

## Efficacy of Addition of Exogenous Lactase to Milk in Adult Lactase Deficiency

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The efficacy of lactase by *Kluyveromyces lactis* in hydrolyzing milk lactose and reducing milk intolerance symptoms was tested in 52 proved lactose malabsorbers. The enzyme was added to milk administered to the patients, and H<sub>2</sub> breath excretion (as an index of carbohydrate malabsorption), was determined by gas chromatograph technique, and milk intolerance symptoms were recorded. H<sub>2</sub> mean excretion was 78.3 ± 5.49 ppm after administration of intact whole milk 500 ml (test A), 43.5 ± 4.99 ppm when lactase 2000 U was added to milk 500 ml immediately before administration (test B); 36.7 ± 5.01 ppm when milk 500 ml was incubated for 12 h with lactase 1000 U (test C), and 29.7 ± 4.35 ppm when the incubation was prolonged for 24 h (test D). Symptoms score was: test A = 5.85 ± 0.56, test B = 3.71 ± 0.45, test C = 2.77 ± 0.63, test D = 1.7 ± 0.68. A correlation index of  $r = 0.44$  ( $p < 0.01$ ) was obtained between reduction in H<sub>2</sub> mean excretion and reduction in symptoms score of a single individual. The addition of this lactase to milk seems to be effective in correcting lactose malabsorption, thus representing a convenient approach in milk intolerance.

### INTRODUCTION

Primary lactase deficiency is present in most of the world's adult population, with different prevalence, ranging from 10% to 100% in various ethnic groups (1).

It is known that, in up to 50% of malabsorbers (2), this deficiency may cause severe gastrointestinal symptoms, including abdominal distension, cramps, flatulence, and watery stools. For this reason, milk is eliminated from the diet of many lactose malabsorbers, with nutritional disadvantages, chiefly in patients needing a good calcium intake (3).

It is worthwhile to note that the severity of symptoms after milk ingestion in lactose malabsorbers may vary widely, depending upon the degree of intestinal lactase deficit, the oral load of milk, and the adaptation mech-

anisms of gut. It is reported that lactase activity is not inducible (4, 5), but it is also known that the constant ingestion of milk may induce some tolerance to non-absorbed lactose (6). Thus, intolerance symptoms often present a varied pattern of expression, thus resembling irritable bowel syndrome (7).

Several strategies to overcome lactose intolerance and milk rejection have been conceived. The primary approach is the complete elimination of milk and dairy products from the diet. This treatment resolves symptoms immediately, but may represent a risk factor in precipitating bone diseases, such as postmenopausal osteoporosis (8). In addition, the elimination of milk and its lactose-containing derivatives cannot be complete, because of the widespread use of lactose in many other foods.

Advances in technology have provided several lactases from yeasts and fungi that can be added to milk before its consumption, producing an efficient hydrolysis of lactose (9, 10). This "enzyme-replacement therapy" for adult lactose malabsorbers with deficient intestinal lactase activity has been studied by many authors with satisfactory results (11-14).

In the present study, we evaluated the lactose hydrolysis efficiency of a liquid enzyme preparation, added to milk immediately before consumption, or previously incubated with milk, in lactose malabsorbers, in order to define the most convenient use and the correlation between the improvement of lactose absorption evaluated by H<sub>2</sub> breath test, and the reduction of milk intolerance symptoms.

### MATERIALS AND METHODS

#### Enzyme preparation

We used a soluble lactase ( $\beta$ -galactosidase) produced from yeast *Kluyveromyces lactis* in liquid form (Lactaid by Iketon, Italy), providing 250 neutral lactase units (NLU) per drop (1 NLU is the quantity of enzyme that, incubated at 25°C and pH 7.5 with *O*-nitrophenyl- $\beta$ -D-galactopyranoside, produces 1  $\mu$ mol of *O*-nitrophenyl per minute).

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### Lactase activity *in vitro*

Lactase activity *in vitro* was studied at different enzyme concentrations, and with different periods of incubation and temperatures.

In the first experiment, we incubated 250-ml samples of whole milk with increasing doses of lactase (ranging from 250 to 1250 units) at 4°C for 12 h. In the second experiment, 250 ml of milk were incubated at 4°C with 1000 units of lactase, and enzymatic activity was assessed at intervals of 0, 15, 30, 60, 120, and 350 min and at 12 h and 24 h. The third experiment consisted of the incubation of 250 ml of milk with 1000 units of lactase for 30 min at 4°C, 10°C, 20°C, 30°C, and 37°C. Since the milk used contains 48 g/L of lactose (molecular weight 342.31), 100% hydrolysis of the sugar would produce 25.3 g/L of glucose (molecular weight 180.16); starting from this value, percentages of hydrolysis were calculated (10). Glucose levels were determined by the colorimetric enzymatic method (glucose kit 510-A by Sigma Chem., St. Louis, MO).

