Effect of the microbial lactase (*EC* 3.2.1.23) activity in yoghurt on the intestinal absorption of lactose: an in vivo study in lactase-deficient humans*

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Breath hydrogen excretion was measured in eight lactase (*EC* 3.2.1.108)-deficient volunteers ingesting 18 g lactose in the form of milk, yoghurt and heated yoghurt. Total excess hydrogen excretion (area under curve) was significantly lower after yoghurt and heated yoghurt, than after milk: 103 (SE 29), 191 (SE 32), and 439 (SE 69) respectively ($P < 0.001$). The oro-caecal transit time of fermentable components from yoghurt and heated yoghurt (mainly lactose) was longer than that from milk: 165 (SE 17), 206 (SE 19), *v.* 103 (SE 19) min ($P < 0.01$). An intestinal perfusion technique was used in the same subjects after ingestion on two consecutive days of 18 g lactose in yoghurt and heated yoghurt. Significantly less lactose was recovered from the terminal ileum after yoghurt than after heated yoghurt meals: 1740 (SE 260) *v.* 2825 (SE 461) mg ($P < 0.05$), and approximately one-fifth of the lactase activity contained in yoghurt reached the terminal ileum. These findings indicate that more than 90% of the lactose in yoghurt is digested in the small intestine of lactase-deficient subjects and suggest that both the lactase activity contained in the viable starter culture and a slow oro-caecal transit time are responsible for this excellent absorption.

Lactose digestion: Lactase deficiency: Dairy products

It is now well established from breath hydrogen studies that lactase (*EC* 3.2.1.108)-deficient subjects absorb lactose more efficiently from fresh yoghurt (Y) than from heated yoghurt (HY) or milk (Kolars *et al.* 1984; Savaiano *et al.* 1984; Dewit *et al.* 1988). This is believed to be due to the presence of lactase of microbial origin in yoghurt (Kolars *et al.* 1984; Pochart *et al.* 1989). However, neither the precise gain in lactose absorption from Y compared with HY, nor the fate of Y microbial lactase beyond the duodenum are known. The aims of the present study, performed in lactase-deficient healthy humans, were therefore to quantify directly the intestinal absorption of lactose after ingestion of Y and HY, and to ascertain the fate and role of Y lactase in the small intestine.

MATERIALS AND METHODS

Subjects

Eight healthy volunteers (four men and four women, seven white and one black) who had not recently taken antibiotics or laxatives were studied. Their ages ranged from 19 to 27 years (mean 22 years), and all gave written informed consent to the protocol which was

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approved by the Local Ethics Committee. The subjects were identified as lactose malabsorbers because their breath H_2 concentrations exceeded 20 parts per million (ppm) after ingestion of 50 g lactose in 250 ml water (Newcomer *et al.* 1975).

Test meals

In all experiments we used the same commercial unflavoured Y (Yoplait, Sodima) and whole milk. The HY was obtained by heating commercial yoghurts for 1 h in a stirred water bath at 70°, allowing the temperature of the yoghurt to rise to 65° for the last 20 min. HY was then immediately cooled to 4° and was consumed within 24 h of heating. The lactose, galactose, glucose and lactase contents of milk, Y and HY are indicated in Table 1. In HY, bacterial lactase (*EC* 3.2.1.23) was very low, and kinetic studies showed us that under in vitro optimal conditions of pH and temperature it could not hydrolyse more than 10% of the lactose present in the meal.

Methods

End alveolar breath samples were obtained on three consecutive days from all eight non-smoking subjects in the fasting state and then half-hourly for 9 h after ingestion in a random order of the following test meals (one on each of the three days): 400 ml whole milk, 450 g commercial unflavoured Y, and 450 g HY. Each test meal contained 18 g lactose and was ingested at 4°.

