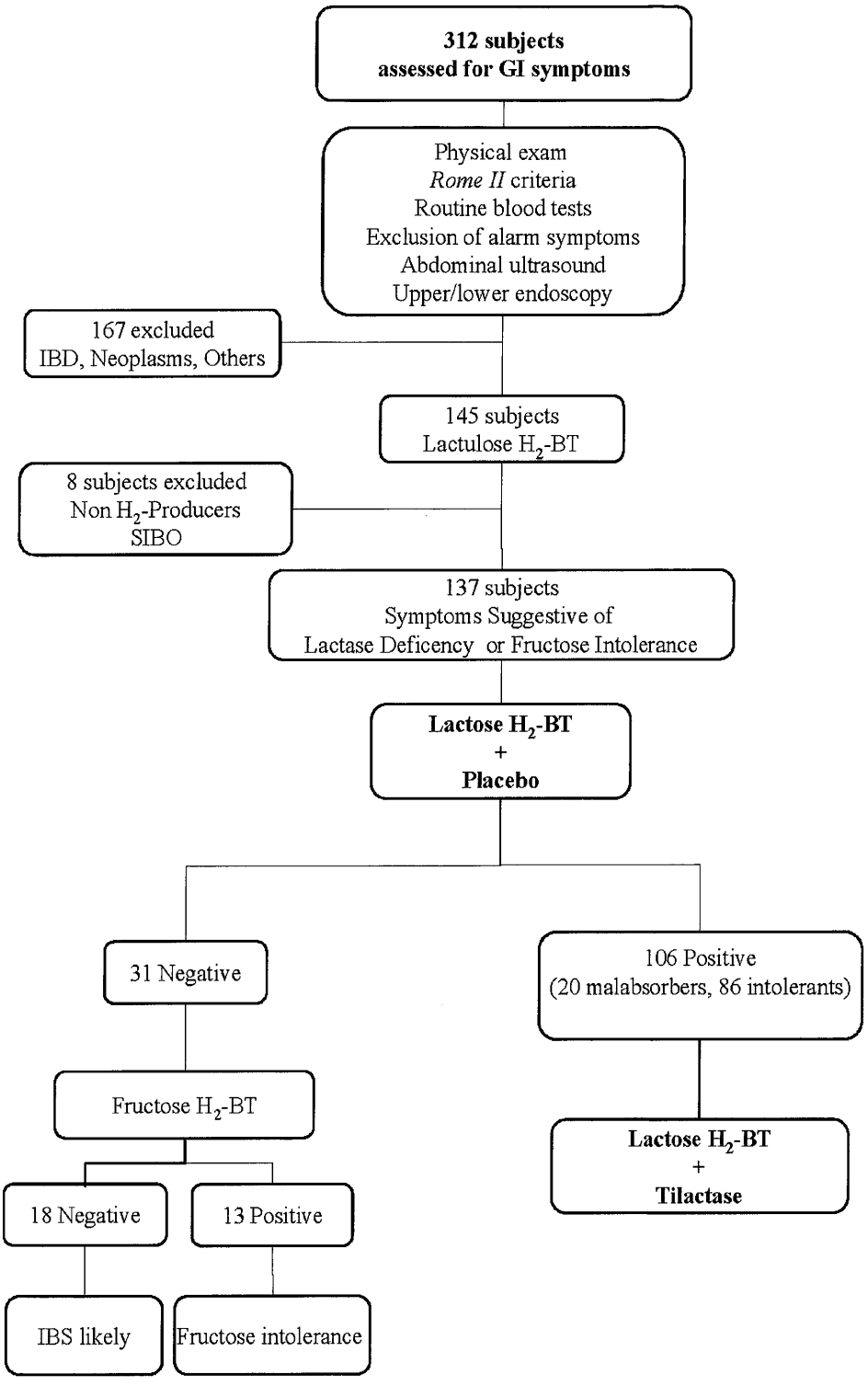
ingestion of 500 ml of unskimmed cow's milk with placebo, low dose (6750 u) or standard dose (11250 u) of tilactase enzymes. Symbols and bars indicate means while vertical lines indicate SEM. Malabsorbers: A) A significant ($P<0.01$) decrease of H_2 levels existed between 90 and 360 minutes with low dose tilactase enzymes and between 180 and 360 minutes after standard dose tilactase enzymes. B) Significant decrease of integrated area under curve (AUC) with low and standard doses of tilactase enzymes, compared with placebo. A trend existed also between either doses of tilactase enzymes. Intolerants: C) a significant ($P<0.01$) decrease of H_2 levels existed between 60 and 360 with low dose tilactase enzymes and between 90 and 360 minutes after standard dose tilactase enzymes. D) Significant decrease of integrated area under curve (AUC) with low and standard doses of tilactase enzymes, compared with placebo. A trend existed also between either doses of tilactase enzymes.

