Clin Chem Lab Med 2003; 41(8):1056–1063 © 2003 by Walter de Gruyter · Berlin · New York

Assessment of Hypolactasia and Site-Specific Intestinal Permeability by Differential Sugar Absorption of Raffinose, Lactose, Sucrose and Mannitol

Jan Hessels[1]*, Harry H. M. Eidhof[1], Jan Steggink[1], Wilfried W. H. Roeloffzen[2], Kalung Wu[2], Guy Tan[3], Jan van de Stadt[4] and Leo van Bergeijk[2]

[1] Clinical Laboratory,
[2] Department of Internal Medicine,
Twenteborg Hospital, Almelo, The Netherlands
[3] Department of Internal Medicine, Streekziekenhuis Midden Twente, Hengelo, The Netherlands
[4] Laboratory for Pathology, Oost-Nederland, Enschede, The Netherlands

The sugar absorption test is a non-invasive test for investigating intestinal permeability by simultaneous measurement of four probe sugars. In this study, we evaluated the utility of raffinose, lactose, sucrose and mannitol as probe sugars and calculated their urinary recovery as a percentage of ingested dose (mol/mol) and the recovery ratios of raffinose/mannitol, lactose/raffinose and sucrose/raffinose. The reference ranges for these ratios, established from 39 healthy volunteers, are 0.005–0.015, 0.13–0.63 and 0.09–0.47, respectively. This sugar absorption test was performed in three patient groups. i) In 109 patients with aspecific gastrointestinal symptoms of whom intestinal histology was studied by duodenal biopsies: the urinary raffinose/mannitol recovery ratio highly correlated with gradation of duodenal damage; the sensitivity and specificity of the raffinose/mannitol ratio for detection of intestinal damage were 93% and 91%, respectively, using a cut-off level of 0.020. ii) In 70 patients in whom intestinal lactase activity was investigated by the lactose tolerance test: the urinary lactose/raffinose recovery ratio provided high diagnostic accuracy for hypolactasia (sensitivity 81% and specificity 89% at a cut-off level of 0.70). In analogy with the lactose/raffinose ratio, we suppose that the sucrose/raffinose ratio can be used as a marker of hyposucrasia. iii) In 40 patients with localized small intestinal damage, Crohn's disease of the ileum (n = 21) and celiac disease with histologically proven duodenal damage (n = 19): the raffinose/mannitol recovery ratio was increased in 100% of patients with celiac disease and in 81% of patients with Crohn's disease; increased lactose/raffinose recovery ratio (hypolactasia) and increased sucrose/raffinose (hyposucrasia) were present in 89% and 95% of celiac patients and 19% and 0% of Crohn's disease patients, respectively. The combination of the raffinose/mannitol ratio and sucrose/raffinose ratio appears to be an indication of the distribution of intestinal damage. Clin Chem Lab Med 2003; 41(8):1056–1063

Key words: Intestinal permeability; Hypolactasia; Hyposucrasia; Raffinose; Lactose; Sucrose; Mannitol; Celiac disease; Crohn's disease.

Abbreviations: AUC, area under the curve; GI, gastrointestinal; IBS, irritable bowel syndrome; IEL, intraepithelial lymphocyte; IgA, immunoglobulin A; IQR, interquartile ranges; Man, mannitol; MCTD, mixed connective tissue disease; NSAID, non-steroid anti-inflammatory drug; Lac, lactose; LTT, lactose tolerance test; ROC curve, receiver operating characteristic curve; SAT, sugar absorption test; SLE, systemic lupus erythematosus; Suc, sucrose; Raf, raffinose.

Introduction

The differential sugar absorption test (SAT) has increasingly been applied for detection of small intestinal diseases. The principles of this test are based on the observation that patients with villous atrophy, caused by *e.g.*, celiac disease (1–3), giardiasis (1) or gastroenteritis (4), have a diminished permeability for small sugar molecules such as mannitol, rhamnose and arabinose. The decreased absorption of the monosaccharides is ascribed to reduced intestinal absorptive area and in this way a decrease in the transcellular transport. These patients, however, show an increased permeability of disaccharides such as lactulose (2, 5, 6), cellobiose (1, 3, 7) and the trisaccharide raffinose (8). The oligosaccharides are solely taken up through intercellular tight junctions or cell extrusion zones of the villous tip (9), and not through transcellular transport. The mentioned monosaccharides and oligosaccharides are poorly metabolized and rapidly cleared in the urine (10). Thus, the urinary recovery ratio of oligosaccharide/monosaccharide increases in the case of diminished small intestinal integrity. When both such sugars are administered in the same drink and the percentages of the ingested dose recovered in urine are expressed as a ratio, many interfering factors, such as gastric emptying, dilution by secretions, rate of transit, renal clearance and urine collection, are eliminated (7).

Urinary recovery ratios of cellobiose/mannitol, lactulose/mannitol, lactulose/rhamnose and raffinose/L-arabinose have all been described to be an index for intestinal permeability. The lactulose/mannitol ratio is most widely used in practice. However, because of the promising results described by Lobley *et al.* (8) with re-

*E-mail of the corresponding author:
famjhessels@hetnet.nl

spect to excellent separation of celiac patients from control subjects using raffinose as probe sugar, we have chosen to evaluate the raffinose/mannitol (Raf/Man) ratio as an index for intestinal permeability. Further, raffinose can be measured enzymatically with the same reagents as used for sucrose after pretreatment of urine with α-galactosidase (11). In order to investigate the clinical utility of the Raf/Man ratio for intestinal permeability, the SAT was performed in 109 patients with gastrointestinal (GI) symptoms and their medical records were reviewed. The results were compared with the histological findings of duodenal biopsies.

