Effective in vivo hydrolysis of milk lactose by beta-galactosidases in the presence of solid foods[1-3]

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ABSTRACT The feasibility of enzyme replacement therapy with exogenous, food-grade, microbial enzymes at mealtime to effect intragastrointestinal hydrolysis of the lactose from 360 ml of cow's milk consumed with a solid food feal (breakfast cereals) was investigated in adult Guatemalan lactose-malabsorbers using a hydrogen breath-analysis procedure to quantify the completeness of postprandial carbohydrate absorption. Adding 2 g of a commercial preparation of beta-galactosidase from *Kluyveromyces lactis* at mealtime to milk taken with a refined cereal (cornflakes) and an unrefined cereal (bran) reduced the production of excess breath H_2 attributable to lactose maldigestion to a level not significantly different from that achieved with lactose-prehydrolyzed milk. Sucrase, as expected, had no effect on H_2 production. A beta-galactosidase from *Aspergillus niger* was less effective that the *K. lactis* enzyme for in vivo hydrolysis. Thus, exogenous betagalactosidases can eliminate lactose malabsorption in lactase-deficient individuals even in the presence of solid foods, allowing lactose intolerant persons to consume milk and dairy products without gastrointestinal discomfort. *Am J Clin Nutr 1985;41:000–000.*

KEY WORDS Lactose, lactase deficiency, in vivo hydrolysis, carbohydrates, dietary fiber, lactose intolerance, milk, betagalactosidase, invertase

Introduction

Primary adult lactase deficiency associated with various degrees of lactose malabsorption and milk intolerance affects the majority of the world's population, especially concentrated in non-white ethnic groups (1, 2). Today, a host of food-grade, beta-D(−)galactosidase enzyme preparations from various microbial organisms are commercially available. They have been conventionally used to hydrolyze the lactose in milk to its constituent monosaccharides—glucose and galactose—by in vitro incubation. Such prehydrolysis of lactose has been shown to reduce the symptoms of milk intolerance in lactose-malabsorbers (3–8). However, the inconvenience and premeditation involved in the in vitro pretreatment of milk with beta-galactosidases presents severe limitations to its use; only milk consumed at home, or carried from home can be drunk by a milk-intolerant lactose-absorber who employs this strategy.

To overcome these limitations, the notion of adding beta-galactosidases directly to milk at mealtime as a form of "enzyme replacement therapy" for individuals with lactase deficiency has emerged (9–11). Such an approach has been shown to reduce intolerance symptoms when lactose-malabsorbers (9) or post-gastrectomy patients (10) consume dietary amounts of milk. Using the hydrogen

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breath-test as an index of the completeness of carbohydrate absorption, we reported a 40% reduction in colonic fermentation of lactose after adding 0.5 g of a preparation of beta-galactosidase from *Klyveromyces lactis* to 350 ml (12 oz) of milk fed to lactose malabsorbers (11); this presumably represented some degree of in vivo hydrolysis of the lactose by the exogenous enzyme. Subsequently, we have confirmed the effective elimination of excess H_2 production with the same volume of milk when 1.5 g of the enzyme preparation was added prior to consumption (Solomons NW, Rosado JL: Unpublished observations).

Dietary practice suggests, however, that milk is most commonly consumed not as an isolated beverage, but as part of a mixed meal that contains solid foods. A typical situation would be a breakfast containing a glass of fluid milk plus milk added to cereal and fruit. Our initial experience with 0.5 g of enzyme suggested that solid foods inhibit the in vivo activity of exogenous beta-galactosidases (11). In the present report, we demonstrate in healthy subjects with primary lactase deficiency that effective enzymatic activity of exogenous beta-galactosidases is possible even in the presence of food if the proper preparation and dosage are chosen.