Figure 3

Effect of tilactase enzymes in subjects with lactose malabsorption or intolerance. Graphs are shown as time-dependent changes of VAS for bloating and abdominal pain (A, C) and as integrated area under curve (B, D) following the ingestion of 500 ml of unskimmed cow's milk with placebo, low dose (6750 u) or standard dose (11250 u) of tilactase enzymes. Symbols and bars indicate means while vertical lines indicate SEM. Bloating: A) a significant ($P<0.01$) decrease of H_2 levels existed between 30 and 360 minutes with low and standard dose tilactase enzymes. B) Significant decrease of integrated area under curve (AUC) with low and standard doses of tilactase enzymes, compared with placebo. A trend existed also between either doses of tilactase enzymes. Abdominal pain: C) a significant ($P<0.05$) decrease of H_2 levels existed between 30 and 360 minutes with low dose tilactase enzymes and at 30 minutes and between 90 and 360 minutes after standard dose tilactase enzymes. D) Significant decrease of integrated area under curve (AUC) with low and standard doses of tilactase enzymes, compared with placebo. A trend existed also between either doses of tilactase enzymes.

Figure 4

Correlations between symptoms and gastrointestinal symptoms and 3 markers of H_2 excretion in intolerant subjects ($n=86$). A) Integrated score for bloating positively correlated with H_2 peak and integrated H_2 excretion and negatively correlated with time to reach maximum H_2 peak; B) No correlations between integrated score for pain and H_2 peak, integrated H_2 excretion and time to reach maximum H_2 peak; C) Total symptom score (including also bowel movements, see methods) was positively correlated with H_2 peak and integrated H_2 excretion and negatively correlated with time to reach maximum H_2 peak.


312 subjects
assessed for GI symptoms
Physical exam
Rome II criteria
Routine blood tests
Exclusion of alarm symptoms
Abdominal ultrasound
Upper/lower endoscopy
167 excluded
IBD, Neoplasms, Others
145 subjects
Lactulose H2-BT
8 subjects excluded
Non H2-Producers
SIBO
137 subjects
Symptoms Suggestive of
Lactase Deficency or Fructose Intolerance
Lactose H2-BT
+
Placebo
31 Negative
106 Positive
(20 malabsorbers, 86 intolerants)
Fructose H2-BT
Lactose H2-BT
+
Tilactase
18 Negative
13 Positive
IBS likely
Fructose intolerance

