Lactose digestion by human jejunal biopsies: the relationship between hydrolysis and absorption

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SUMMARY The relationship between lactose hydrolysis and absorption of released glucose was investigated by determining the kinetics of lactose digestion by jejunal biopsies incubated *in vitro*. Lactase activity in intact biopsies correlated with conventional assay of tissue homogenates ($r=0.85$, $p<0.001$), and glucose uptake from 28 mM lactose was directly proportional to lactase activity ($r=0.95$, $p<0.001$) in 21 subjects with normal lactase levels, six with hypolactasia (primary or secondary to coeliac disease) and two with lactose intolerance but normal lactase activity. Kinetic analysis at 0.56–56 mM lactose in five normal subjects showed saturable kinetics for hydrolysis (app $K_m=33.9\pm2.2$ mM; app $V_{max}=26.5\pm1.1$ nmol/min/mg dry weight) but glucose uptake could be fitted to a model either of saturable uptake (app $K_t=47.2\pm0.3$ mM; app $J_{max}=14.1\pm0.2$ nmol/min/mg) or saturable uptake plus a linear component (app $K_t=21.3\pm1.15$; app $J_{max}=4.59\pm0.12$; app $K_d=0.093\pm0.010$ nmol/min/mg/mM). The proportion of glucose taken into the tissue did not significantly exceed 50% of the total released at any lactose concentration suggesting the lack of an efficient capture mechanism for the released glucose. The results suggest that lactose hydrolysis is the rate limiting step in the overall absorption of glucose from lactose *in vitro*, and that the relationship between hydrolysis and absorption is the same in normal subjects and in hypolactasic subjects.

The relationship between lactose hydrolysis and absorption of the released monosaccharides is not fully understood. From perfusion studies with lactose and an equimolar mixture of glucose and galactose, Gray and Santiago¹ concluded that hydrolysis was rate limiting to the overall process of lactose absorption. McMichael, Webb and Dawson,² however, found no correlation between mucosal lactase activity *in vitro* and lactose hydrolysis *in vivo*, and concluded that hydrolysis was rate limiting only when disaccharidase activity was very low.

Lactose intolerance correlates poorly with indirect measures of lactase activity – for example, the oral lactose tolerance test,³ and also with mucosal lactase activity measured directly.^{4–6} This may be explained by variations in the load of lactose presented to the intestine, variable gastric emptying and intestinal motility, and differences in compensa-

tory mechanisms of the colon⁷ but there remains a possibility that lactase activity measured in mucosal homogenates does not accurately reflect the ability of intact tissue to digest lactose.

In this study, lactose absorption by jejunal biopsies has been investigated using a technique which measures initial rates of monosaccharide uptake.⁸ We have compared lactose hydrolysis in intact tissue with that in tissue homogenates and also determined the relationship between lactose hydrolysis and subsequent absorption of glucose in the same tissue.

Methods

PATIENTS

Fourteen adults and 15 children who were undergoing routine investigation for gastrointestinal symptoms, short stature or failure to thrive were studied. The final diagnoses, clinical details and lactase activities are given in Table 1. Lactase deficiency (activity <0.65 U/g wet weight determined using non-incubated homogenised tissue) was found

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Table 1 Clinical details and lactase activities of the patients

Patient	Age (yrs)	Sex	Lactase activity (normal > 1.0 U/g wet wt) (hypolactasia < 0.65 U/g wet wt)	Diagnosis
Adults				
1	44	F	10.44	Irritable bowel syndrome
2	37	F	9.02	Irritable bowel syndrome
3	45	F	5.35	Irritable bowel syndrome
4	34	F	9.97	Irritable bowel syndrome
5	20	F	4.56	Irritable bowel syndrome
6	20	F	4.88	Irritable bowel syndrome
7	58	F	2.83	Irritable bowel syndrome
8	59	F	6.50	Pernicious anaemia
9	29	F	2.41	Congenital short stature
10	55	M	8.59	Post vagotomy diarrhoea
11	61	F	2.36	Pancreatic steatorrhoea
12	21	M	3.29	Crohn's disease
13	49	M	0.61	Hypolactasia
14	68	F	0.00	Untreated coeliac disease
Children				
15	11	M	0.86	Diarrhoea
16	17 mths	M	1.32	Previous gastroenteritis
17	15	F	7.83	Abdominal pain
18	11	M	3.22	Congenital short stature
19	8	M	3.56	Abdominal pain and diarrhoea
20	3	M	0.96	Failure to thrive
21	3	F	9.31	Failure to thrive
22	3½	M	4.92	Previous gastroenteritis
23	6	F	2.10	Diarrhoea
24	14	F	0.04	Coeliac disease in relapse
25*	8	M	5.58	Lactose intolerance
26*	12	F	1.56	Lactose intolerance
27*	14	M	1.37	Lactose intolerance
28*	8	F	4.21	Lactose intolerance
29*	4	M	1.24	Lactose intolerance