### Subjects

Sixty-seven subjects, ranging in age from 20 to 65 yr (mean 43.5 yr), 14 males and 53 females, entered the study. They were screened among the patients referred to our Gastroenterology Unit for gastrointestinal symptoms, and all 67 had the following features: 1) gastrointestinal complaints related to milk ingestion; 2) absence of gastrointestinal diseases (all the subjects underwent upper gastrointestinal radiology, barium enema, or colonoscopy, abdominal ultrasonography, stool cultures, fecal occult blood testing, serum chemistry analysis); 3) no ingestion of antibiotics for the previous 2 wk. An informed consent was obtained from each subject before entering the study.

### Breath collection

Lactose malabsorption was determined by H<sub>2</sub> breath analysis procedure (15, 16). After an overnight fast, end expiratory breath samples were collected before milk ingestion and every 10 min over a 6-h period. The patients were instructed not to smoke. During the test, the hydrogen concentration in each sample was immediately determined by gas chromatography, using Microlyzer model 12 (Quintron Instruments Co., Milwaukee, WI) (17).

### Lactose absorption tests

In the screening phase of the study, each subject was tested for lactose absorption capacity by determining H<sub>2</sub> production after an oral dose of whole cow's milk 500 ml (test A). Assuming that cow's milk contains lactose 5%, this dose provided 25 g lactose.

Only subjects who, in this screening test, showed a rise in breath H<sub>2</sub> concentration >20 parts per million (ppm) above the baseline level for three or more con-

secutive breath collection intervals underwent the following breath tests: one with whole cow's milk 500 ml with 2000 units (8 drops) of liquid enzyme preparation, added within 5 min before milk ingestion (test B); the second test using 500 ml of whole cow's milk incubated under refrigeration at 4°C with 1000 units of lactase. Incubation time was prolonged for 12 h for 40 subjects (test C), and for 24 h for the remaining 12 subjects (test D). The producer advised 4 drops (1000 units) in 500 ml of milk incubated for 24 h at 4°C to obtain lactose hydrolysis of more than 90%.

An interval of at least 72 h was allowed between successive tests, to avoid the effect of acidification described by Perman (21). Subjects underwent the last two tests in random order, without any information about the content of milk.

After each test, the subjects were asked to fill out a questionnaire, to assess a semiquantitative score of symptoms. Scores were assigned as follows: abdominal pain, abdominal distention, bloating, and flatulence: for each item 0 if absent, 1 if mild, 2 if moderate, 3 if severe; bowel movements: 0 if absent, 1 if present, 2 in case of two or more; stool consistency: 0 if normal, 1 if loose, 2 if watery. Overall theoretical score ranged from 0 to 16. Mean symptomatological score, previously assessed in a group of 23 known lactose absorbers tested with whole cow's milk, proved to be  $1.99 \pm 0.74$  (mean  $\pm$  SD) (unpublished personal observations).

### Data analysis

The breath excretion of hydrogen was quantified as peak of concentration expressed in ppm of H<sub>2</sub>, and as mean concentration (in ppm), defined as mean of the five greatest increments in H<sub>2</sub> concentration above baseline (2, 18).

Differences between mean data were analyzed by two-tailed Student's *t* test for paired and unpaired data, where appropriate. The evaluation of variations in symptoms frequency were made by  $\chi^2$  method. Data concerning H<sub>2</sub> concentrations and symptoms score are expressed as mean  $\pm$  SE.

## RESULTS

Lactase activity observed *in vitro* is shown in Fig. 1. When milk was incubated for 12 h at 4°C with graded doses of lactase, the maximal percentage of hydrolysis reached 47% (Fig. 1A). Incubation of milk at 4°C with 1000 units of lactase for periods ranging from 15 min to 24 h has been found to produce 60.5% hydrolysis of lactose (Fig. 1B). Fig. 1C shows increasing percentages of hydrolysis depending on temperature of incubation when milk is incubated for 30 min with lactase 1000 U/250 ml. Under these conditions, the maximum of hydrolysis (25%) was reached at 37°C. Temperatures exceeding 37°C were not tested, because it is known that lactase has a temperature optimum of 37°C (10).



