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Enzyme Replacement for Lactose Malabsorption Using a Beta-D-Galactosidase

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We evaluated 10 healthy symptomatic lactose malabsorbers for effect of an oral beta-D-galactosidase derived from *Aspergillus oryzae* (Lactrase, Kremers Urban Company, Milwaukee, WI, U.S.A.) on symptom and breath hydrogen response to challenge with 50 g lactose. Basally and at 30-min intervals for 8 h after lactose challenge, end-alveolar breath samples were collected and analyzed for hydrogen using gas chromatography. Symptoms were scored at 30 min and hourly for 8 h, rating bloating, cramps, nausea, pain, diarrhea, and flatulence. Four challenges were performed on 4 separate days with at least 3 days between challenges. The first two challenges served as baselines. Just before ingestion of 50 g powdered lactose dissolved in 200 ml water, beta-D-galactosidase capsules were given orally as a 250-mg dose for the third challenge and a 500-mg dose for challenge 4. Hydrogen excretion, quantified by using a trapezoidal method for computing area under the discontinuous curve of breath hydrogen concentration, was decreased in subjects receiving beta-D-galactosidase (baseline I, 346.0 ppm/h; baseline II, 367.2 ppm/h; 250-mg galactosidase, 208.2 ppm/h; 500-mg galactosidase, 178.0 ppm/h; $p \leq 0.05$). Other analyzed parameters of H_2 excretion were also decreased. Analysis of symptom response scores showed a dose-related decrease for bloating and flatus ($p \leq 0.05$) and no statistical difference in the other assessed symptoms. We conclude that beta-D-galactosidase from *Aspergillus oryzae*, when given just before ingestion of lactose by lactose malabsorbers, can produce a dose-dependent reduction (statistically significant for the 500-mg dose) in breath hydrogen excretion, bloating, and flatus.

Key Words: Lactose intolerance—Enzyme replacement—Lactrase—Lactose malabsorption—*Aspergillus oryzae*—Galactosidase.

Beta-galactosidases have hydrolytic activity in vivo when given as enzyme replacement therapy (1), and several such products derived from various fungal sources are marketed in the United States (2,3). Little data, however, are available about their effect on objective determinants of lactose absorption, particularly when given at mealtime, just prior to lactose ingestion (1,2,4–6). We evaluated a new beta-D-galactosidase, Lactrase (Kremers Urban Co., Milwaukee, WI, U.S.A.), derived from *Aspergillus oryzae* because of its several unique properties, including stability over a wide pH range, optimal activity at body temperature, and its capsule dosage form, convenient for mealtime administration (5,6).

We evaluated the effects of Lactrase on breath hydrogen (H_2) excretion, which is an objective determinant of carbohydrate absorption (7–11), and symptom response to lactose challenge.

METHODS

Study Population

Ten healthy lactose malabsorbers formed the study group. Lactose malabsorption was previously defined by screening breath H_2 tests with an H_2 rise of greater than 20 parts per million (ppm) above the baseline value after ingestion of 50 g lactose. All had symptoms referable to lactose intolerance. There were 6 men and 4 women, with a mean age was 43.5 ± 11.6 (SD) years. Subjects were fasting, nonsmoking, and NPO overnight and during testing. The evening meal prior to testing was requested to be low fiber, low carbohydrate, and lactose restricted. No subject had a history of chronic small bowel disease or gastrointestinal resection. Subjects denied use of oral antibiotics or cathartics for 4 weeks prior to and during the study period.

Lactose Challenge Studies

On 4 separate days, with at least 3 days between challenges, subjects ingested 50 g powdered lactose dissolved in 200 ml water. Studies 1 and 2 served as

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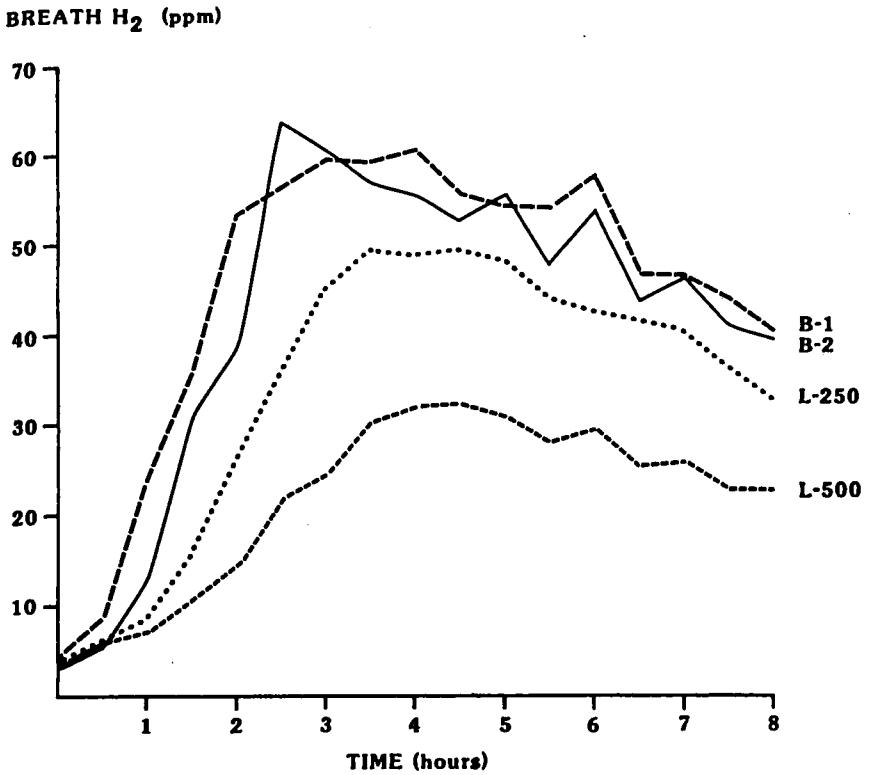