The disaccharides lactose and sucrose are digested by intestinal lactase (EC 3.2.1.23) and sucrase (EC 3.2.1.48). Lactose (β-D-galactopyranosyl-α-D-glucopyranoside) is degraded into glucose and galactose and sucrose (β-D-fructofuranosyl-α-D-glucopyranoside) into glucose and fructose. The enzymes are localized on the villous enterocytes of the intestinal brush border. Lactase is present at the highest concentration in the first years of life (until about 5 years) and declines in adult life to about 10% of that found in the newborn period, whereas sucrase is present at a constant concentration during life. Lactase activity is found in a proximal to distal gradient in the small intestine with high activity in the jejunum and low activity in duodenum and ileum (12). Low intestinal lactase levels will result in dumping and fermentation of lactose in the colon and often leads to intestinal discomfort and diarrhea (13). There are well-known racial and ethnic differences in adulthood lactase activities, with relatively high activity present in Northern European populations and very low activity in the majority of Asian and African populations (primary hypolactasia). Adulthood lactase activity is believed to be regulated at the transcriptional level (14). Secondary hypolactasia is the result of mucosal damage. The degree of hypolactasia, measured as enzyme activity in distal duodenal biopsies, is related to the degree of villous atrophy (15, 16). Lactase activity can be determined directly by measuring its activity in intestinal biopsies or indirectly by non-invasive lactose tolerance tests (LTT). In the latter tests, lactose degradation or absorption is measured after a standard dose of lactose (25 or 50 g lactose). The rate of lactose degradation can be monitored by changes in blood glucose (17) or H_2 production released during lactose fermentation (lactose breath hydrogen test). Lactose absorption can be monitored by urinary lactose excretion in a SAT (4, 8, 18, 19). Various investigators have shown that a combined SAT, using lactose/lactulose or lactose/raffinose (Lac/Raf), can potentially recognize hypolactasia in humans (8, 18, 19) and in rats (20). The principle of this differential SAT is based on the finding that recovery of intact disaccharides in urine is inversely related to their rate of intestinal hydrolysis. In order to investigate the clinical utility of the Lac/Raf ratio as a marker of hypolactasia, both the SAT and the LTT (plasma glucose response) were performed in 70 patients with GI symptoms.

Since raffinose is not metabolized in the small intestine its absorption is independent of the location of the intestinal damage. On the contrary, sucrose and lactose are degraded by sucrase and lactase in intact proximal small intestine. As assumed above, proximal or entire damage of the gut associated with hypolactasia and hyposucrasia will result in higher increased sucrose and lactose relative to raffinose absorption with concomitant increased Lac/Raf and sucrose/raffinose (Suc/Raf) recovery ratios. However, when intestinal damage is solely limited to the ileum, lactose and sucrose will proximally be degraded with concomitant normal/low Lac/Raf and Suc/Raf and increased Raf/Man recovery ratios. In order to investigate these assumptions, the SAT was performed in patients with Crohn's disease of the ileum (n = 21) and celiac disease with abnormal duodenal biopsy (n = 19).

Raffinose, lactose, sucrose and mannitol concentrations in urine were measured by enzymatic analysis (11) and the Raf/Man, Suc/Raf and Lac/Raf recovery ratios were calculated from the recoveries of the ingested dose. Reference values have been established from 39 healthy volunteers.

Materials and Methods

Reagents

D(–) mannitol was obtained from OPG (Utrecht, The Netherlands), D(+) raffinose pentahydrate from Fluka (Buchs, Switzerland), lactose monohydrate and sucrose from Bufa (Uitgeest, The Netherlands). The reagents used for enzymatic measurement of the probe sugars have previously been described by us (11). Plasma glucose concentration was determined on a Hitachi analyzer with a standard glucose dehydrogenase assay of Roche (Mannheim, Germany). Reagents for determination of immunoglobulin A (IgA) gliadin and endomysium were obtained from Pharmacia (Woerden, The Netherlands).

Subjects and clinical examination

The SAT was performed in a group of 109 adult patients (35 male, 74 female; median age 49 years, range 22–83 years) with GI symptoms during the period of 1996 until 1999. The diagnostic approach consisted of anamnesis and physical examination and on indication determination of IgA gliadin and/or endomysium antibodies; stool examination for fat excretion and microscopic analysis for parasites and cysts using the Ridley concentration method; dietary restriction for lactose or lactose challenge; sonography of the upper abdomen and X-ray of the small and large bowels. When no pathological process could be found the patients were diagnosed as having irritable bowel syndrome (IBS) when they fulfilled the criteria for IBS as described by Manning *et al.* (21). Gastroduodenoscopies with biopsies were taken in all cases and their medical records were reviewed. The second group consisted of 70 patients (24 male, 46 female, median age 47 years, range 18–78 years) with gastrointestinal symptoms suspect for lactose intolerance. Both the SAT and, within 3–5 days, the LTT were performed in these patients. The third group consisted of 40 patients (11 male, 29 female, median age 41 years, range 18–76 years) with previously established diagnosis; 21 patients with Crohn's disease of the ileum (abnormal X-ray of ileum) and 19 patients with celiac disease (presence of gliadin or endomysium antibodies in serum and abnormal duodenal biopsy). A group of 39 apparently healthy volun-

teers (13 males, 25 females, median age 37 years with a range of 24–57 years) represented the reference group.