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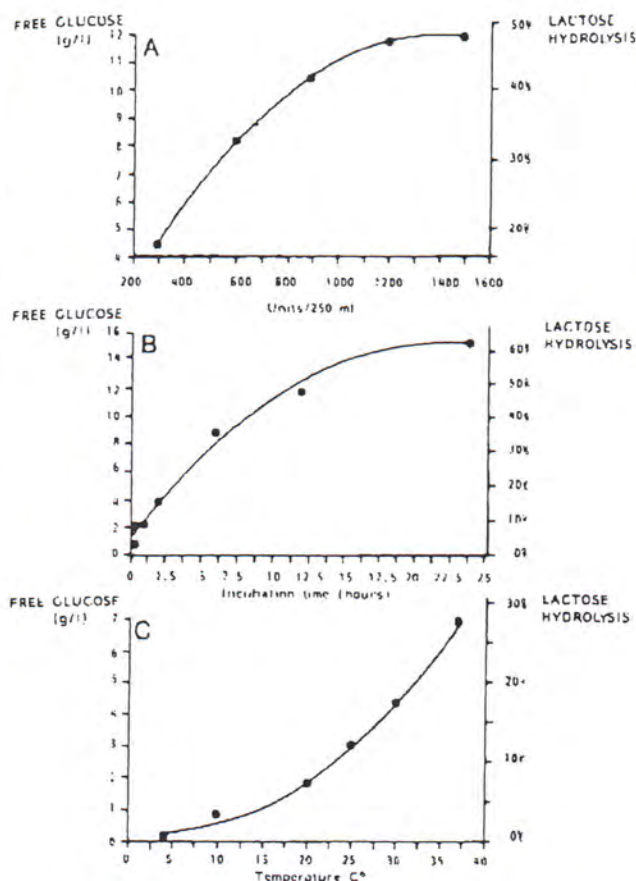


FIG. 1. Hydrolytic activity of lactase by *Kluyveromyces fragilis* tested under different conditions. Activity is expressed as g/l of glucose produced and as corresponding percentage of lactose hydrolysis. 1) Milk incubated for 12 h at 4°C with increasing concentrations of lactase. 2) Milk incubated with lactase 1000 U/250 ml at 4°C for periods ranging from 15 min to 24 h. 3) Milk incubated 30 min with lactase 1000 U/250 ml at different temperatures (from 4°C to 37°C).

### Lactose absorption

Of the 67 subjects who entered the study, only 52 (nine males and 43 females), that is 78%, were proved to be lactose malabsorbers on the basis of the first breath test (test A) and underwent the further steps of the protocol.

In this test, the maximal peak in H<sub>2</sub> concentration above baseline was 103.7 ± 8.80 ppm, with a mean value in five periods of 78.3 ± 5.49 ppm.

When lactase was added to milk shortly before ingestion (test B), we had a significant reduction in H<sub>2</sub> maximal peak (62.8 ± 7.61 ppm;  $p < 0.0005$ ) and in H<sub>2</sub> mean concentration (43.5 ± 4.99 ppm;  $p < 0.0005$ ) (Fig. 2).

Incubation of milk with lactase for 12 h (test C) lowered the same parameters to 42.4 ± 7.29 ppm (peak), and to 36.7 ± 5.01 (mean). Exclusively with regards to these 40 subjects, with test A we obtained 101.5 ± 11.08 ppm for H<sub>2</sub> peak and 87.5 ± 6.03 ppm for H<sub>2</sub> mean. With test B, the same values were 61.7 ± 9.97 ppm and 56.3 ± 6.22 ppm, respectively. Differ-

ences in all parameters obtained with the three tests proved to be statistically significant (minimum significance level,  $p < 0.02$ ). Results and significances of this group are summarized in Table 1.

After incubation of milk with lactase for 24 h (test D), peak H<sub>2</sub> was 40.6 ± 6.17 ppm, and mean H<sub>2</sub> was 29.7 ± 4.35 ppm. In these 12 patients, we obtained 108.0 ± 11.20 ppm (peak H<sub>2</sub>) and 91.3 ± 8.13 ppm (mean H<sub>2</sub>) with test A; the same parameters were 64.5 ± 5.75 ppm (peak H<sub>2</sub>) and 43.8 ± 4.21 ppm (mean H<sub>2</sub>) with test B (Table 2).