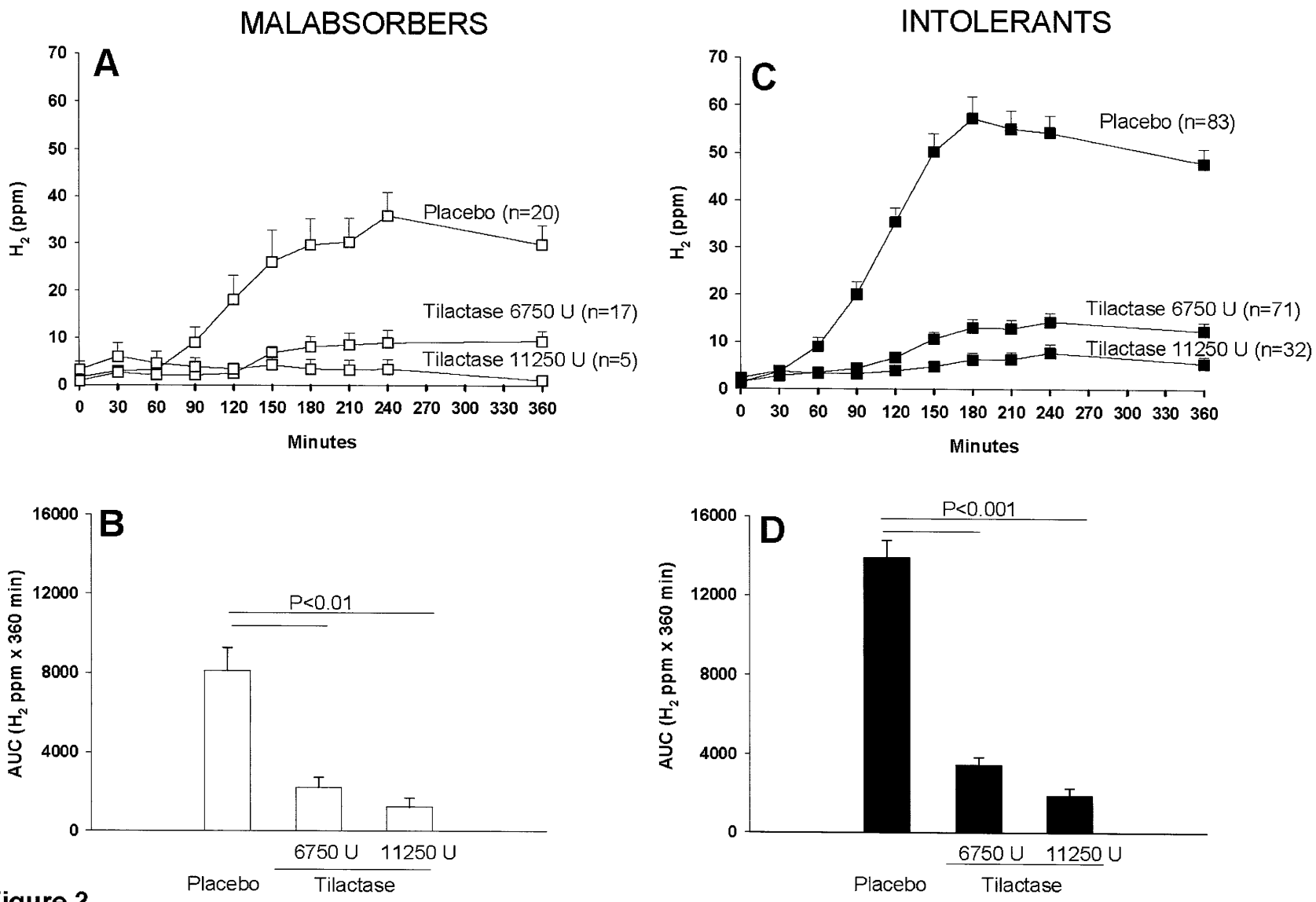

MALABSORBERS
INTOLERANTS
A
B
C
D
H2 (ppm)
Minutes
Placebo (n=20)
Tilactase 6750 U (n=17)
Tilactase 11250 U (n=5)
Placebo (n=83)
Tilactase 6750 U (n=71)
Tilactase 11250 U (n=32)
AUC (H2 ppm x 360 min)
P<0.01
P<0.001
Placebo
Tilactase
6750 U
11250 U