Subjects and methods

The H_2 breath-analysis test for carbohydrate malabsorption

Carbohydrate malabsorption was determined using a modification of the H_2 breath-analysis test previously reported for use in adult subjects in our laboratory (12). A sample of mixed, expired air was collected by having the subject breath through a one-way Hans Rudolph valve into a 5-L rubber anesthesia bag. The samples were then delivered into a plastic syringe, fitted with a three-way stopcock. The H_2 concentration was determined with a Microlyzer Model 12 breath H_2 analyzer (13, 14) (Quintron Instruments Co, Milwaukee, WI, USA). Breath was collected before, and at 60-min intervals postprandially over 6 h. Subjects were classified as "lactose-malabsorbers" if they manifested an increment in breath H_2 concentration of $\geq$20 ppm after ingestion of 18 g of lactose as 360 mL of fluid whole cow's milk. The protcol was approved by the Committee on the Use of Humans as Experimental Subjects of MIT.

Experiment #1

Ten healthy adult lactose-malabsorbers received four different test meals in a randomized order separated by at least 72 h. All meals consisted of 40 g of cornflakes, one banana, one hard-cooked egg, and 360 mL of cow's milk: *Meal A*, intact, whole milk; *Meal B*, milk incubated for 24 h at 4°C with 0.25 g of 'LactAid' (SugarLo Co, Pleasantville, NJ, USA), a beta-galactosidase derived from *Kluyveromyces lactis*[6]; *Meal C*, milk to which 2 g of LactAid was added *immediately prior* to the meal; *Meal D*, milk to which 266 mg of 'Lactase N' (GB Fermentation Products Co, Kingstree, SC, USA), a beta-galactosidase derived from *Aspergillus niger*, was added *immediately prior* to the meal. Both enzyme preparations have about 11% protein by weight.

Experiment #2

Ten proven lactose-malabsorbers, including five who participated in Exp #1, were studied in another protocol in which five different meals were presented in a randomized order. All meals consisted of 28 g of bran cereal, one banana, and 360 ml of cow's milk, but the milk varied among the meals as follows: *Meal E*, intact, whole milk; *Meal F*, milk prehydrolyzed with 0.25 g of LactAid during 24 h of incubation; *Meal G*, milk to which 8000 IU of sucrase (invertase, Sigma Chemical Co, St. Louis, MO, USA) had been added; *Meal H*, milk to which 1 g of LactAid was added *immediately prior* to the meal; *Meal I*, milk to which 2 g of LactAid were added *immediately prior* to the meal.

Data analysis

Descriptive statistics of mean ± SEM for the absolute breath H_2 concentrations and for the *change* in breath H_2 concentration was calculated for each treatment. Also an estimate of excess breath H_2 excretion was calculated by integrating the area under the 6-h curve of breath H_2 concentration change by triangulation, and expressing the volume in arbitrary units, ppm · hr, as previously described (15). Comparisons between treatments were performed using the Student t test for paired samples (16).

Results

The composite curves of breath H_2 concentrations and the changes in breath H_2 concentration serially over 6 h for the cornflakes-based meal and the various forms of milk (*Meals A–D*) in Exp #1 are shown in Figure 1. The respective mean areas under the breath H_2 curves were, in ppm · hr: *Meal A*, 73 ± 22; *Meal B*, 11 ± 4; *Meal C*, 12 ± 7; and *Meal D*, 36 ± 14. Prehydrolyzed milk and mealtime-treated milk containing 2 g of LactAid produced a significant decrease in the excretion of H_2 as compared to the meal containing intact milk ($p < 0.01$). No

[6] This 24-h incubation in known to effect a >90% hydrolysis of lactose to its constituent monosaccharides: galactose and glucose.