On the 4th day, each subject was intubated with a triple-lumen tube, tracted by a mercury bag (Phillips & Giller, 1973). One lumen was used to inflate the bag in order to hasten tube progression. When the bag had reached the caecum, as confirmed fluoroscopically, subjects were asked to stay in a semi-recumbent position. The second lumen was used to sample ileal contents 15 cm above the ileo-caeal junction, and the third, 25 cm proximal to the aspiration port, was used for perfusion. On the morning of the 5th and 6th days, a 2 ml/min infusion of polyethyleneglycol 4000 (PEG: 10 g PEG 4000/l saline (154 mM-sodium chloride) at 37°) was started. After 1 h of equilibration, fasting subjects ingested in a random order either the Y or HY meal in 4–10 min. Before ingestion, 30 μCi ^{14}C-labelled PEG and 10^8 bacterial spores of *Bacillus stearothermophilus* germinating only at 65° (Merck, Darmstadt, FRG) were added to the meals as meal and bacterial markers respectively (Besnier *et al.* 1983; Pochart *et al.* 1989) Ileal contents were constantly collected on ice by manual aspiration in order to aspirate as much fluid as possible during the 9 h after the beginning of the meals. Samples were separated into 30-min portions. The pH of ileal fluid was measured immediately after collection and samples were frozen at −20° to prevent bacterial degradation of carbohydrates. Maintenance of the intestinal tube position was checked fluoroscopically at the end of all studies.

Analysis

H_2 concentrations in expiratory gas samples were measured using an electrochemical cell (Exhaled Hydrogen Monitor, GMI Medical Ltd, Renfrew, Scotland). In ileal fluid, pH was measured with a pH meter (Radiometer, Copenhagen, Denmark), PEG by turbidimetry (Hydén, 1955), and ^{14}C-labelled PEG by a scintillator counter (Intertechnique, Paris). The spore concentration of *Bacillus stearothermophilus* in each ileal sample was determined in duplicate as follows: six successive 1:10 dilutions of each sample in sterile saline were performed. Each diluted portion (1 ml) was combined with 15 ml sterile standard agar gelose (Plate Count Agar) at 65°, and incubated aerobically for 24 h at 65°, and bacterial colonies were counted. In milk, yoghurts and ileal samples, the lactose, galactose and glucose contents were assessed enzymically (Boehringer Biochemicals, Mannheim, FRG). Lactase activity was measured in Y, HY and ileal samples as follows: after adequate

Table 1. *Carbohydrate and lactase* (EC *3.2.1.23) content of the three test meals*

(Mean values with their standard errors for ten determinations on each product)

	Yoghurt		Heated yoghurt		Whole milk	
	Mean	SE	Mean	SE	Mean	SE
Lactose (g/l)	39·9	0·4	39·8	0·2	45·0	0·5
Galactose (g/l)	10·1	0·1	10·1	0·1	0	
Glucose (g/l)	0·02	0·1	0·1	0·1	0	
Lactase (IU/g)	1·7	0·1	0·3	0·1	0	

dilution in 0·1 M-sodium phosphate (pH 7) and sonication for 1 min in an ice bath, a 1 ml portion of each sample was incubated with 4 ml 0·005 M-ortho-nitrophenyl-β-D-galactopyranoside (ONPG). The reaction, which proceeded at 37°, was stopped after 10 min by adding 5 ml of cold 0·5 M-sodium carbonate solution. Absorbance was read at 420 nm in a spectrophotometer and compared with the absorbance read on a standard curve prepared with ortho-nitrophenol (ONP). The lactase (*EC* 3.2.1.23) activity unit (IU) was defined as the amount needed to hydrolyse 1 μmol ONPG/min at 37°. To study the influence of freezing on lactase activity and lactose content in ileal samples, both were measured before and after freezing in the first two experiments, and were not found to be modified. High levels of lactose, galactose or glucose might theoretically inhibit weakly the lactase activity measured with ONPG (Itoh *et al.* 1980; Greenberg & Mahoney 1982; Mahoney, 1985). To ensure that this phenomenon did not occur, the lactase activity was measured in a series of ileal samples before and after dialyzing overnight in phosphate buffered saline; dialyzing eliminated the sugars from the samples but did not modify the lactase activity.