* These patients had abnormal breath hydrogen tests and stool chromatograms following an oral lactose load.

in two patients with coeliac disease (nos 14 and 24) and one with acquired lactose intolerance (no. 13). Borderline lactase deficiency (0.65–1.0 U/g wet weight) was found in two patients (nos 15 and 20) who had no evidence of lactose intolerance and normal breath hydrogen tests. Two children (nos 16 and 22) were thought to have had transient lactose intolerance due to gastroenteritis but were asymptomatic and had normal lactase activity at the time of biopsy. Five children (nos 25–29) had continuing lactose intolerance with abnormal breath hydrogen tests and positive stool chromatograms for sugars after an oral lactose load, but had normal lactase levels by conventional assay; three of these were subsequently shown to be hypolactasic. Histology was normal in all patients except those with coeliac disease who showed partial villous atrophy. This study was approved by the ethical committees of the Central and North Manchester Health Authorities.

BIOPSY INCUBATION

Peroral jejunal biopsies were obtained by Crosby capsule and immediately transferred to ice cold Krebs-Ringer solution, pH 7.4, containing 20 mM HEPES and 1 mg/ml bovine serum albumin (BSA), and gassed with 95% O₂/5% CO₂. Single biopsies were subdivided, one portion examined histologically, and one blotted and frozen at –20°C for disaccharidase analysis. The remaining tissue specimens were pre-incubated in the same buffer for 10 minutes, then transferred to 2 ml of buffer containing 28 mM lactose, (labelled with 5 µCi [1-¹⁴C-D-glucose]-lactose), and 7.5 µCi [³H]-polyethylene glycol 4000 (PEG) for incubation (10 min) at 37°C in 25 ml conical flasks gassed with 95% O₂/5% CO₂ and shaken at 200 cycles/min.

In five adults, multiple biopsies were taken using the Quinton-Rubin hydraulic instrument and subdivided to yield tissue specimens for incubation in buffer containing 0.56–56 mM lactose, 112 mM

Na⁺ and sufficient mannitol to maintain the osmolality at 310 mOsm/kg.

After incubation (10 min), the medium was removed by suction filtration and collected separately. The biopsy specimens, retained on membrane filters (Schleicher and Schuell ST69, pore diameter 1.2 µm), were washed twice with 20 ml ice cold 150 mM sodium chloride, transferred individually to conical PTFE-glass homogenisers, and snap-frozen at -50°C to prevent metabolism. Each piece was subsequently homogenised in 1.6 ml of water and aliquots used to measure:

(1) *Tissue dry weight* was determined by drying a 400 µl aliquot on to a preweighed glass fibre disc (Whatman GF/D, 2.5 cm diameter). Discs were dried at 70°C over P₂O₅ and weighed on a micro-balance against a tared reference disc.

(2) *Tissue radioactivity* was determined by dual channel liquid scintillation counting of a 400 µl aliquot dissolved in 2.5 ml tissue solubilizer (NCS, Amersham International) before addition of a toluene based scintillant containing 4 g/l 2,5-diphenyl oxazole and 250 mg/l 1,4-di-2-(5-phenyloxazoly)-benzene. Pre-incubation medium counts were measured using 20 µl aliquots plus 380 µl of water dissolved in NCS.

