Influence of *Lactobacillus acidophilus* Added to Milk on Lactose Malabsorption in Humans

S. E. Gilliland and H. S. Kim

Story in Brief

Consumption by lactose malabsorbers of milk containing viable cells of *Lactobacillus acidophilus* from a frozen concentrated culture significantly reduced lactose malabsorption. The degree of lactose malabsorption was measured using a breath hydrogen test (BHT). The effect of *L. acidophilus* on lactose malabsorption was determined by comparing the amount of hydrogen excreted in the breath of six human test subjects in each experimental group at the start of the trial and after consuming milk containing either 0, 2.5×10^6, 2.5×10^7, or 2.5×10^8 *L. acidophilus* per ml daily for 6 days. Consumption of milk without cells of *L. acidophilus* did not affect lactose malabsorption, but consumption of milk containing 2.5×10^6 or 2.5×10^8 *L. acidophilus* per ml significantly reduced malabsorption. However, milk containing an intermediate level (2.5×10^7/ml) did not influence malabsorption. The lack of a significant effect in the latter group of test subjects was probably due to large increases in excreted hydrogen on day 7 as compared to day 0 by two of the six test subjects. An additional trial showed that the beneficial effect of *L. acidophilus* on lactose malabsorption did not require that the milk be consumed daily. The reduced malabsorption was not due to hydrolysis of the lactose prior to consumption, which indicated that the beneficial effect must have occurred in the digestive tract after consuming milk containing *L. acidophilus*.

Introduction

The inability to absorb lactose, which is one of the several causes for milk intolerance in humans, has been associated with deficiency of lactase in the intestines. In lactase-deficient individuals the ingestion of one or two glasses of milk often leads to a gastrointestinal disorder which is called lactose intolerance and includes such symptoms as cramps, flatulence, and diarrhea. These symptoms discourage lactose malabsorbers from consuming milk.

The objectives of this study were to determine if the addition of cells of *L. acidophilus* to milk can prevent or reduce lactose malabsorption in humans and, if so, to determine whether or not the effect is dependent on consuming the milk over a period of time.

Materials and Methods

Frozen concentrated cultures of *L. acidophilus* NCFM (of human origin) were obtained from Marschall Products, Miles Laboratories, Inc. (Madison, WI) and stored at -196 C in liquid nitrogen. The milk containing the desired numbers of *L. acidophilus* was prepared by adding the needed amount of thawed concentrated culture to pasteurized whole milk obtained

from the Dairy Processing Plant in the Animal Science Department of Oklahoma State University. The frozen concentrated culture was thawed by submerging the container (5 g cryogenic vial) in one liter of tap water at 30-35 C until thawing was complete. Total populations of *L. acidophilus* in milk were confirmed by plate counts using lactobacilli MRS agar (Speck, 1976). The milk was stored in a refrigerator until used (not more than 3 days).

The breath hydrogen test (BHT) used to measure lactose malabsorption in human test subjects was a slight modification of that reported by Levitt and Donaldson (1970). Breath hydrogen was quantitated using a Varian Model 920 Gas Chromatograph equipped with a thermal conductivity detector, a 1 ml sample loop, and a six port gas sampling valve. Separation of hydrogen from other gaseous components of the breath was obtained using a column (12 ft long, 1/16 in. inside diameter) packed with 60-80 mesh 5A molecular sieve (Supeloco, Inc., Bellofonte, PA). Separation was achieved at a column temperature of 53 C with argon as the carrier gas at a flow rate of 18 cc/min. The detector temperature was 107 C. When a breath sample was to be analyzed, a 1-ml sample from the sample bag was transferred into a sample loop via the gas sampling valve by vacuum created with a 25-ml gastight syringe. Concentrations of hydrogen (ppm) were determined by comparing the area of the hydrogen peak obtained from each sample with a standard curve prepared using mixtures of hydrogen (99.998 percent purity) and nitrogen.

The breath hydrogen test was conducted in the morning following a 12-hr fasting period. The sample bags were flushed with nitrogen and evacuated by vacuum prior to each use. While standing, the subject inhaled deeply, retained his breath for 10 sec, then exhaled into the sample bag. Following the initial breath sample the test subjects consumed the indicated milk product (5 ml/kg body wt) as the lactose dose. Additional breath samples were collected by the same procedure 60 min after consuming the test dose and thereafter at 30-min intervals for 2 to 3 hr. The collected samples were analyzed immediately for hydrogen content by gas chromatography, and the average hydrogen content (ppm) of the three consecutive samples exhibiting the greatest levels of hydrogen was used as the hydrogen concentration in the breath of each subject.