Calculations

Total excess H_2 excreted in breath after test meals was determined by integrating the area under the H_2 concentration curve located between a sharp increase (>5 ppm) and the return of H_2 to baseline values (Flourié *et al.* 1986). Oro-caecal transit time was defined as the time elapsing between the beginning of meal ingestion and the sharp increase in breath H_2.

The volume flowing through the terminal ileum for each 30-min period of sampling was calculated by unlabelled PEG dilution, and standard formulas were corrected for the volume of marker fluid infused, assuming that it was not absorbed in the 25 cm segment between the infusion and aspiration sites (Phillips & Giller, 1973). Ileal flow of ^{14}C-labelled PEG and spores ingested in the meal were calculated from the amounts of ^{14}C-labelled PEG and spores aspirated, after correction for recovery of the ileal marker. The amounts of lactose, galactose, glucose and lactase in each ileal sample were determined from the calculated ileal volume (without subtraction of the perfused volume) and from the carbohydrate and lactase concentrations in the sample. The total ileal content for 9 h was obtained by the summation of results for individual periods.

Statistical analysis

Results are expressed as means with their standard errors. Values were compared by analysis of variance using the SAS software.

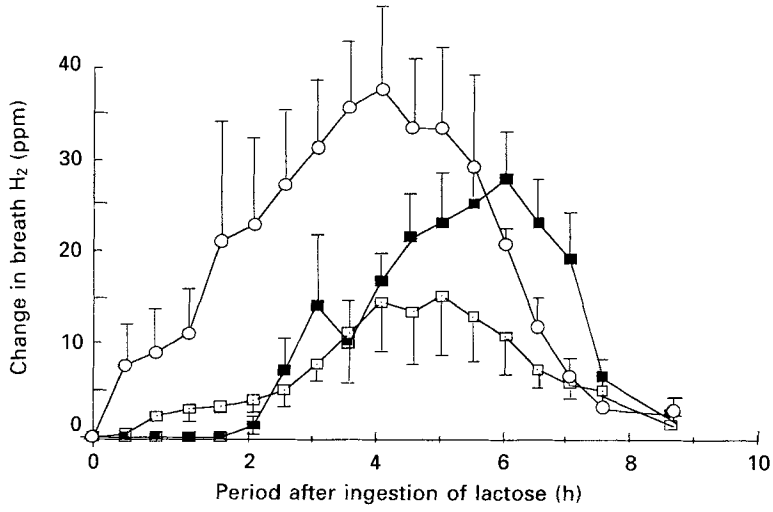

Fig. 1. Changes in breath hydrogen after ingestion of 18 g lactose in milk (○), heated yoghurt (■) or yoghurt (□) by lactase (*EC* 3.2.1.108)-deficient subjects. Values are means with their standard errors represented by vertical bars for eight subjects. For details of procedures, see p. 72.

RESULTS

Breath H_2 excretion after meals

Fig. 1 shows the changes in breath H_2 after ingestion of milk and yoghurts. The area under the curve was significantly higher for milk than for HY and Y ($P < 0{\cdot}001$) (Table 2). The difference between the area under the curve for Y and HY did not reach statistical significance when the variance of the three treatments was considered ($P = 0{\cdot}08$). A significantly shorter oro-caecal transit time was measured after milk than after HY and Y ($P < 0{\cdot}05$) (Table 2).

Water flow and pH of the distal ileum and recovery of meal markers

The mean flow of water through the terminal ileum for the 9 h after Y ingestion was not significantly different from that calculated after HY, i.e. 645 (SE 161) *v.* 569 (SE 156) ml. The pH of the ileal fluid was not affected by the Y or HY meals in comparison with basal fasting values (range 6·6–7·8).

The time-course of passage through the ileum of ^{14}C-labelled PEG and spores followed roughly the same pattern, and did not differ for Y and HY meals (Fig. 2). The ^{14}C-labelled PEG and the spores of test meals always appeared in ileal samples within 1 h of the beginning of meal ingestion and sometimes as early as 30 min. The percentages of ^{14}C-labelled PEG and spores ingested in Y which were recovered from the terminal ileum during the 9 h aspiration period were 96 (SE 3) and 99 (SE 10) respectively (ranges 90–105 and 85–120). They were not significantly different from those calculated after HY, i.e. 96 (SE 3) and 95 (SE 12) respectively (ranges 87–104 and 85–120).