Test protocol and laboratory methods

After a 12-hour fast, subjects emptied their bladder and ingested the test solution. This solution contained 7 g raffinose pentahydrate (11.8 mmol) and 2 g mannitol (11.1 mmol), 20 g lactose monohydrate (55.5 mmol) and 20 g sucrose (58.5 mmol) in 150 ml of water. Lactose and sucrose were added as probe sugars and served as osmotic fillers; the solution had an osmolarity of about 900 mosmol/l. A hyper-osmolar solution discriminates better between normal and damaged mucosa of the small bowel in patients with celiac disease (22). Further, 0.1 g methylparahydroxybenzoic acid was added as a preservative, and the solution could be kept at room temperature for 1 year. After 2 hours subjects were stimulated to drink water. Urine was collected during the following 5 hours after intake of the sugar solution in a container with 1 ml of 2% chlorhexidine solution. After measuring the urine volume an aliquot was stored at −20 °C until analysis. No symptoms were observed by the absorption test in subjects of the reference group. Some patients with an abnormal absorption test had an increase in stool frequency after the test but in no instance a change in fluid management was required. Urinary recovery of the probe sugars was calculated from the amount excreted in 5 hours collected in urine expressed as a percentage of the oral dose (mol/mol), with which the Raf/Man, Lac/Raf and Suc/Raf recovery ratios were calculated.

For the LTT, blood was collected for plasma glucose measurement after an overnight fast. Thereafter, subjects ingested the lactose solution (50 g lactose in 100 ml water) and blood was collected at 15, 30, 60 and 90 min. A plasma glucose increase of more than 1.4 mmol/l within 60 min was designated as a normal response and less than 1.4 mmol/l as a flat response. When glucose increase was highest at 90 min this was designated as a delayed response.

Table 1 Mean and 95% reference interval for urinary recovery (%) of mannitol, raffinose, sucrose and lactose, and recovery ratios of raffinose/mannitol (Raf/Man), sucrose/raffinose (Suc/Raf) and lactose/raffinose (Lac/Raf) from 39 healthy volunteers.

	Mean	95% Reference interval
Recovery (%)		
Mannitol	17.9	8.3–27.6
Raffinose	0.17	0.09–0.25
Sucrose	0.041	0.018–0.071
Lactose	0.062	0.028–0.095
Recovery ratio		
Raf/Man	0.010	0.005–0.015
Suc/Raf	0.28	0.09–0.47
Lac/Raf	0.38	0.13–0.63

Biopsy

Small bowel biopsies were performed by gastroduodenoscopy at the level of the pars descendens duodeni. Three specimens were collected in formaldehyde solution. The biopsies were histologically examined by one of the authors (JvdS), who was blinded for the results of the SAT. The intestinal mucosa has been classed into grades I to III as previously described by Uil *et al.* (6) according to the classification of Marsh (23). Grade I: normal mucosal architecture and normal number of intraepithelial lymphocytes (IELs); grade II: type 1 infiltrative lesion (normal mucosal architecture but with an increased number of IELs) + type 2 hyperplastic lesion (in addition to increased IELs there is an increase in crypt depth without a reduction in villous height); grade III: type 3 destructive lesion (a reduction in villous height to crypt depth ratio, normally between 5:1 and 3:1; hyperplasia and increased IELs may be present).

Statistical analysis

Reference ranges were calculated using the parametric IFCC method (24). Statistical differences between Raf/Man, Lac/Raf and Suc/Raf recovery ratios from patient groups and controls were calculated by the non-parametric Mann-Whitney U-test. Correlation between Lac/Raf (SAT) and glucose response (LTT) was calculated by Pearson correlation statistics. All statistical analyses were performed by using the statistical package Analyse-it (Analyse-it Ltd, Leeds, UK).

Table 2 Diagnosis of 109 patients subdivided according to duodenal histology and urinary raffinose/mannitol recovery ratio.

Duodenal histology	n	Diagnosis
Grade I and raffinose/mannitol ratio ≤0.020	61	IBS (11), dyspepsia (5), hernia diaphragmatica (2), NSAID-induced GI symptoms (3), iron deficiency anemia e.c.i. (3), post-cholecystectomy syndrome (2), cholelythiasis (2), bacterial overgrowth (2), lung carcinoma (1), diverticulosis (3), hypothyroidism (3), type 2 diabetes (2), SLE (1), lactose intolerance (4), colitis ulcerosa (1), colonic polyps (1), giardiasis (1), atrophic gastritis with ulcera (2), helicobacter pylori infection (1), no diagnosis (6), spontaneous recovery (5)
Grade I and raffinose/mannitol ratio >0.020	18	Bacterial overgrowth (2), iron deficiency anemia e.c.i. (2), giardiasis (1), vitamin B_{12} deficiency e.c.i. (2), stenosis of truncus celiacus (1), lung carcinoma (1), chronic pancreatitis (1), Crohn's disease (2), no diagnosis (6)
Grade II and raffinose/mannitol ratio ≤0.020	3	Diverticulosis (1), post-cholecystectomy syndrome (1), spontaneous recovery (1)
Grade II and raffinose/mannitol ratio >0.020	12	NSAID-induced GI symptoms (1), celiac disease with gluten-free diet (1), Crohn's disease (1), MCTD (1), giardiasis (4), entamoeba histolytica (1), Isospora belli (1), gastroenteritis (1), no diagnosis (1)
Grade III and raffinose/mannitol ratio >0.020	15	Celiac disease (9), giardiasis (4), Crohn's disease (1), spontaneous recovery (1)

Results

The mean values and reference ranges for urinary recovery of raffinose, lactose, sucrose and mannitol and for the Raf/Man, Lac/Raf and Suc/Raf recovery ratios obtained from 39 healthy volunteers are shown in Table 1.