Figure 2

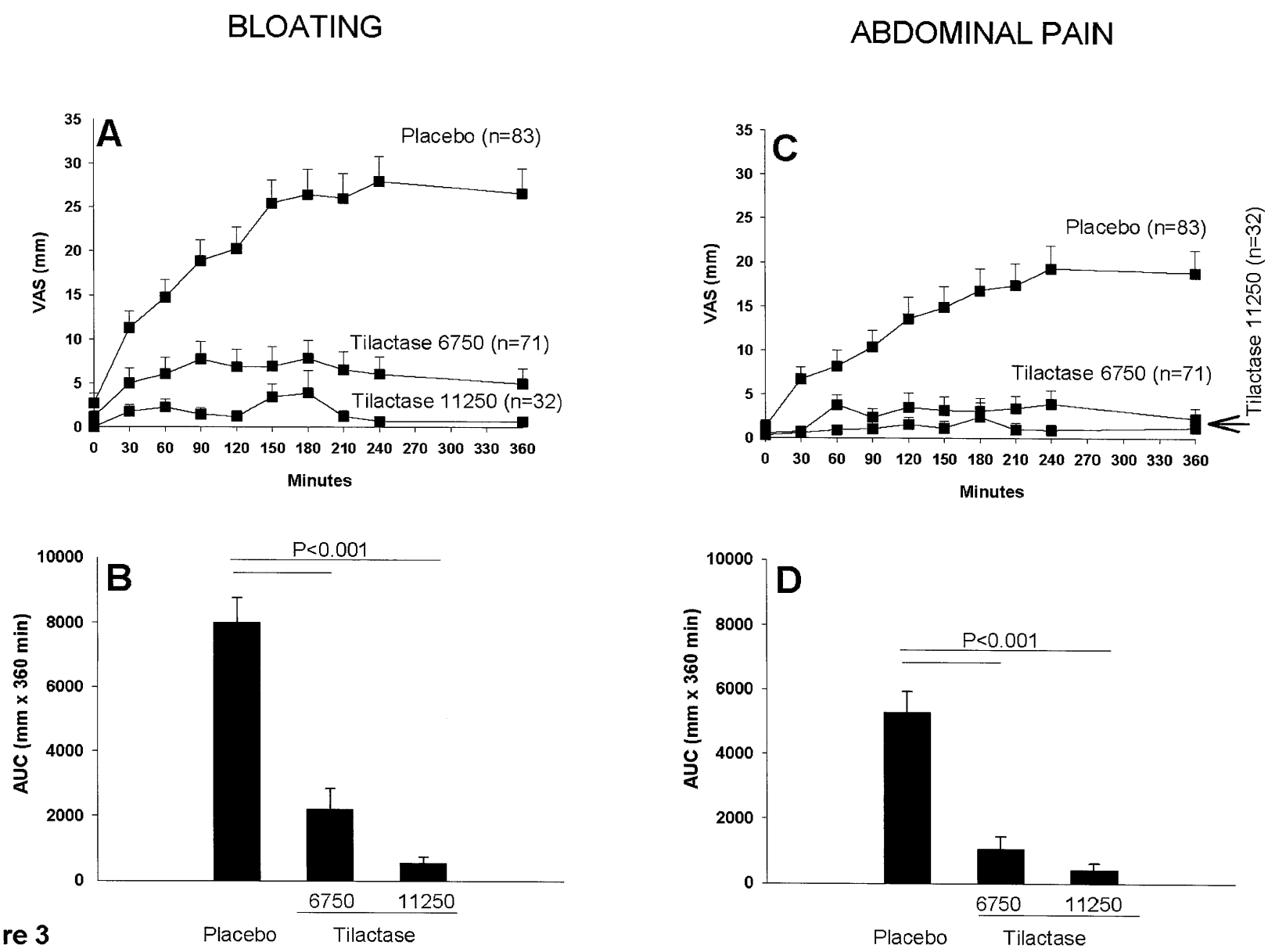

BLOATING
ABDOMINAL PAIN
A
Placebo (n=83)
Tilactase 6750 (n=71)
Tilactase 11250 (n=32)
VAS (mm)
Minutes
C
Placebo (n=83)
Tilactase 6750 (n=71)
Tilactase 11250 (n=32)
VAS (mm)
Minutes
B
P<0.001
AUC (mm x 360 min)
6750
11250
Placebo
Tilactase
D
P<0.001
AUC (mm x 360 min)
6750
11250
Placebo
Tilactase