Carbohydrate recovery from the terminal ileum

Lactose, galactose and glucose recovery in the terminal ileum followed roughly the same pattern as that of ^{14}C-labelled PEG and spores. The mean amounts of lactose not absorbed by the small intestine during the 9 h post-ingestion period were significantly lower after Y than after HY ($P < 0{\cdot}05$) (Table 3, Fig. 3). The mean amounts of galactose and glucose

Table 2. *Breath hydrogen excretion after ingestion of 18 g lactose in yoghurt, heated yoghurt or milk in eight lactase* (EC *3.2.1.108*)*-deficient subjects*

(Mean values with their standard errors)

	Area under curve		Oro-caecal transit time (min)	
	Mean	SE	Mean	SE
Yoghurt (Y)	103	29	165	17
Heated yoghurt (HY)	191	32	206	19
Milk (M)	439	69	103	19
SED (14 df)	110		55	
Significance of comparisons:				
Y *v.* M	$P < 0.001$		$P < 0.01$	
Y *v.* HY	$P = 0.08$		NS	
HY *v.* M	$P < 0.001$		$P < 0.001$	

SED, standard error of the difference; NS, not significant.

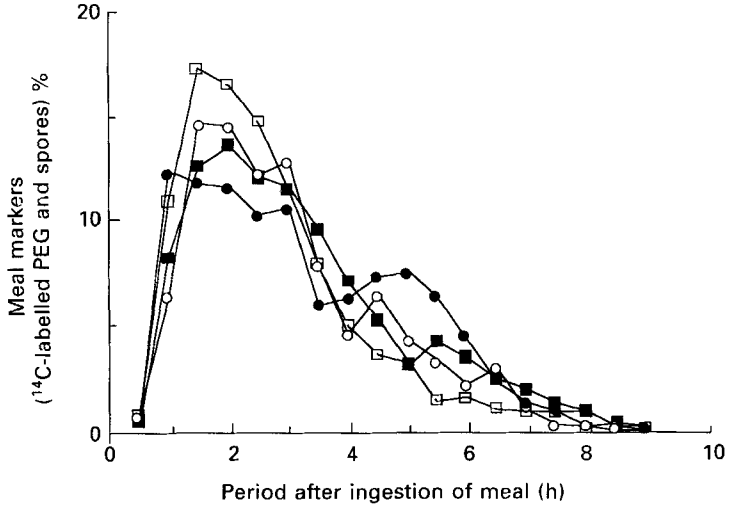

Fig. 2. Time-courses of the passage of the meal markers ^{14}C-labelled polyethylene glycol (○, ●) and bacterial spores (□, ■) through the ileum after ingestion of yoghurt (○, □) or heated yoghurt (●, ■) by eight lactase-deficient subjects. For details of procedures, see p. 72.

present in the ileal samples did not differ between Y and HY (Table 3). When the values were expressed as hexose units (mmol of lactose $\times 2$ + mmol of galactose and glucose), the mean amounts of hexoses malabsorbed by the small intestine were significantly lower after Y than after HY ($P < 0.05$) (Table 3).

Lactase recovery from the terminal ileum

After ingestion of Y, a significant and important increase in lactase flow rate was observed which followed the same time course as the meal markers (Fig. 4). In contrast, compared with late values, the lactase activity of ileal fluid was not affected by HY (Fig. 4). The mean amounts of lactase recovered during the 9 h were significantly higher ($P < 0.01$) after Y (192 (SE 37), range 70–333 IU) than after HY (47 (SE 7), range 21–74 IU).