Test subjects determined to be lactose malabsorbers were selected from international students (20 to 31 yr of age) at Oklahoma State University. Only those lactose malabsorbers excreting at least 30 ppm hydrogen were accepted as test subjects. All were apparently healthy with no recent history of gastrointestinal disturbance and were not currently using oral antibiotics. None of the subjects had consumed commercially available acidophilus milk. All subjects gave consent for participating in the study after being informed of the nature and the purpose of the study. The individuals were not told whether or not the milk they were to consume contained cells of *L. acidophilus*.

Twelve test subjects who were determined to be lactose malabsorbers were randomly assigned to one of two groups. One group was assigned milk containing 2.5×10^8 cells of *L. acidophilus* per ml (acidophilus group) and the other was assigned milk without the cells of *L. acidophilus* (control group). Using the assigned milk, subjects consumed 0.8 oz (5 g) per 2.2 lb (1 kg) body wt twice daily for 6 days. The milk was delivered to the subjects with instructions to keep it refrigerated until consumed. Sufficient milk for

3 days was delivered each time; thus, none of the milk was used beyond 3 days after preparation.

The intensities of lactose malabsorption for each subject in both groups were determined on day 0 using the BHT in which pasteurized whole milk was used as the test dose. On day 7, the BHT was repeated. The lactose test dose for the subjects in the control group was again pasteurized whole milk. The test dose for the subjects in the acidophilus group was pasteurized whole milk containing 2.5×10^8 *L. acidophilus* per ml. Neither the test subjects nor the analyst knew which subjects received control milk or milk containing lactobacilli until the trial was completed. Comparison of breath hydrogen content of test subjects on day 0 and day 7 for each group was made by treating the data as a paired experiment for statistical analysis.

Additional trials were conducted to determine if the number of cells of *L. acidophilus* in the milk was critical. Milk containing different populations of *L. acidophilus* cells was evaluated in a manner similar to that in the first trial. Twelve test subjects (not used in the previous trial) who were determined to be lactose malabsorbers were randomly assigned to one of two groups and drank the assigned milk for 6 days. One group was assigned milk containing 2.5×10^7 cells of *L. acidophilus* per ml, and the other received milk containing 2.5×10^6 cells of *L. acidophilus* per ml. Control milk was used as the test dose for the BHT on day 0, and the milk containing lactobacilli as assigned was used as the test dose on day 7. Neither the test subjects nor the analyst knew which subjects received milk containing 2.5×10^7 cells or 2.5×10^6 cells of *L. acidophilus* per ml. The data was treated for statistical evaluation as in the first trial.

To determine if consumption of milk containing cells of *L. acidophilus* had an immediate effect on lactose malabsorption, milk containing 2.5×10^6 cells of *L. acidophilus* per ml was evaluated using a randomized complete block design. A BHT was done on five new subjects twice (day 0 and day 7) using pasteurized whole milk as the lactose test dose. The BHT was repeated on days 14 and 21 using milk containing 2.5×10^6 *L. acidophilus* per ml as the test dose. No milk was consumed by any of the subjects during the periods between the breath hydrogen tests.

To determine if lactose in refrigerated milk containing cells of *L. acidophilus* was hydrolyzed prior to consumption, the amounts of lactose were quantitated periodically during refrigerated storage of the milk using an enzymatic method (Taylor, 1970)

Results

Concentrations (ppm) of breath hydrogen before (day 0) and after (day 7) from each of six subjects consuming pasteurized whole milk (control) twice daily for 6 days are shown in Table 1. While there was some individual variation, the average values for day 0 and day 7 were essentially the same. Statistical analysis of the data revealed no significant difference ($P>0.50$) between day 0 and day 7. For the group that consumed milk containing 2.5×10^8 *L. acidophilus* per ml, the breath hydrogen was significantly ($P<0.01$) lower on day 7 than on day 0 (Table 2). All subjects, except for the second one, exhibited much lower levels of hydrogen on day 7 than on day 0. Thus, daily consumption of pasteurized whole milk containing 2.5×10^8 cells of *L. acidophilus* per ml reduced lactose malabsorption in humans.