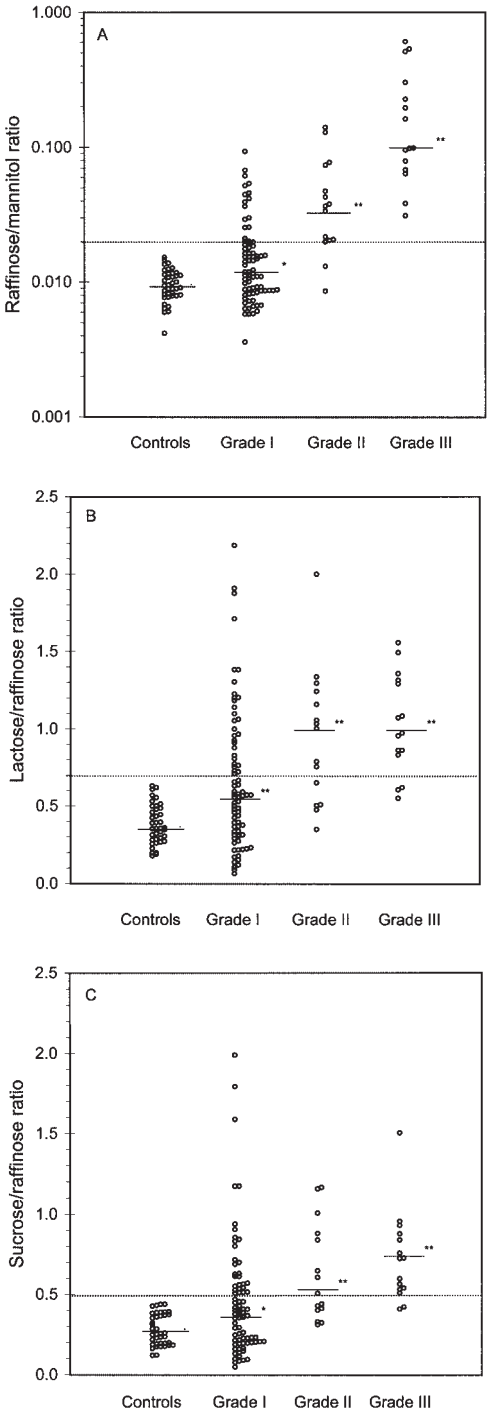

Figure 1 Urinary recovery ratios of raffinose/mannitol (A), lactose/raffinose (B) and sucrose/raffinose (C) from controls (n = 39), patients with grade I (n = 79), grade II (n = 15) and grade III (n = 15) duodenal biopsies (see text for explanation of histological gradation). The uninterrupted lines represent median values and the dotted lines the cut-off values. Statistically significant differences compared with controls (Mann-Whitney U-test) are reproduced as * $p < 0.01$, ** $p < 0.001$.

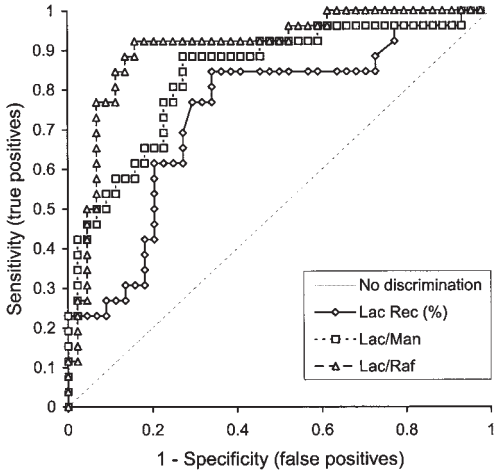

Figure 2 Receiver operating characteristic (ROC) curve of urinary lactose recovery (%), lactose/mannitol (Lac/Man) and lactose/raffinose (Lac/Raf) recovery ratios (SAT). Hypolactasia was established by plasma glucose increase (normal response ≥ 1.4 mmol/l) post-lactose load (LTT).

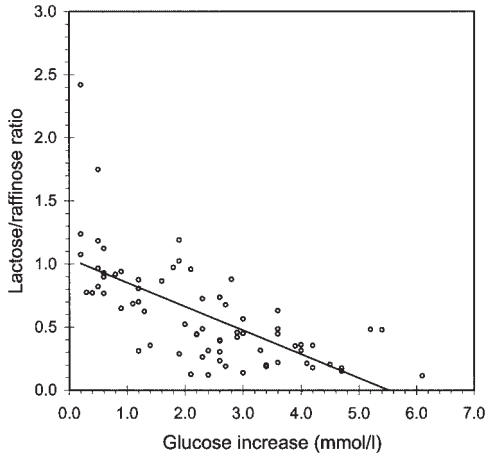

Figure 3 Correlation between plasma glucose response (LTT) and lactose/raffinose recovery ratio (SAT). Correlation was calculated by Pearson correlation statistics.