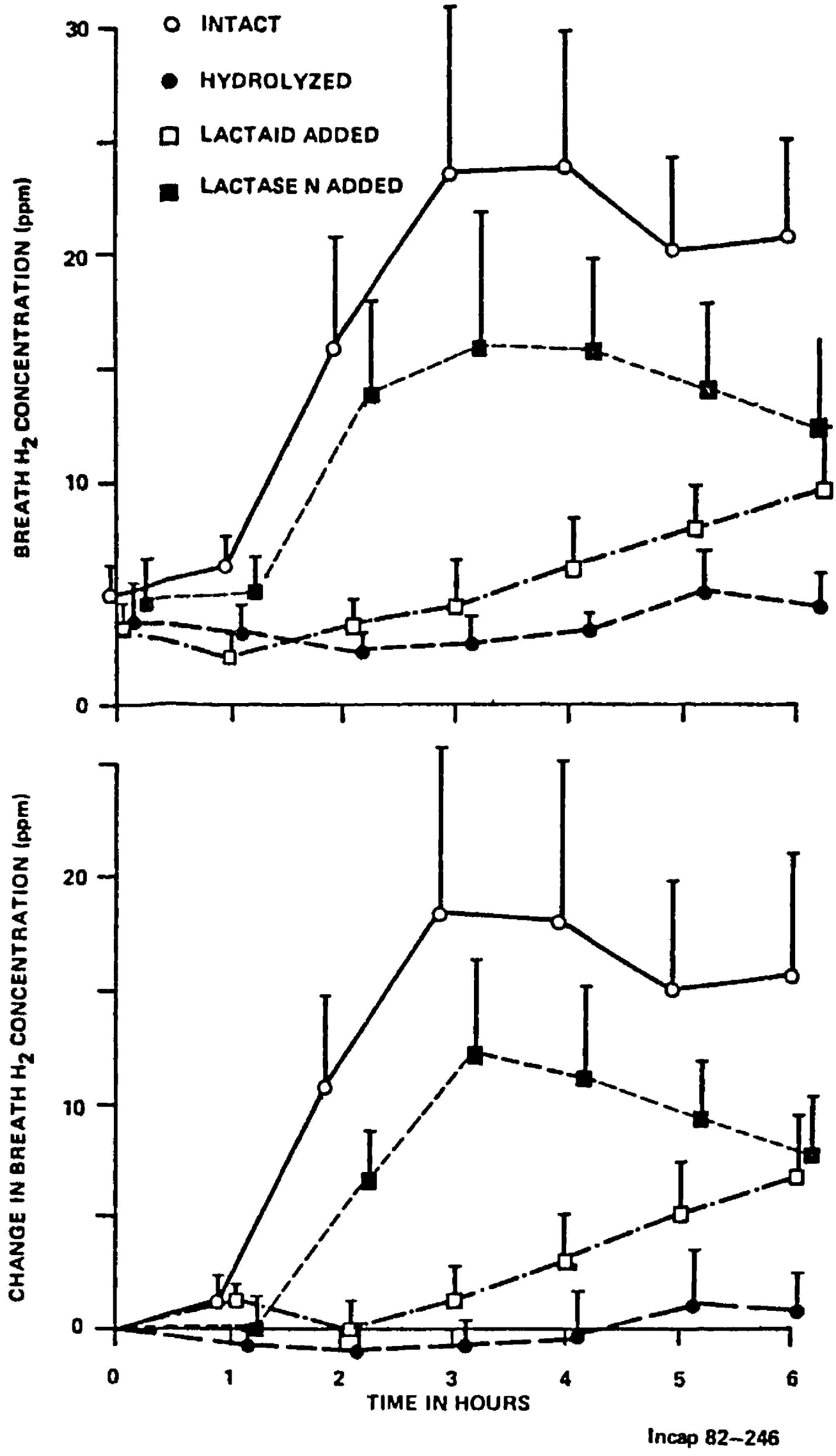

FIG 1. The mean (±SEM) of concentration of breath H_2 measured serially over 6 h in 10 lactose-malabsorbers who consumed the various forms of 360 ml volumes of milk along with cornflakes, banana and egg for the respective treatments: Meal A, with intact, whole milk (O); Meal B, with 24 h incubated, prehydrolyzed milk (●); Meal C, with 2 g of LactAid added to milk (□); Meal D, with 266 mg of 'Lactase N' added to the milk (■). The upper graph shows the absolute H_2 concentration, and the lower graph the *changes* in breath H_2 concentration. Areas under the curve are calculated from the change in breath H_2 concentration. Both enzyme preparations have about 11% (w/w) protein.

statistically significant difference in excess breath H_2 excretion between the 24-h and the immediate pretreatments with LactAid were seen. Lactase N produced no reduction in breath H_2 excretion as compared to intact milk ($p < 0.1$), and excess H_2 production was significantly greater with the mealtime addition of the *A. niger* enzyme than with the *K. lactis* preparation ($p < 0.05$).