(3) *Sugar assay*: boiled aliquots of medium and tissue were analysed fluorimetrically for glucose and galactose using hexokinase/glucose-6-phosphate dehydrogenase/NAD⁹ or galactose dehydrogenase/NADP.¹⁰ Lactose was assayed in tissue homogenates only using β-galactosidase/galactose dehydrogenase/NADP.¹⁰

(4) *Brush-border enzymes*: 225 µl aliquots of homogenate were diluted four-fold in MOPS-mannitol buffer containing 2% BSA and frozen at -20°C for subsequent determination of lactase by a fluorimetric adaptation of the method of Dahlqvist,¹¹ using 4-hydroxyphenylacetic acid in place of *o*-dianisidine; β-fucosidase, α-glucosidase and alkaline phosphatase by fluorimetric assays with the appropriate 4-methylumbelliferyl compounds as substrates; and protein by a fluorescamine method.¹² Incubation media were analysed for β-fucosidase, alkaline phosphatase and α-glucosidase.

Kinetic parameters were derived with the aid of a curve fitting program available from the CERN computer program library.¹³ The data were fitted to models of saturable uptake (Michaelis-Menten kinetics) with or without a linear component of uptake.

CALCULATION

Intracellular glucose uptake was calculated from the equation:

$$\begin{aligned} & \text{glucose uptake} \\ &= \left(\frac{\text{tissue } ^{14}\text{C dpm}}{\text{medium } ^{14}\text{C dpm}/\mu\text{l}} - \frac{\text{tissue } ^3\text{Hdpm}}{\text{medium } ^3\text{Hdpm}/\mu\text{l}} \right) \\ & \quad \times \text{sugar concentration (mM)} \end{aligned}$$

For lactose incubations, this assumes that all intracellular ¹⁴C activity (obtained by correcting total ¹⁴C activity for that present in the PEG space) is derived from glucose, and that lactose does not significantly penetrate this space. This assumption was checked by comparing the radiometrically measured uptake of glucose with the glucose measured fluorimetrically in 119 tissue specimens. There was good correlation between the two measures of glucose (*r*=0.96, *p*<0.001) and the slope of the line was not significantly different from 1, implying that the assumption is correct. Furthermore, fluorimetric measurements of tissue lactose did not exceed those expected from the PEG space.

Lactase activity of intact tissue was calculated from the radiometrically determined intracellular glucose and the fluorimetrically determined glucose in the medium as follows:—

$$\text{lactase activity (U/g dry wt)} = \frac{\text{intracellular glucose (nmol)} + \text{medium glucose (nmol)}}{\text{incubation time (min)} \times \text{dry weight (mg)}}$$

Results

LACTASE ACTIVITY IN INTACT TISSUE

The histology of biopsies was normal after incubation and the specific activities (U/g dry weight) of all brush-border enzymes measured were not significantly changed compared with paired unincubated biopsies from the same individuals. Preliminary experiments showed that lactose hydrolysis was linear throughout the incubation period and was not significantly different when calculated in terms of either galactose or glucose released. Tissue uptakes of glucose and galactose were also linear with time but at low lactose concentrations, uptake of galactose was at the limits of fluorimetric detection and thus radiometric measurements of glucose uptake were used in the kinetic studies.

The lactase activity of intact tissue and of homogenates of the same tissue specimens after incubation were compared in 23 subjects (Fig. 1). There was a highly significant correlation between the two measures of lactase activity (*r*=0.84, *p*<0.001) but in intact tissue we detected only 25% of the activity measured in homogenised tissue. Control experi-

Fig. 1 Relationship between lactase activity in 23 specimens incubated intact and in the same specimens incubated after homogenisation. (●), patients with normal histology and disaccharidase activity; (○), patients 25–29 with lactose intolerance; (■), patients 14 and 24 with coeliac disease; (□), patient 13 with hypolactasia. Line ($y = 0.25x + 1.30$) is the least squares fit to the data and the intercept is not significant.

ments where tissue homogenates were assayed at pH 7.4 and at pH 6.0 showed the same reduction in lactase activity at the lower pH.

The lactase activity measured in intact tissue in normolactasic subjects ranged from 2.7 to 18.0 U/g dry weight. The two patients with coeliac disease and one with acquired alactasia were hypolactasic by the assay of intact tissue. Of the five subjects with

clinical lactose intolerance but previously normal lactase activity, three were hypolactasic by assay of intact tissue and repeat conventional assay of tissue homogenates confirmed this; the other two were normolactasic in both assays (Fig. 1). The relationship between assay of intact tissue and assay of tissue homogenates was no different for these eight subjects than for the normolactasic subjects (Fig. 1).