The group of 109 patients with GI symptoms was subdivided with respect to histological classification and their diagnoses are presented in Table 2. Figure 1 shows the Raf/Man (1A), Lac/Raf (1B) and Suc/Raf (1C) recovery ratios from controls and patients. This Figure shows that the degree of small intestinal damage (controls; n = 39, grade I; n = 79, grade II; n = 15 and grade III; n = 15) significantly correlates with the Raf/Man (medians 0.010, 0.012, 0.037 and 0.099, respectively), the Lac/Raf (medians 0.35, 0.57, 1.00 and 0.97, respectively) and the Suc/Raf recovery ratios (medians 0.26, 0.39, 0.51 and 0.72, respectively). The sensitivity of the Raf/Man recovery ratio for detection of duodenal damage corresponding with grade II and III biopsies is 93% (28/30). The specificity, calculated from the control group is 100% and calculated from the group with GI symptoms without duodenal damage (grade I) is 77% (61/79). It is striking to observe that notably the Lac/Raf and Suc/Raf recovery ratios in the group with grade I

biopsies is highly variable with values below and above the reference ranges (Figure 1B and C). Within this group, Raf/Man ratios are also variable, meanwhile less visible, with log scale with values above the reference range.

The results of the LTT could not be interpreted in 3/74 cases because of diabetes mellitus (basal plasma glucose level >6.9 mmol/l) and in another case because the basal glucose level was higher than the post-load glucose level. Figure 2 shows the receiver operating characteristic (ROC) curve for the percentage lactose recovery (area under the curve (AUC) 0.74), Lac/Man (AUC 0.84) and Lac/Raf recovery ratios (AUC 0.90). A cut-off value of 0.70 for Lac/Raf provides the highest diagnostic accuracy (sensitivity 81% and specificity 89%) for prediction of hypolactasia. This cut-off value is close above the upper reference limit (Table 1). Figure 3 shows a statistically significant correlation ($p < 0.001$; Pearson correlation coefficient −0.67; 95% CI: −0.51 to −0.78) between plasma glucose increase (LTT) and Lac/Raf ratio (SAT). Figure 4 demonstrates that normolactasia (glucose increase of ≥1.4 mmol/l) was observed in 63% (44/70) and hypolactasia (glucose increase of <1.4 mmol/l or delayed glucose response) in 37% (26/70) of the cases; the Lac/Raf ratio of the normolactasia group (median 0.39; range 0.11–1.19) is significantly lower than that of the hypolactasia group (median 0.88; range 0.31–2.42). This Figure also demonstrates that all patients with a delayed glucose response (n = 8) had an increased Lac/Raf ratio.

Figure 5 shows the statistically significant differences in recovery ratios between controls (n = 39), celiac patients (n = 19) and patients with Crohn's disease (n = 21) for Raf/Man ratio (medians 0.010, 0,195 and 0.039, respectively), Lac/Raf ratio (medians 0.35, 1.00 and 0.23, respectively) and Suc/Raf ratio (medians 0.26, 0.67 and 0.13, respectively).

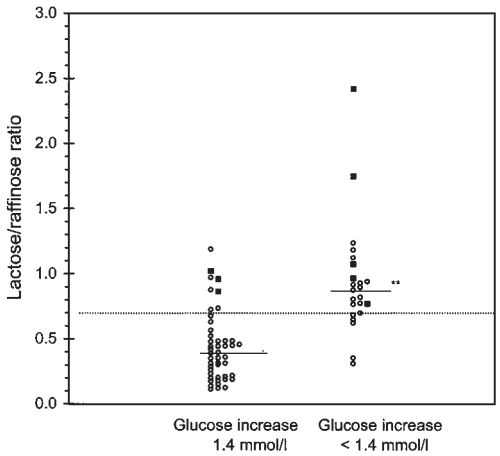

Figure 4 Lactose/raffinose recovery ratio (SAT) in patients with normolactasia (glucose increase ≥ 1.4 mmol/l) and hypolactasia (glucose increase < 1.4 mmol/l). Patients with delayed response are expressed as %. The uninterrupted lines represent median values and the dotted line the cut-off value. Statistically significant difference between both groups (Mann-Whitney U-test) is reproduced as ** $p < 0.001$.