Data for the bran cereal studies (*Meals E–I*) are shown in Figure 2. The respective mean areas under the breath H_2 concentration curves were, in ppm · hr; *Meal E*, 83 ± 26; *Meal F*, 19 ± 17; *Meal G*, 113 ± 18; *Meal H*, 60 ± 9; *Meal I*, 51 ± 15. Although the immediate-addition curves were not as superimposable on the prehydrolyzed milk treatment as they had been for the cornflakes in Exp #1, once again, no significant differences between 24-h and immediate pretreatments with LactAid was observed with the bran cereal meals. Both replacement dosages of LactAid produced a significant reduction in the 6-h breath H_2 excretion volume as compared to the sucrase ($p < 0.05$). There was a qualitatively greater production of H_2 with the prehydrolyzed milk and the bran cereal (*Meal F*) as compared to prehydrolyzed milk and cornflakes (*Meal B*). This is presumably due to the colonic fermentation of the dietary fiber components in the unrefined breakfast cereal.

Discussion

Lactase deficiency is widespread, and since lactose ingestion produces untoward manifestations of gastrointestinal discomfort in many lactase-deficient individuals, they may reduce or restrict their intake of milk and dairy products with a consequent diminution in total consumption of calcium, riboflavin, vitamin D, and other nutrients abundant in these foods. Thus, strategies to permit lactose-intolerant persons to consume milk have been sought, many based on the pretreatment of milk with food-grade, microbial beta-galactosidases. The inconvenience of this approach, however, led several groups to examine mealtime replacement therapy with "lactases" (9–11), analagous to the treatment of pancreatic insufficiency with pancreatic extracts (17). Quantitative evidence of in vivo hydrolysis was lacking in some studies (9, 10), and it was of obvious importance to determine whether food interfered with beta-galactosidase replacement therapy.

In the present study, we demonstrate that a fourfold increase of the dosage of LactAid to milk at mealtime as part of a cornflakes and egg breakfast (11) produced quantitative elimination of lactose-related breath H_2 excretion, equivalent to that effected by prehydrolyzed milk. The *A. niger* enzyme, Lactase N, had no effect at the dosage chosen. These two enzymes have markedly different temperature and pH optima for in vitro hydrolysis (9), with the *K. lactis* beta-galactosidase having optimal conditions of pH (6.8) and temperature (37°C) approximating those found in the small intestine. This might explain its greater efficacy. This enzyme also reduced postprandial colonic fermentation of lactose when added to the bran cereal breakfast. The elimination of excess hydrogen excretion due to lastose was not so quantitative as with the cornflakes, however. If physical or chemical factors in food are responsible for the inhibition of in vivo enzymatic function, we might speculate that binding might be a mechanism, since bran has a higher amount of dietary fiber.

That the effect of addition of beta-galactosidase to milk at mealtime is truly *enzymatic*, rather than non-specific, is strongly suggested by the comparison with a hydrolase specific for sucrase (Invertase). We have also observed that heating the enzyme-added milk to 70°C eliminated effective reduction in breath H_2 excretion by lactose-malabsorbers (unpublished observations).

Preservation of the ability and desire of individuals with primary or acquired lactase deficiency to consume milk and dairy products has nutritional advantages with regard to calcium intake and dietary diversity. The addition of appropriate doses of food-grade beta-galactosidases at mealtime can provide effective hydrolysis of milk lactose even in the presence of solid foods. Thus, even the most lactose-sensitive malabsorber should now be able to enjoy meals containing breakfast cereals either by using prehydrolyzed milk or by adding exogenous beta-galactosidases as replacement therapy for their deficient intestinal lactase activity.