Table 1. Effect of daily consumption of pasteurized whole milk on lactose malabsorption in humans

Subject	PPM H_2 in Breath	
	Day 0	Day 7
1	70.6	60.1
2	34.5	29.3
3	59.1	74.4
4	38.0	39.4
5	30.1	28.1
6	35.5	34.4
Average	44.6[a]	44.3[a]

[a]Not significantly different ($P>0.50$).

In the second trial the effect of daily consumption of pasteurized whole milk containing 2.5×10^6 cells of *L. acidophilus* per ml on lactose malabsorption also was significant ($P<0.025$) (Table 3). Each test subject included in this group excreted less hydrogen on day 7 than on day 0.

The group of six test subjects that daily consumed pasteurized whole milk containing 2.5×10^7 *L. acidophilus* per ml for 6 days did not reveal a significant ($P>0.35$) reduction in breath hydrogen (Table 4). Three of the six subjects (2, 3 and 4) excreted more hydrogen on day 7 than on day 0. The remaining subjects exhibited lower levels of breath hydrogen on day 7 than on day 0.

Table 2. Effect of daily consumption of pasteurized whole milk containing 2.5×10^8 cells of *L. acidophilus* per ml on lactose malabsorption in humans

Subject	PPM H_2 in Breath	
	Day 0	Day 7
1	47.0	12.1
2	56.0	53.0
3	33.8	13.9
4	45.9	19.4
5	49.1	33.9
6	53.5	38.0
Average	47.6[a]	28.4[a]

[a]Significantly lower on day 7 ($P<0.01$).

Table 3. Effect of daily consumption of pasteurized whole milk containing 2.5×10^6 cells of *L. acidophilus* per ml on lactose malabsorption in humans

Subject	PPM H_2 in Breath	
	Day 0	Day 7
1	49.0	43.3
2	58.5	23.0
3	72.8	44.8
4	37.7	23.8
5	72.7	69.7
6	43.9	38.8
Average	58.8[a]	40.6[a]

[a]Significantly lower on day 7 ($P<0.025$).

Table 4. Effect of daily consumption of pasteurized whole milk containing 2.5×10^7 cells of *L. acidophilus* per ml on lactose malabsorption in humans

Subject	PPM H_2 in Breath	
	Day 0	Day 7
1	34.6	22.7
2	31.5	50.0
3	34.5	51.6
4	41.1	46.1
5	38.3	33.9
6	40.1	9.5
Average	36.7[a]	35.6[a]

[a]No significant difference ($P>0.35$).

To determine if daily consumption of milk containing *L. acidophilus* was necessary to reduce lactose malabsorption, an additional trial was conducted (Table 5). In this trial the subjects did not consume milk with or without lactobacilli during the periods between breath hydrogen tests. Concentrations of breath hydrogen for the test subjects were determined on days 0 and 7 (control milk as test dose) and on days 14 and 21 (milk containing 2.5×10^6 *L. acidophilus* per ml as test dose). For the 2 days (0 and 7) on which control milk was used as the test dose, there was not a significant difference in BHT results ($P>0.70$). There also was not a significant difference ($P>0.95$) between the concentrations of hydrogen in the breath of the subjects for the days (14 and 21) on which milk containing *L. acidophilus* was used as the test dose. However, the overall average of breath hydrogen concentrations on days 14 and 21 was significantly lower than that for days 0 and 7 ($P<0.01$). Thus, the presence of *L. acidophilus* (2.5×10^6/ml) in the milk used as a test dose for the BHT significantly reduced lactose malabsorption, and it was not necessary to consume the milk daily for it to be beneficial in this regard.

The lactose content of the milk did not change during 7 days of refrigerated storage. No free glucose due to the hydrolysis of lactose was detected. The population of *L. acidophilus* in milk during refrigerated storage also did not change.

Table 5. Immediate effect of consuming milk containing *L. acidophilus* on lactose malabsorption in humans[a]

	PPM H_2 in Breath			
	Control Milk		Milk containing *L. acidophilus*[b]	
Subject	Day 0	Day 7	Day 14	Day 21
1	51.3	46.1	17.4	35.7
2	57.9	40.9	45.6	45.3
3	39.5	38.8	21.3	21.6
4	54.8	66.0	58.2	38.7
5	35.2	37.0	30.3	29.9
Averages	47.7[c]	45.8[c]	31.1[c]	34.2[c]
Overall averages	46.8[d]		32.7[d]	

[a]No milk with or without *L. acidophilus* was consumed during the periods between breath hydrogen tests.
[b]2.5×10^6 cells of *L. acidophilus* per ml.
[c]Day 0 not significantly different from day 7 ($P>0.70$); day 14 not significantly different from day 21 ($P>0.95$).
[d]Significantly lower for *L. acidophilus* milk ($P<0.01$).