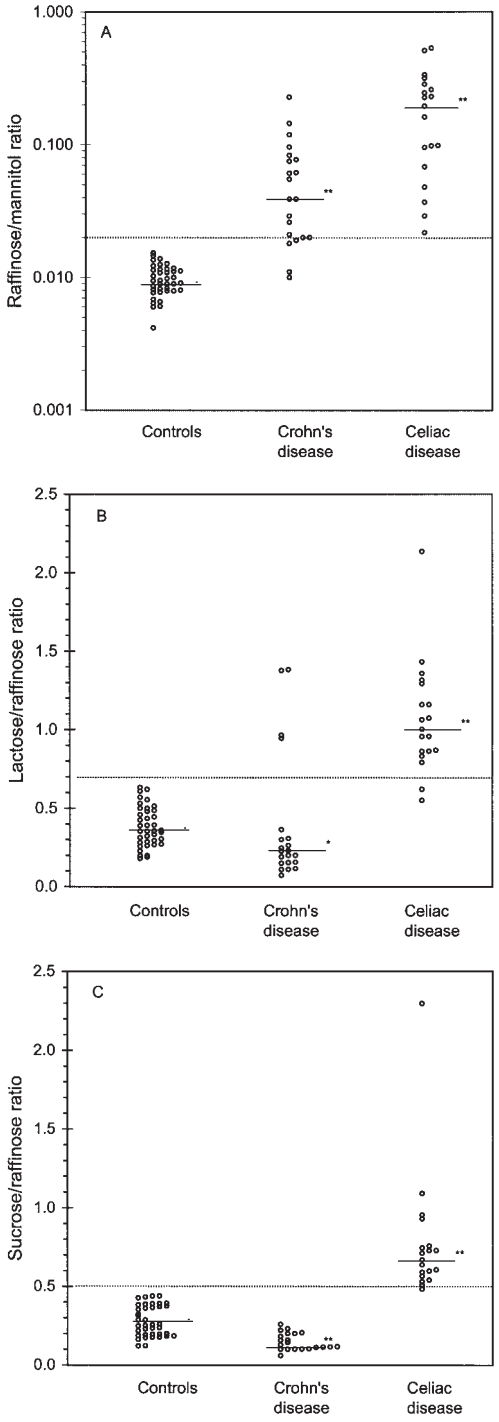

Figure 5 Urinary recovery ratios of raffinose/mannitol (A), lactose/raffinose (B) and sucrose/raffinose (C) from controls (n = 39), patients with Crohn's disease (n = 21) and patients with celiac disease (n = 19). The uninterrupted lines represent median values and the dotted lines the cut-off values. Statistically significant differences compared with controls (Mann-Whitney U-test) are reproduced as * $p < 0.01$, ** $p < 0.001$.

Discussion

The recovery ratios, calculated from the percentage recovery in 5 hours collected urine, of two probe sugars has often been described as an index for small intestinal integrity. The trisaccharide raffinose (O-α-D-

galactopyranosyl(1,6)-α-D-glucopyranosyl-β-D-fructofuranoside) is a plant sugar and fulfills the requirements for a probe sugar (8). Raffinose is absorbed by unmediated diffusion and is not present to a significant extent in fasting urine (8, 10). Absorption of raffinose in the small intestine is about 100-fold less than of mannitol, normally being 0.17% and 18% of the ingested dose, respectively (Table 1). This is comparable with the excretion of raffinose described by Lobley *et al.* (8). The absorption of raffinose is lower than described for lactulose, giving lower Raf/Man (mean 0.010) than lactulose/mannitol ratios (mean about 0.020) (2, 5, 22). This is most likely attributed to the larger molecular size of raffinose compared with lactulose. The 3- and 4-fold lower urinary excretion of lactose and sucrose can be explained by the fact that their rate of absorption is dependent on both degradation by intestinal enzymes and intestinal permeability.

There is a good correlation between the Raf/Man recovery ratio and the degree of villous atrophy with almost complete separation between controls and patients with grade II/III biopsies (Figure 1) and between controls, patients with Crohn's disease and celiac disease (Figure 5). With ROC curve analysis a cut-off value of 0.020 provides an optimal combination of highest sensitivity and specificity. The excellent sensitivity of 93% for the Raf/Man ratio makes this test an ideal screen for small intestinal damage. This may influence the necessity of duodenal biopsies. Although no histological abnormalities were observed in the duodenal biopsies (grade I) the Raf/Man ratio was increased in 18/79 cases, resulting in a specificity of 77%. Three possible explanations for this somewhat disappointing specificity can be given: i) The Raf/Man ratio is not specific for small intestinal damage but may also be increased in other diseases. This could be a possible explanation for only 1 patient with pancreatitis. ii) The Raf/Man ratio is more sensitive than the small intestinal biopsy. It has been shown, although the intestinal biopsy may be normal in routine microscopy, that there is often a change in morphometry when analyzed with a computerized measuring facility (25). This might, at least partly, be an explanation especially for mild disturbances in the villous layer and slightly increased Raf/Man ratios; iii) The gastrointestinal damage is located in the distal part of the small intestine. In that case the Raf/Man ratio will be increased despite normal duodenal histology (see below).

Several authors have suggested that sucrose is a marker of gastric damage (26, 27). However, Meddings *et al.* (20) have shown that the highest sucrose excretion rates were observed in rats with severe damage in the entire gastrointestinal tract induced with high dose indomethacin. Considering the much smaller surface area and shorter contact time of the probe sugars in the stomach it may be concluded that the degree of sucrose excretion rate is mainly dependent on small intestinal integrity and not, or to a much lesser extent, to gastric damage. Because of the normally present high sucrase and lactase activity in proximal intestine and diminished activity during villous atrophy (16), increased sucrose and lactose excretion rates would be expected in intestinal disorders associated with proximal damage. The disaccharides sucrose and lactose are believed to be absorbed *via* intercellular tight junctions in the same way as raffinose. Thus, the recovery ratios Lac/Raf and Suc/Raf are solely dependent on intestinal lactase and sucrase activity. Lactose/lactulose (or lactose/raffinose) and sucrose/lactulose (or sucrose/raffinose) have previously been proposed to be markers for hypolactasia and hyposucrasia, respectively (4, 8, 18, 19). However, until now no literature has been available about comparison of the SAT and the LTT. In the present study we demonstrate a highly significant inverse correlation between glucose increase following LTT and Lac/Raf recovery ratio (Figure 3). A cut-off value of 0.70 for the Raf/Man ratio provides good diagnostic accuracy for detection of hypolactasia with a sensitivity and specificity of 81% and 89%, respectively. As supposed, the Lac/Raf ratio is a better measure for hypolactasia (highest AUC) than percentage lactose recovery or Lac/Man ratio (Figure 2). Exploration of hypolactasia is of importance for diagnostic purposes and for follow-up in patients with celiac disease (15, 28). In analogy, with an arbitrary chosen cut-off value of 0.50 (close above upper reference limit; Table 1) the Suc/Raf recovery ratio is proposed to be a good marker for hyposucrasia.