KINETICS OF LACTOSE DIGESTION

The concentration dependence of both lactose hydrolysis and glucose absorption from lactose determined in five normal subjects was curvilinear with lactose hydrolysis exceeding glucose absorption at all lactose concentrations (Fig. 2). The curve fitting programme showed that both lactose hydrolysis and glucose uptake could be fitted with equal precision either to a model of single saturable

Fig. 2 Concentration dependence of lactose hydrolysis (○) and uptake of glucose from lactose (●) in specimens from five normal adults. Values are the mean (± 1 S.E.) hydrolysis/uptake rate at lactose concentrations between 0.56–56 mM. The curves are the computer-derived best fit to the data.

Table 2 Kinetic constants (means ± 1 SE) for lactose hydrolysis and glucose uptake derived from the data in Figure 2 by a computerised curve-fitting program.¹³

	app K_m/K_t (mM)	app V_{max}/J_{max} (nmoles/min/mg)	app K_d (nmoles/min/mg/mM)
Lactose hydrolysis			
Single component	33.9 $\pm$ 2.2	26.5 $\pm$ 1.1	—
Two component	1.04 $\pm$ 1.31	1.16 $\pm$ 0.79	0.38 $\pm$ 0.07
Glucose uptake			
Single component	47.2 $\pm$ 0.3	14.1 $\pm$ 0.2	—
Two component	21.3 $\pm$ 1.15	4.59 $\pm$ 0.12	0.093 $\pm$ 0.010

uptake or to one of single saturable uptake plus a linear component. The derived kinetic constants are given in Table 2: those for two component lactose hydrolysis appear physiologically and biochemically improbable.

Fig. 3 Capture efficiency for glucose uptake from lactose at different lactose concentrations, calculated as intracellular/total glucose for the same subjects as in Figure 2 (see Methods). There is no significant difference between the values from 2.8 to 56 mM lactose.

Fig. 4 Relationship between uptake of glucose from lactose and lactase activity in 29 biopsies incubated intact in 28 mM lactose. The symbols are described in the legend to Figure 1. the line ($y=0.55x$) is the least squares fit to the data forced through the origin.

The proportion of the total glucose released by hydrolysis which was taken up by the tissue increased with lactose concentration up to 5.6 mM and above that remained constant (Fig. 3).

Figure 4 shows that in biopsies incubated at a lactose concentration of 28 mM, uptake of glucose was directly related to tissue lactase activity ($r=0.95$, $p<0.001$). This relationship was true for normal subjects, those with hypolactasia and those with clinical lactose intolerance.

Discussion

The use of human jejunal biopsies to study sugar absorption has largely been confined to the study of monosaccharide uptake,¹⁴⁻¹⁷ although London *et al* investigated lactose metabolism in biopsies using a steady-state incubation technique.¹⁸ The method described here has been validated previously for the measurement of initial rates of monosaccharide absorption⁸ and the present results show that it is applicable to the measurement of lactose hydrolysis, thus enabling both processes to be measured in the same piece of tissue.

The assumption that all intracellular ¹⁴C-radioactivity is due to glucose requires comment, as small amounts of lactose have been shown to penetrate the mucosa *in vivo*,¹⁹ and penetration via the cut edges of the tissue will occur *in vitro*. In practice, such penetration is unlikely to be significant because of the multiplying effects of villi and microvilli, which produce a mucosal to serosal surface area ratio of the order of 200:1.²⁰ Furthermore, frozen section autoradiography has confirmed that sugars enter the mucosa predominantly at the tips of the villi.²¹ The observation that no intracellular lactose could be detected in the current study is consistent with the results of London *et al*¹⁸ who found no intracellular accumulation during incubation of biopsies for up to 90 minutes. It is likely that in the 10 minute incubation of our experiments, any lactose permeation would have been below the limits of detection or compensated by the extracellular fluid space correction.