FIG. 1. Effect of Lactrase on H_2 excretion after 50-g lactose challenge. B-I and B-II, baselines; L-250, 250 mg Lactrase given prior to lactose challenge; L-500, 500 mg Lactrase given prior to lactose challenge. Results are expressed as the mean of 10 study subjects.

baselines (B-I, B-II). Just prior to lactose challenge, Lactrase capsules were given orally as a 250-mg dose for study 3 (L-250) and as a 500-mg dose for study 4 (L-500). Lactrase, as provided by Kremers Urban Company, contained 125 mg of standardized lactase enzyme dispersed in maltodextrins, a starch-like filler.

Hydrogen Excretion

End-alveolar breath samples were collected using an alveolar gas collection system (Ga Sampler, Quintron Instrument Company, Milwaukee, WI, U.S.A.), which discards the first portion of expired air and captures the rest of a single expiration in a gas-impermeable bag. Two 30-ml samples were collected at baseline and after challenge every 30 min for 8 h. Samples were analyzed for H_2 content by gas chromatography (Microlyzer, Model 12, Quintron Instrument Co.).

Symptom Scoring

Symptoms were assessed by a self-administered questionnaire. Bloating, flatus, cramps, nausea, and abdominal pain were rated at 30 min and then hourly for 8 h after lactose challenge and were graded as 0 (none) to 4 (severely distressing). The number of stool motions experienced were recorded at each grading session. Cumulative scores for the 8-h study period were added for group comparisons.

Data Analysis

Symptoms scores and breath hydrogen data were compared using Fisher's test of least significant difference. A p-value less than 0.05 was considered to be statistically significant. Data are expressed as the mean ± standard error of the mean.

RESULTS

Breath H_2 excretion results are shown in Fig. 1. Mean baseline H_2 values were not different between challenge study groups (Table 1). The breath hydrogen excretion data were compared between groups using various parameters: (a) peak H_2 rise above mean fasting baseline value (peak H_2 rise), (b) the sum of the highest 5 increments above fasting baseline H_2 (Hi 5 H_2), (c) cumulative H_2 excretion over the 8-h testing period (CUM H_2), and (d) the area under the discontinuous curve of H_2 excretion calculated by a trapezoidal method (H_2 AUC). These parameters have been used in other studies for group comparisons, with CUM H_2 and H_2 AUC suggested as reliable parameters for such comparisons (12). Peak H_2 rise, Hi 5 H_2, CUM H_2, and H_2AUC were not different for baselines (B-I and B-II) or 250-mg Lactrase (L-250) study groups (Table 1). The 500-mg Lactrase group subjects, however, have significantly lower H_2 excretion as assessed by all four parameters ($p \leq 0.05$).

Cumulative symptom response scores are shown in Table 2. Symptom scores were significantly lower for bloating and flatus in L-250 and L-500

TABLE 1. *Effect of Lactrase on various parameters of H_2 excretion after lactose challenge*[a]

	Challenge group			
	B-I	B-II	L-250	L-500
Baseline H_2 (ppm)	3.2 ± 0.7	4.4 ± 0.8	4.1 ± 0.7	3.8 ± 0.4
Peak H_2 rise (ppm)	75.2 ± 14.9	81.3 ± 10.9	56.1 ± 13.0	33.3 ± 5.4[b]
Hi 5 H_2 (ppm)	67.2 ± 13.4	64.0 ± 10.2	52.6 ± 10.8	32.6 ± 4.4[b]
CUM H_2 (ppm)	366.2 ± 81.0	392.9 ± 59.7	294.6 ± 60.8	188.0 ± 25.5[b]
H_2 AUC (ppm/h)	346.0 ± 77.8	367.2 ± 56.9	280.3 ± 56.3	178.0 ± 24.1[b]

[a] Data expressed as mean ± SEM for 10 study subjects.
[b] $p < 0.05$.
Peak H_2 rise, maximal postlactose increment above fasting basal H_2; Hi 5 H_2, mean of the highest 5 increments; CUM H_2, cumulative H_2 excretion over 8 h; H_2 AUC, area under the discontinuous curve computed by a trapezoidal method.

groups. No differences were observed for the other assessed symptoms.