Primary hypolactasia is defined as genetically determined low lactase and normal sucrase activity. Increased lactose/lactulose ratio and decreased intestinal lactase activity (or low lactase/sucrase activity ratio) is invariably observed in patients with proven primary hypolactasia (19). In our study, 6/70 patients had the combination of increased Lac/Raf, normal Suc/Raf and normal Raf/Man ratios (data not shown), highly suggestive for primary hypolactasia and 6/6 patients had a flat LTT (median glucose increase 0.6 mmol/l; range 0.3–0.9 mmol/l). Potentially, this differential SAT may be able to distinguish between primary and secondary hypolactasia. As we have not measured intestinal enzyme activities, further studies are necessary to support this hypothesis.

Secondary hypolactasia and hyposucrasia are caused by decreased enzyme activity as a consequence of intestinal damage: all patients (n = 8) with delayed glucose response (highest glucose at 90 min) had increased Raf/Man, Lac/Raf and Suc/Raf ratios (data not shown), compatible with decreased enzyme activity secondary to intestinal damage. Additionally, hypolactasia and hyposucrasia were observed in 80% (12/15) and 87% (13/15) of patients with grade III biopsies (Figure 1B and C) and 89% (17/19) and 95% (18/19) of celiac disease patients (Figure 5B and C), respectively. These observations are compatible with those described by Meddings *et al.,* who documented increased sucrose/lactulose ratios in duodenal damage in rats treated with low doses of aspirin or indomethacin (20). However, our results are significantly better than described by others: about half of the celiac patients had an abnormal/flat LTT (28) or increased lactose/lactulose ratio (19), despite severely depressed enzyme activities in

small intestinal biopsies. In another study, only 65% of celiac patients with villous atrophy, 15% with giardiasis and 6% with villous atrophy associated with non-celiac histology, had low intestinal disaccharidase levels (29). This is generally ascribed to the fact that mucosa is most damaged proximally in the small bowel. The biopsy specimen only reflects the condition at the ligament of Treitz, while LTT and SAT measure the lactose-hydrolyzing capacity of the entire small intestine (19, 28). Another possibility might be that raffinose is a better probe sugar than lactulose because of its lower urinary recovery in controls and smaller reference range than lactulose. Further, urinary probe sugars at micromolar concentrations need to be measured with high accuracy, because errors are multiplied when calculating ratios. This can be achieved by using enzymatic methods (11). Possibly, chromatographic methods are not sufficient for this purpose.

It is worthwhile to mention that 19% (13/70) of patients in the LTT study had increased Lac/Raf and Suc/Raf ratios, despite normal Raf/Man ratios, indicating hypolactasia and hyposucrasia without intestinal damage. An appropriate flat glucose response was observed in 9/13 patients (median 1.1 mmol/l; range 0.2–2.8 mmol/l). Further, 19% (15/79) of patients with grade I biopsies had the same pattern, which was not observed in controls, patients with celiac disease, Crohn's disease or grade II or III biopsies. Neither primary nor secondary causes can explain the loss of both lactase and sucrase activity without intestinal damage. It has been shown that duodenal secretions, notably bile salts, can rapidly release all enzymes from the brush border (30). Furthermore, carbohydrate ingestion, notably sucrose and fructose, induces the transcription of lactase and sucrase genes (31, 32) and malnutrition suppresses their transcription (33). The nutritional status of our patients had not been recorded. In addition, there are indications that atrophic gastritis in the elderly may play a role in lactose intolerance (34, 35). On the other hand, increased Lac/Raf and Suc/Raf ratios can also be ascribed to decreased raffinose recovery. We have established that 6/13 patients had raffinose recovery less than the lowest reference limit of 0.09% (Table 1). It may be speculated that this decrease is caused by accelerated intestinal transit. As lactose and sucrose recovery are already close to zero, only raffinose recovery is affected by increased transit time, causing increased Lac/Raf and Suc/Raf ratios. Further investigations are necessary to elucidate the causes of this proposed "tertiary" hypolactasia and hyposucrasia.

The marked differences in Lac/Raf and Suc/Raf ratios between celiac and Crohn's disease (Figure 5) can be ascribed to differences in location of intestinal damage: only Crohn's disease patients with an abnormal X-ray of the ileum and celiac patients with abnormal duodenal biopsy were included. In the case of distal intestinal damage, sucrose and lactose are already enzymatically degraded before reaching the ileum, resulting in low Suc/Raf and Lac/Raf ratios. On the contrary, proximal (or entire) intestinal damage will result in increased Suc/Raf and Lac/Raf ratios (Figure 5B and C). Thus, the combination of increased Raf/Man and normal/low Suc/Raf ratio (21/21 patients; Figure 5C) is compatible with distal intestinal damage, which is an additional diagnostic property of the differential SAT. This combination is not specific for Crohn's disease, but is also observed in some patients with giardiasis (data not shown). Establishment of the distribution of intestinal damage may be of importance for the choice of therapy for Crohn's patients. The prolonged released formula (*e.g.*, Pentasa®) is also active in the small intestine, whereas the targeted released formula (*e.g.*, Asacol®) disintegrates mainly in the colon (36). Interestingly, 4/21 Crohn's patients during recovery of disease had increased Lac/Raf and normal Suc/Raf ratios. It is known that sucrase activity is more rapidly recovered than lactase activity, providing a relative lactase deficiency.