Our results might have been biased by metabolism of absorbed glucose. However, the rate of lactose hydrolysis in control experiments was the same when expressed either as galactose or glucose released. Because galactose is poorly metabolised, this suggests no significant loss of glucose from the overall system. Thus glucose metabolism appears negligible, in accord with the results of Desjeux *et al* who used a similar *in vitro* technique.¹⁷

The kinetics of lactose hydrolysis have not previously been studied in intact jejunal biopsies. Our results suggest that hydrolysis follows Michaelis-

Menten kinetics with a K_m of 33.9 ± 2.2 mM comparable with that of 17–23 mM and 21 mM obtained for purified preparations of human lactase.^{22, 23} Lactose concentrations in this study were chosen to give maximum information about the uptake of released glucose, and the majority of concentrations lie below the estimated K_m for lactose hydrolysis which must therefore remain approximate. This distribution of concentrations also explains why it is possible to fit a model of two-component hydrolysis to the data. The kinetic constants thus obtained, however, have little physiological meaning and bear no relationship to K_m values for purified lactase.^{22, 23} This emphasises the need to interpret computer models of kinetic data with caution.²⁴

The kinetics of uptake of glucose released by hydrolysis could be fitted to a model of a single saturable transport system either with or without a linear uptake system. Incubation at higher concentrations might help to distinguish between these possibilities, but we favour the two component model as this has been demonstrated for glucose uptake when presented as the monosaccharide in rat *in vivo*²⁵ and in man *in vivo*²⁶ and *in vitro*.^{8, 16} Furthermore, the apparent J_{max} derived for glucose uptake from lactose assuming a saturable component only (14.1 ± 0.2 nmol/min/mg) was greater than that which we have previously observed for the active component of glucose uptake when presented as the monosaccharide (8.4 ± 1.1 nmol/min/mg),⁸ which is difficult to explain in kinetic terms.

A number of additional factors might influence the relationship between the amount of lactose hydrolysed and glucose uptake. In the current study, diffusional effects were not significant because a high shaking rate was chosen to minimise the unstirred water layer.²⁷ Significant galactose inhibition of transport was also unlikely because, although the galactose concentration present at the glucose transporting site in this tissue could not be measured, if one assumes a K_i for galactose of 10 mM (its previously derived K_i value²⁸) then a steady-state galactose concentration of 100 mM would be necessary to account for the change in the previously observed K_i for glucose from 1.81 mM⁸ to that of 21.3 mM found in this study. From the rate of hydrolysis depicted in Figure 2, we calculate that this concentration could be achieved only at the end of 10 minute incubations in 56 mM lactose and assuming that no galactose was absorbed by the tissue, conditions which were not present in the current study. Furthermore, because the time course of glucose uptake remained linear throughout the incubation period, galactose released by hydrolysis was not significantly inhibitory to uptake.

Theoretically, glucose uptake from lactose could also be affected by the efficiency of capture of the monosaccharides. Crane,²⁹ and Parsons and Pritchard³⁰ have postulated a spatial arrangement of the brush border such that after disaccharide hydrolysis, translocation of hexose into the tissue is favoured rather than its back diffusion to the medium. Our study lends no support to this concept for lactose hydrolysis *in vitro* as at low concentrations, efficiency of glucose capture was low and rose to a maximum of only 50% at 5.6 mM, thereafter remaining more or less constant. There was thus an equal chance of back diffusion to the medium and no favoured delivery to the glucose transport site.

Although changes in capture efficiency may affect uptake at low lactose concentrations, it seems likely from our results that glucose uptake from lactose is determined primarily by the rate of lactose hydrolysis because Figure 4 clearly shows that at a given lactose concentration glucose uptake was directly related to the lactase activity of the tissue. Furthermore, if lactose hydrolysis is rate limiting to glucose absorption from lactose, then analysis of the active glucose transport step alone should yield kinetic parameters closely akin to those of lactose hydrolysis.³¹ Thus the app K_i of glucose uptake (21.3 mM) was in broad agreement with the app K_m of lactose hydrolysis (33.9 mM).

Our results refute the hypothesis that lactase activity measured in mucosal homogenates does not reflect the ability of intact tissue to hydrolyse lactose. Although in intact tissue we measured only 25% of the activity in tissue homogenates, this was because of the higher pH of the assay necessary for maintenance of tissue integrity and is consistent with the known pH profile for brush border lactase.^{22, 23} The important point is that the relationship between the two assays of lactase (intact *vs* homogenised tissue) was the same in the normolactasic subjects, those with hypolactasia (primary or secondary), and in those with lactose intolerance of uncertain aetiology but with normal lactase levels. Furthermore, the relationship between the uptake of glucose from lactose and tissue lactase activity was also similar in these groups. Thus, the explanation for the poor correlation between lactose intolerance and mucosal lactase activity,^{4, 6} does not lie in a functional disorder of the lactase in the brush border membrane.

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