DISCUSSION

Early lactase enzyme preparations available for clinical use were beta-galactosidases manufactured from *Kluyveromyces lactis* yeast (13). These were offered as a dry powder in individual dose packets and could hydrolyze 70% of lactose in 1 q of milk in 24 h. A liquid enzyme preparation became available that provided longer shelf-life and more practical use. Lactases from *Aspergillus niger* and *Saccharomyces lactis* became available for pretreatment of milk before consumption, but had the disadvantage of imparting a noticeably sweeter taste to milk and requiring 24 h for hydrolysis, thus making these enzymes usable for milk only when taken at home or carried from home (1). Powder and liquid forms were also used to hydrolyze lactose, but the preparations were not suitable for use with other lactose-containing foods, particularly solids such as cheese.

Despite discouraging prior work with mealtime administration of enzyme preparations, "enzyme replacement therapy" given immediately before milk and solid lactose food ingestion was investigated (1,2). The effectiveness of in vivo lactose hydrolysis was demonstrated using enzymes of yeast source, *Kluveromyces lactis* (Lact Aid, Sugar Lo Co., Pleasantville, NJ, U.S.A.), and fungal source, *Aspergillus niger* (Lactase N, G. B. Fermentation Industries, Kingstree, SC, U.S.A.). Our interest was stimulated by the availability of a galactosidase from *Aspergillus oryzae* (Lactrase, Kremers Urban Co.) having unique properties that would favor mealtime administration and would hydrolyze lactose from foods other than milk. Characteristics of three commercial beta-galactosidases are outlined in Table 3. Temperature and pH characteristics of Lactrase seemed optimal for body conditions and its capsule form appeared to be ideal for mealtime use (2) (S.S. Wagle, personal communication).

Our investigation was designed to limit variables by using powdered lactose in water and measuring carbohydrate absorption objectively using breath

TABLE 2. *Lactrase effect on symptom response scores after lactose challenge*

	Challenge group[a]			
	B-I	B-II	L-250	L-500
Bloating	8.4 ± 1.6	9.0 ± 2.1	6.4 ± 2.1[b]	2.7 ± 0.7[b]
Flatus	9.6 ± 2.1	10.4 ± 2.1	7.6 ± 2.2[b]	6.1 ± 1.7[b]
Cramps	3.3 ± 1.6	3.6 ± 1.3	3.9 ± 2.0	1.1 ± 0.3
Nausea	1.3 ± 0.5	1.9 ± 1.1	1.1 ± 0.5	0.3 ± 0.2
Abdominal pain	4.7 ± 1.7	3.2 ± 1.2	5.3 ± 2.1	1.6 ± 0.8
Diarrhea	2.7 ± 1.3	4.1 ± 0.8	4.8 ± 1.5	2.2 ± 0.9
Number of motions	3.5 ± 1.2	6.3 ± 2.1	6.1 ± 2.0	3.4 ± 1.3

[a] Sum of symptom scores; data expressed as mean ± SEM of 10 study volunteers.
[b] $p < 0.05$. B-I and B-II, baseline studies; L-250, 250 mg Lactrase given prior to lactose challenge; L-500, 500 mg Lactrase given before challenge.

TABLE 3. *Characteristics of available beta-D-galactosidase preparations*

Brand	Source	pH optimum	T° optimum	Form	Retail price[a]
Lact-Aid	*Kluyveromyces lactis*	6.8	37°C	Liquid	$0.12
				Tablet	0.13
Lactrase-N	*Aspergillus niger*	4.4	60°C	Powder	NA
Lactrase	*Aspergillus oryzae*	4.5–5.5	37°C	Capsule	$0.34

[a] Retail prices per usual recommended dose are provided, but preparations are not comparable.

H_2 methods. These data confirm the in vivo hydrolysis of lactose by Lactrase taken immediately prior to lactose challenge. There was a quantitative, dose-dependent reduction in H_2 excretion after Lactrase, as documented by comparison of various assessed parameters. In addition, reductions in symptom response scores were also seen, with statistically significant decreases in bloating and flatus. Other investigators have shown similar results in children and adults ingesting milk (4–6). Additional work is underway comparing different potencies of the enzyme and types of lactose challenge. It remains to be seen whether or not other symptoms, such as abdominal discomfort, cramps, nausea, or diarrhea, will be ameliorated with higher doses of Lactrase.

We conclude that Lactrase beta-galactosidase from *Aspergillus oryzae* hydrolyzes lactose in vivo and is associated with a reduction in symptoms. It can be effectively and conveniently administered at mealtime. Other preparations of lactase enzyme supplements are available (Table 3), but few have been carefully studied. Prices vary, but lactase content and bioactivity also vary dramatically. As it is not practical and would be expensive to prescribe lactrase for every meal, we advise restriction of lactose-containing foods for symptomatic lactose malabsorbers. We suggest Lactrase for social situations when the lactose content of the meal is unknown or with lactose meals known to produce symptoms.

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