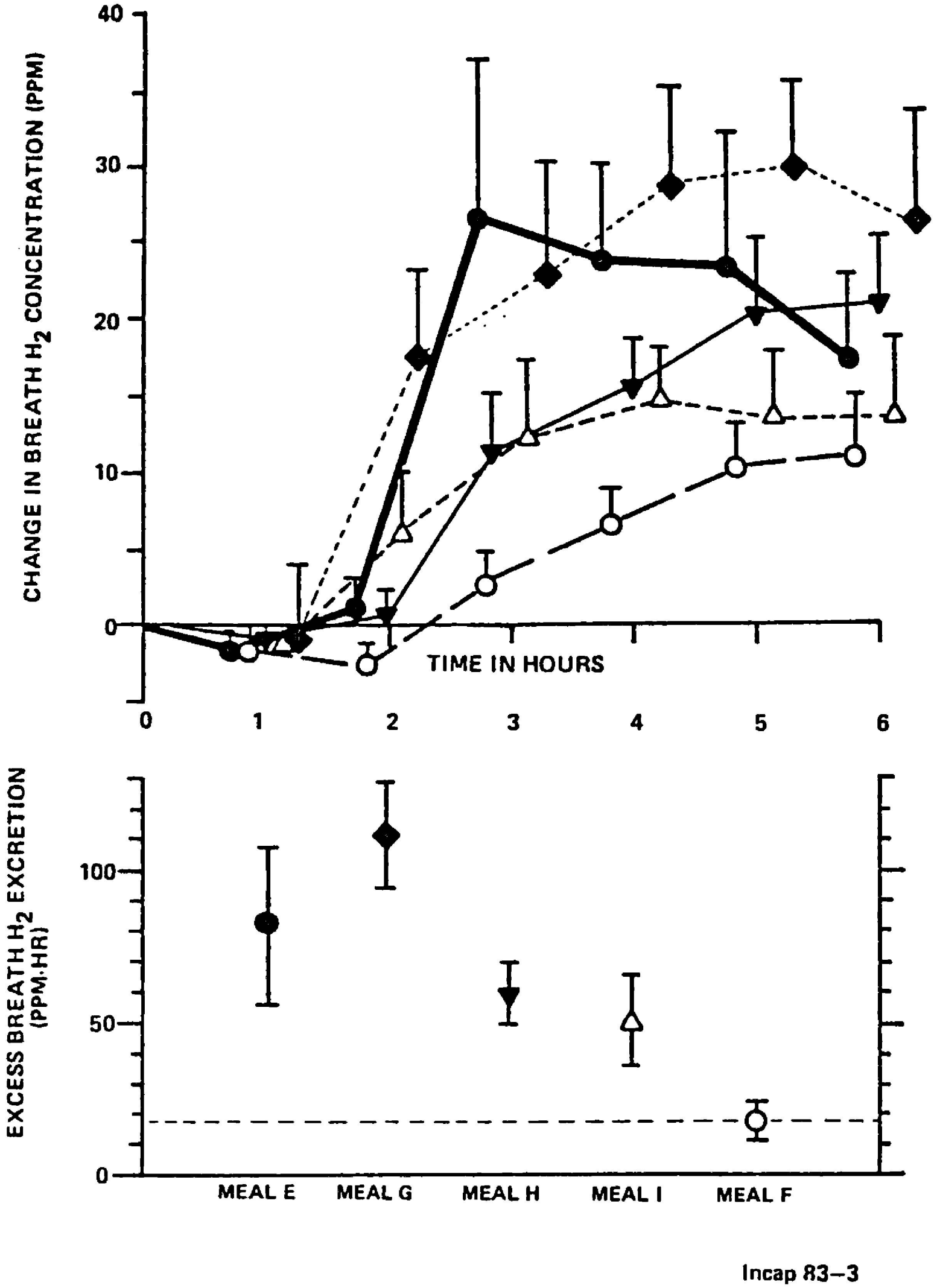

FIG 2. The upper graph shows the mean (±SEM) for the changes in breath H_2 concentration in the 10 lactose-malabsorbers who consumed the meal of bran cereal with various forms of milk in a 360 ml volume: Meal E, intact whole milk (●); Meal F, with 24-h incubated, prehydrolyzed milk (O); Meal G, with 8000 IU sucrase added to the milk (♦); Meal H, with 1 g of LactAid added to the milk (▼); Meal I, with 2 g of LactAid added to the milk (Δ). The lower panel presents the mean ± SEM of the excretion volumes of pulmonary H_2 above basal levels, expressed in ppm · hr. The dotted line passing through the mean of Meal F represents the contribution to total breath H_2 of the non-absorbed carbohydrates in the solid foods of the test meal of bran cereal.

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