Table 3. *Amounts of unabsorbed sugars recovered in 9 h from the terminal ileum in eight lactase* (EC *3.2.1.108*)*-deficient subjects ingesting 18 g lactose in yoghurt and heated yoghurt*

(Mean values with their standard errors, ranges in parentheses)

	Lactose (mg)		Galactose (mg)		Glucose (mg)		Hexoses (hexose units)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Yoghurt (Y)	1740 (294–2534)	260	725 (127–1380)	178	333 (85–1048)	112	14·9 (2·8–23·4)	2·4
Heated yoghurt (HY)	2825 (494–4225)	461	493 (20–1086)	113	209 (12–463)	60	19·5 (3·0–28·7)	3·0
SED (14 df)	635		234		236		3·45	
Significance of comparisons Y *v.* HY	$P=0·011$		$P=0·09$		$P=0·3$		$P=0·033$	

SED, standard error of the difference.

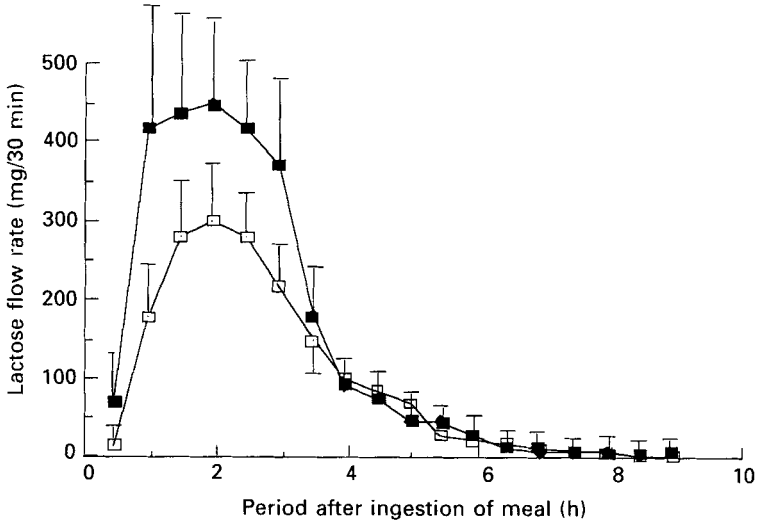

Fig. 3. Lactose flow rate through the ileum after ingestion of yoghurt (□) or heated yoghurt (■). Values are means with their standard errors represented by vertical bars for eight lactase-deficient subjects. For details of procedures, see p. 72.

DISCUSSION

The present work aimed to quantify directly the lactose absorption from Y and HY in lactase-deficient subjects, and to define the fate and role of Y microbial lactase in the human small intestine.

Previous breath H_2 tests showed that lactose is significantly better absorbed from Y than from HY, and from HY than from milk (Kolars *et al.* 1984; Savaiano *et al.* 1984; Dewit *et al.* 1988). The present work confirms this finding. Moreover, direct measurement of the residual sugars recovered from the terminal ileum after ingestion of 450 g yoghurt (i.e. 155 hexose units) showed that only 9·6% of the sugar load was malabsorbed. We did not

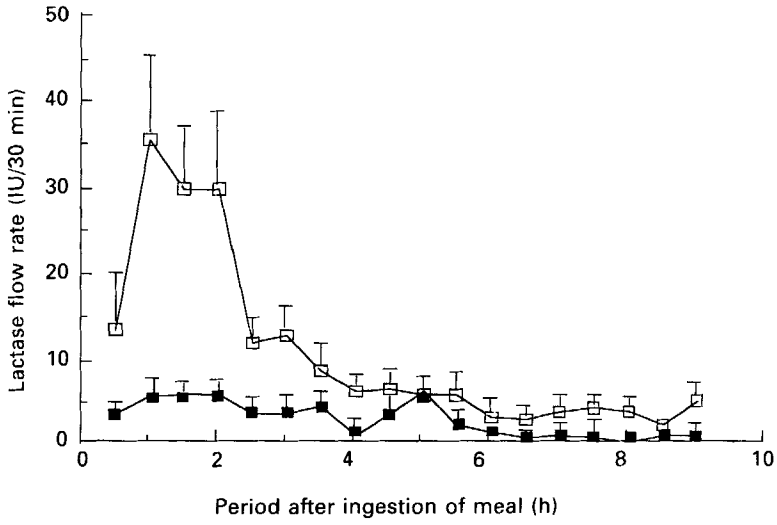

Fig. 4. Lactase flow rate through the ileum after ingestion of yoghurt (□) or heated yoghurt (■) by lactase (*EC* 3.2.1.108)-deficient subjects. Values are means with their standard errors represented by vertical bars for eight subjects. For details of procedures, see p. 72.