Figure 3

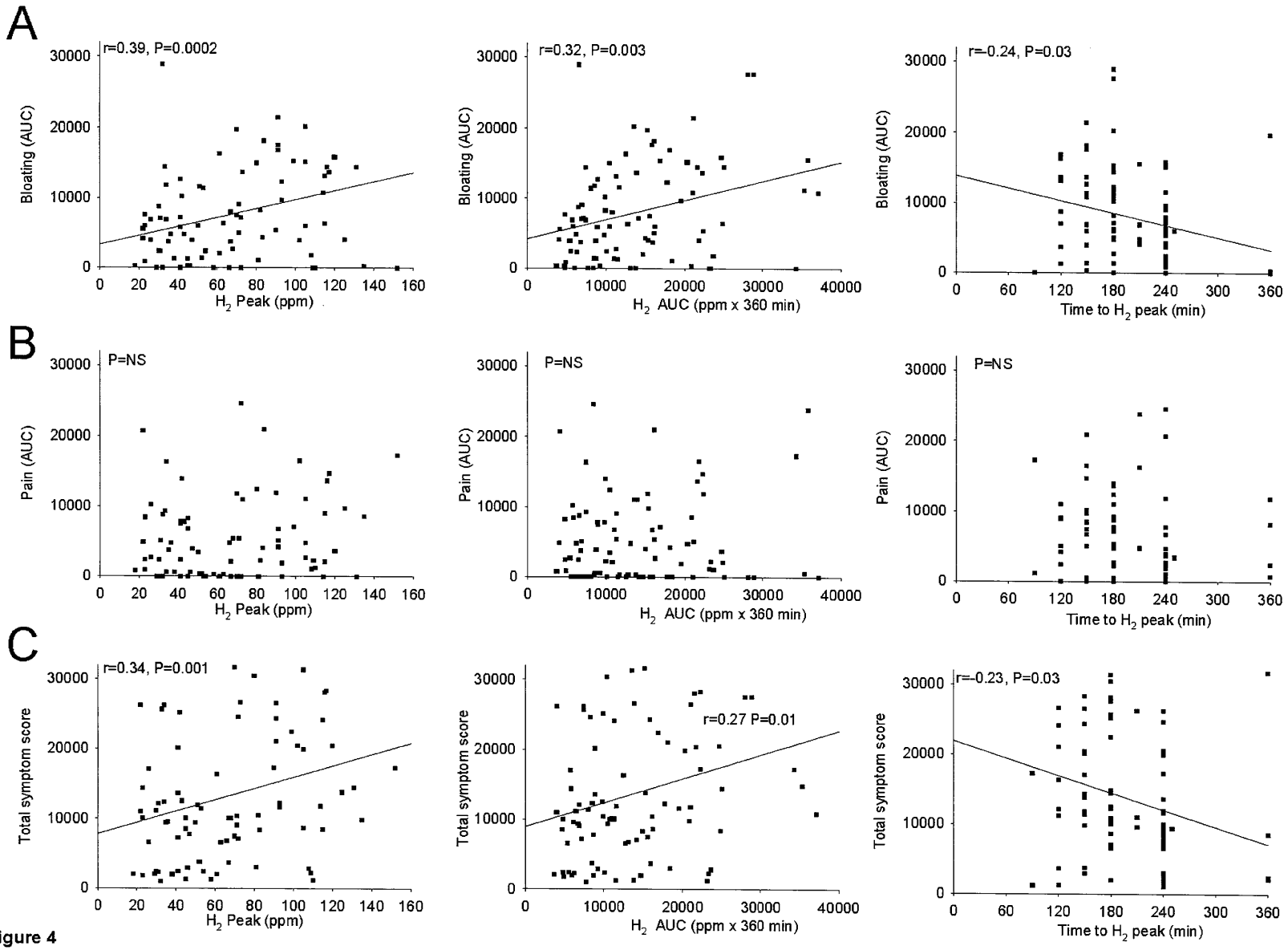

A
r=0.39, P=0.0002
Bloating (AUC)
H2 Peak (ppm)
r=0.32, P=0.003
H2 AUC (ppm x 360 min)
r=-0.24, P=0.03
Time to H2 peak (min)
B
P=NS
Pain (AUC)
C
r=0.34, P=0.001
Total symptom score
r=0.27 P=0.01
r=-0.23, P=0.03

Figure 4

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