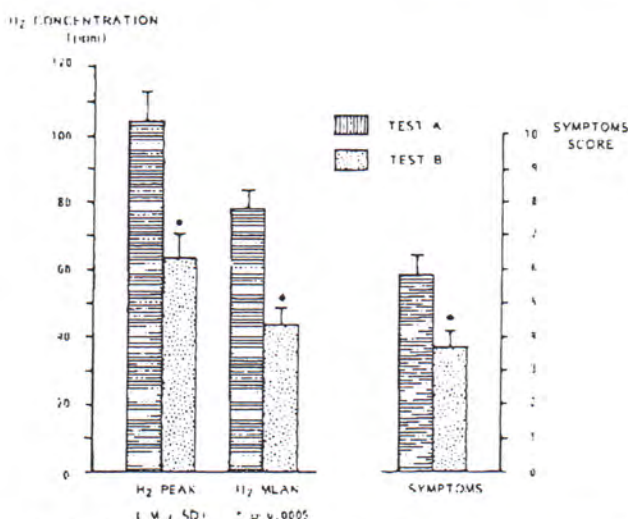


FIG. 2. Values of H<sub>2</sub> in expired air (peak and mean excretion) expressed in parts per million (ppm) and symptoms score obtained from 52 proved lactose malabsorbers during test with milk alone (test A) and test with lactase added to milk shortly before ingestion (test B) (mean ± SE).

TABLE 1

Values of H<sub>2</sub> maximal (Peak) and Mean Excretion Expressed in Parts per Million (mean ± SE) Obtained from 40 Lactose Malabsorbers during H<sub>2</sub> Breath Test\*

	Test A	Test B	Test C
H <sub>2</sub> Peak†	101.5 ± 11.08	61.7 ± 9.97	42.4 ± 7.29
H <sub>2</sub> Mean‡	87.5 ± 6.03	56.3 ± 6.22	36.7 ± 5.01

\* With milk alone (test A), milk with lactase added shortly before ingestion (test B), and milk incubated with lactase for 12 h (test C).

† H<sub>2</sub> peak: A-B  $p < 0.005$ ; A-C  $p < 0.0005$ ; B-C  $p < 0.025$ .

‡ H<sub>2</sub> mean: A-B  $p < 0.005$ ; A-C  $p < 0.005$ ; B-C  $p < 0.01$ .

TABLE 2

Values of H<sub>2</sub> Maximal (Peak) and Mean Excretion Expressed in Parts per Million (mean ± SE) Obtained from 12 Lactose Malabsorbers during H<sub>2</sub> Breath Test\*

	Test A	Test B	Test D
H <sub>2</sub> Peak	108.0 ± 11.2	64.5 ± 5.75	40.6 ± 6.17
H <sub>2</sub> Mean	91.3 ± 8.13	43.8 ± 4.21	29.7 ± 4.35

\* With milk alone (test A), milk with lactase added shortly before ingestion (test B), and milk incubated with lactase for 24 h (test D).

† H<sub>2</sub> peak: A-B  $p < 0.005$ ; A-D  $p < 0.0005$ ; B-D  $p < 0.005$ .

‡ H<sub>2</sub> mean: A-B  $p < 0.0005$ ; A-D  $p < 0.0005$ ; B-D  $p < 0.005$ .



The percentage of subjects who had H<sub>2</sub> excretion fully normalized (concentration <20 ppm in any determination) were 21% with test B, 40% with test C, and 42% with test D.

Symptoms

With test B, we observed a significant reduction of symptoms with regard to abdominal distension ( $p < 0.02$ ) and bloating ( $p < 0.01$ ); no significant difference was found in abdominal pain and flatulence. Test C caused a reduction of abdominal pain ( $p < 0.05$ ), abdominal distension ( $p < 0.001$ ), and bloating ( $p < 0.01$ ), but no difference in flatulence. No better results were obtained with test D.

Roughly 6% of the subjects were free of all symptoms during test A, 38% during test B, 75% during test C, and 83% during test D.

Only four subjects experienced diarrhea, so that factor dropped out of the symptoms analysis.

Global score of symptoms attained to the following mean values: test A =  $5.85 \pm 0.56$ , test B =  $3.71 \pm 0.45$ , test C =  $2.77 \pm 0.63$ , test D =  $1.7 \pm 0.68$ . Symptoms values of tests B, C and D showed a  $p < 0.0005$  versus test A values.

In Table 3, symptoms values of single subgroups with statistical significances are summarized.

To evaluate with more certainty whether prolonged incubation with lactase (24 h) offers any advantage over shorter incubation (12 h), we compared mean values of symptoms and H<sub>2</sub> excretion improvements obtained with these two methods with the same values obtained with test A. Mean reductions obtained in H<sub>2</sub> excretion were  $21.9 \pm 3.88$  ppm with test C, and  $23.3 \pm 5.62$  with test D. Mean reductions in symptoms score with these two tests were  $3.3 \pm 0.47$  and  $3.4 \pm 0.77$ . These values are fully comparable.