Discussion

Many individuals who are classified as lactose malabsorbers on the basis of clinical tests involving a standard lactose dose of 1.8 oz (50 g) can consume dietary amounts of lactose, such as would be contained in a glass of milk, without experiencing symptoms of lactose intolerance (Torun et al., 1979). From a practical standpoint the lactose dose for determining lactose malabsorption should be a smaller dose, such as 0.18 oz (5 g) of milk per 2.2 lb (1 kg) of body wt. For most of the test subjects in this study this resulted in test doses similar to 8 to 9 oz of milk, which contained about 0.42 oz (12 g) of lactose. This amount of milk should not have resulted in the digestive system merely being "overloaded" with lactose.

Since the lactose content of milk containing cells of *L. acidophilus* remained unchanged during refrigerated storage, the reduction of lactose malabsorption was not due to the lactose in milk being hydrolyzed prior to ingestion. Thus, the *L. acidophilus* must have exerted its beneficial effect in the digestive system.

It seems that daily consumption of pasteurized whole milk containing 2.5×10^7 cells of *L. acidophilus* per ml should have shown a significant difference between the hydrogen excretions on days 0 and 7 since milk containing both 2.5×10^8 and 2.5×10^6 cells per ml effectively reduced lactose malabsorption. The unexpected result was probably due to the abnormally increased hydrogen excretion by subjects 2 and 3 on day 7. The possible explanations of this unexpected result included variations in diet and other activities of the test subjects that may have imposed stresses on the digestive system.

Individual variations among the test subjects appeared to influence the degree of hydrogen excretion as well as the effect of consuming milk containing cells of *L. acidophilus*. For some subjects, the effect was very beneficial; for others it was not. Thus, we might expect that every lactose malabsorbing person who consumes milk containing *L. acidophilus* might not realize total relief from symptoms associated with lactose malabsorption.

Literature Cited

Levitt and Donaldson. 1970. J. Lab. Clin. Med. 75:927-945.
Skala and Lamacora. 1971. Nutr. Metab. 13:200-206.
Speck. 1976. Compendium of Methods for the Microbiological Examination of Foods. Am. Pub. Health Assoc.
Taylor. 1970. Australian J. Dairy Tech. 25:7-9.
Torun et al. 1979. Arch. Latinoamer de Nurt. 29:445-494.

Frozen Concentrated Cultures from Cells of *Lactobacillus acidophilus* Grown in Pepsinized Whey Media

S. E. Gilliland and S. L. Mitchell

Story in Brief

Three pepsinized whey based media were compared for growing cells of *Lactobacillus acidophilus* to be used in preparing frozen concentrated cultures. Concentrations of dried sweet whey tested in the media were 2.5, 5.0, and 7.5 percent. The pH of the media was maintained at 6.0 during growth of *L. acidophilus* at 37 C with a neutralizer consisting of 20 perecent Na_2CO_3 in 20 percent NH_4OH. As the level of whey solids was increased in the media, maximum populations attained during growth decreased. The culture reached maximum populations in the media containing 2.5 percent and 5.0 percent whey solids after 12 and 18 hr, respectively, then remained constant for the remainder of the 24-hr incubation period. However, in the medium containing 7.5 percent whey solids, the culture appeared to enter a death phase after only 12 hr of incubation. The maximum population in the 7.5 percent medium was also considerably lower than those in the other two media. Frozen concentrated cultures were prepared from cells of *L. acidophilus* grown in the media containing 2.5 percent and 5.0 percent whey solids and stored in liquid nitrogen at − 196 C. The viability and bile resistance of the cultures during storage in liquid nitrogen and subsequent storage in refrigerated milk was highest for cells which had been grown in the 2.5 percent pepsinized whey medium.

Introduction

Currently there is much interest in the use of *Lactobacillus acidophilus* as a dietary adjunct. Cells of *L. acidophilus*, in the form of a concentrated culture added to refrigerated low-fat milk, may help maintain intestinal health and improve intes-