measure directly carbohydrate malabsorption from milk; however, using the amount of H_2 excreted and the known quantity of hexoses malabsorbed after Y and HY meals, we calculated from the breath H_2 excretion that about 39 (SE 6)% of the hexoses in the milk were not absorbed by our subjects. This value agrees with the findings in the literature for both H_2 breath tests (Kolars *et al.* 1984) and direct measurements (Bond & Levitt, 1976; Debongnie *et al.* 1979). Hydrolysis of lactose from Y reached 90·3%, but significant amounts of galactose and glucose were recovered from the ileal fluid, possibly due to the fact that some of the lactose hydrolysis occurred in vitro. However, glucose recovery was significantly lower than that of galactose, which seems to imply incomplete absorption of the hexoses derived from in vivo lactose hydrolysis near the sampling port (Gray & Santiago, 1966; Rambaud *et al.* 1968).

It has been shown that the lactase activity in Y is not completely denatured during its passage through the stomach, and can be detected in the duodenum (Kolars *et al.* 1984; Pochart *et al.* 1989). This resistance to gastric acidity depends on both the buffering capacity of Y and the integrity of the bacterial cell membrane (Martini *et al.* 1987). However, in the duodenum of lactase-deficient subjects, Y microbial lactase is not active because of the low pH (Pochart *et al.* 1989), and its fate downstream, where a suitable pH occurs, had not previously been known. Our study demonstrates that about one-fifth of the Y bacterial lactase reaches the terminal ileum with a time-course identical to that of lactose. However, it should be pointed out that the better absorption of Y lactose than milk lactose observed here may not only be due to bacterial lactase activity since the percentage of hexoses malabsorbed was only 12·5% from HY *v* 39% from milk. In HY, bacterial lactase was very low, and kinetic studies showed us that under in vitro optimal conditions of pH and temperature it could not hydrolyze more than 10% of the lactose present in the meal. Furthermore, we have previously shown that after ingestion of HY, the lactase activity was negligible in the duodenal contents of lactase-deficient subjects (Pochart *et al.* 1989) so that this hydrolysis rate is unlikely to occur in vivo. The oro-caecal transit time of Y and HY meals, as determined from the breath H_2 tests, was significantly longer than that of whole

milk. Assuming that 10% of the intestinal lactase activity persists in adult lactase-deficient subjects (Asp *et al.* 1971), this delay in gastrointestinal transit might partly explain the better digestion of Y and HY lactose *v.* milk lactose. In lactase-deficient subjects, such a mechanism has indeed already been proposed to explain the better absorption of equivalent amounts of lactose from whole milk compared with skimmed milk, or of lactose taken with a meal or with chocolate or fibre (Welsh & Hall, 1977; Solomons *et al.* 1979, 1985; Nguyen *et al.* 1982; Martini & Savaiano, 1988; Lee & Hardy 1989).

In conclusion, more than 90% of the carbohydrates in Y are digested in the small intestine of lactase-deficient subjects. Such absorption is in part due to the bacterial lactase activity since (a) lactose absorption is significantly lower after HY ingestion, and (b) one-fifth of the lactase activity from ingested Y reaches the terminal ileum at the same time as lactose. However, an improved hydrolysis of lactose by the residual intestinal lactase activity may also be involved, as lactose absorption from HY (in which the bacterial lactase activity is destroyed) remains markedly higher than lactose absorption from milk, and could be due to the slower oro-caecal transit time of Y and HY compared with milk.

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