We also wished to determine whether a reduction in H<sub>2</sub> excretion, as index of improved lactose absorption, effectively parallels symptoms reduction. Figure 3

shows the correlation between differences in symptoms score and in H<sub>2</sub> mean excretion for 40 subjects who underwent test A and C. We obtained an  $r$  of 0.44, with a  $p < 0.01$ .

DISCUSSION

In 52 proved lactose malabsorbers, enzyme replacement therapy proved to effectively improve lactose malabsorption (expressed as reduction of H<sub>2</sub> breath excretion) and to reduce subjective manifestations of discomfort or objective pathologic response to milk ingestion. In addition, as shown in Fig. 3, it is possible to obtain a significant relationship between reduction of H<sub>2</sub> breath excretion and symptoms score.

All experiments permitted us to obtain satisfactory clinical results, even under different conditions. This agrees with the *in vitro* curves of lactase activity that show sufficient lactose hydrolysis in each condition tested. Even if the temperature optimum of the enzyme was 37°C, we obtained an appreciable lactose hydrolysis also at 4°C (Fig. 1). It may be useful to point out that, whereas the percentage of lactose hydrolysis *in vitro* is

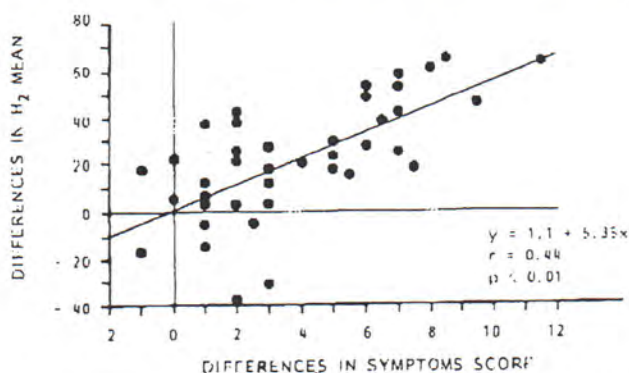


FIG. 3. Differences of H<sub>2</sub> mean excretion and of symptoms score obtained between ingestion of milk without lactase (test A) and milk incubated with lactase for 12 h (test C) in 40 lactose malabsorbers. A positive difference corresponds to a value reduction during test C.

TABLE 3  
Symptoms Scores and Significances in Four Tests\*

	Test A	Test B	Test C	Test D
All subjects	$5.85 \pm 0.56$	$3.71 \pm 0.45$	—	—
	← $p < 0.0005$ →			
Group of 40	$6.27 \pm 0.69$	$3.83 \pm 0.41$	$2.77 \pm 0.63$	—
	← $p < 0.005$ →		← NS →	
	← $p < 0.0005$ →			
Group of 12	$5.12 \pm 0.97$	$3.55 \pm 1.09$	^	$1.70 \pm 0.68$
	← $p < 0.05$ →		← $p < 0.01$ →	
	← $p < 0.005$ →			

\* For H<sub>2</sub> breath test with milk alone (test A) and milk with lactase added shortly before ingestion (test B), particular values relative to subjects who subsequently underwent test with milk incubated with lactase for 12 h (test C) and for 24 h (test D) are given.



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rather low in the first hour, nevertheless, when the enzyme is added to milk shortly before ingestion, we obtain a fairly good reduction of  $H_2$  production and symptoms. This seems to indicate that the enzyme is not immediately inactivated in the stomach, but continues its action in lower tracts of the gut.

The milk preincubated *in vitro* for 24 h, as expected, permitted more efficient lactose hydrolysis than the preincubation for 12 h, but the amelioration of intolerance symptoms was not sufficiently significant to justify the prolongation of the incubation period. Otherwise, this procedure seems to be applicable at home, and it does not sensibly alter the taste of milk as stated by each patient.

When the procedure of preincubation is not possible, we feel that the extemporaneous addition of lactase to milk is convenient, even if this procedure is less efficient in reducing the entity of lactose malabsorption.

On the other hand, it is important to outline that our results suggest that complete quantitative hydrolysis of all lactose does not appear to be a prerequisite for lactose tolerance in malabsorbers. In fact,  $H_2$  production after all tests performed shows in all cases an incomplete lactose hydrolysis with consequent lactose escape from small bowel. We found that 75% of patients studied did not complain of intolerance symptoms after 12 h of milk incubation, and 83% were asymptomatic after 24 h of milk incubation, whereas the percentages of the subjects with complete absorption were only 40% and 42%, respectively.

Those satisfactory results, which confirm those reported in previous papers (2, 12, 13), indicate good bioavailability of the lactase used, maybe also due to the liquid form of preparation, which makes this therapy an effective and rational approach in milk-intolerant individuals.

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