The knowledge that increased Raf/Man and low/normal Suc/Raf ratios are caused by distal intestinal damage explains a great part of the supposed lack of specificity of the Raf/Man ratio. Within the group of grade I biopsies, 11/18 patients had increased Raf/Man and low/normal Suc/Raf ratios. Taking this into account, the specificity for detection of intestinal damage of the combination of Raf/Man and Suc/Raf ratios becomes 91% (72/79) and the positive predictive value 80% (28/35). The sensitivity is 93% (28/30) and the negative predictive value 97% (72/74). These diagnostic characteristics appear to be sufficient for routine clinical use in gastroenterology.

It is interesting to mention that we have measured extremely high Lac/Raf ratios (>10) in lactating women and women in the third trimester of pregnancy. In addition, it is important that patients empty their bladder before drinking the test solution, because sucrose and lactose may be present in fasting urine. Thus, endogenous lactose can gives false positive results with this method.

In conclusion, this study demonstrated that i) Raf/Man recovery ratio is an excellent measure for intestinal damage with high sensitivity and specificity; ii) Lac/Raf recovery ratio is a good index for hypolactasia and iii) the combination of Raf/Man with Suc/Raf ratio provides information of the distribution of intestinal damage. In selective proximal damage the Suc/Raf ratio is high and declines as damage moves distally.

References

1. Juby LD, Rothwell J, Axon AT. Cellobiose/mannitol sugar test – a sensitive tubeless test for coeliac disease: results on 1010 unselected patients. Gut 1989; 30:476–80.
2. Van Elburg RM, Uil JJ, Mulder CJ, Heymans HS. Intestinal permeability in patients with coeliac disease and relatives of patients with coeliac disease. Gut 1993; 34:354–7.
3. Cobden I, Dickinson RJ, Rothwell J, Axon AT. Intestinal permeability assessed by excretion ratios of molecules: results in coeliac disease. Br Med J 1978; 2:1060–1.
4. Noone C, Menzies IS, Banatvala JE. Scopes JW. Intestinal permeability and lactose hydrolysis in human rotaviral gastroenteritis assessed simultaneously by non-invasive differential sugar permeation. Eur J Clin Invest 1986; 16:217–25.

5. Juby LD, Rothwell J, Axon AT. Lactulose/mannitol test: an ideal screen for celiac disease. Gastroenterology 1989; 96:79–85.
6. Uil JJ, van Elburg RM, van Overbeek FM, Meyer JW, Mulder CJ, Heymans HS. Follow-up of treated coeliac patients: sugar absorption test and intestinal biopsies compared. Eur J Gastroenterol Hepatol 1996; 8:219–23.
7. Cobden I, Hamilton I, Rothwell J, Axon AT. Cellobiose/mannitol test: physiological properties of probe molecules and influence of extraneous factors. Clin Chim Acta 1985; 148:53–62.
8. Lobley RW, Burrows PC, Warwick R, Dawson DJ, Holmes R. Simultaneous assessment of intestinal permeability and lactose tolerance with orally administered raffinose, lactose and L-arabinose. Clin Sci 1990; 79:175–83.
9. Turner JR, Rill BK, Carlson SL, Carnes D, Kerner R, Mrsny RJ, *et al.* Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. Am J Physiol 1997; 273:C1378–85.
10. Menzies IS. Absorption of intact oligosaccharide in health and disease. Biochem Soc Trans 1974; 2:1042–7.
11. Hessels J, Snoeyink EJM, Platenkamp AJ, Voortman G, Eidhof HHM. Assessment of intestinal permeability: enzymatic determination of urinary mannitol, raffinose, sucrose and lactose on Hitachi analyzer. Clin Chem Lab Med 2003; 41:33–8.
12. Newcomer AD, McGill DB. Distribution of disaccharidase activity in the small bowel of normal and lactase-deficient subjects. Gastroenterology 1966; 51:481–8.
13. Rings EHHM, Grand RJ, Büller HA. Lactose intolerance and lactase deficiency in children. Curr Opinion Pediatr 1994; 6:562–7.
14. Van Beers EH, Rings HHM, Taminiau JAJM, Heyman HSA, Einerhand AWC, Dekker J, *et al.* Regulation of lactase and sucrase-isomaltase gene expression in the duodenum during childhood. J Pediatr Gastroenterol Nutr 1998; 27:37–46.
15. Nieminen U, Kahri A, Savilahti E, Färkilii MA. Duodenal disaccharidase activities in the follow-up of villous atrophy in coeliac disease. Scan J Gastroenterol 2001; 36:507–10.
16. Heitlinger LA, Rossi TM, Lee P, Lebenthal E. Human intestinal disaccharidase activities: correlation with age, biopsy technique, and degree of villous atrophy. J Pediatr Gastroenterol Nutr 1991; 12:204–8.
17. Newcomer AD, McGill DB. Lactose tolerance tests in adults with normal lactase activity. Gastroenterology 1966; 50:340–6.
18. Maxton DG, Catt SD, Menzies IS. Combined assessment of intestinal disaccharidases in congenital asucrasia by differential urinary disaccharide excretion. J Clin Pathol 1990; 43:406–9.
19. Bjarnason I, Batt R, Catt S, Macpherson A, Maxton D, Menzies IS. Evaluation of differential disaccharide excretion in urine for non-invasive investigation of altered intestinal disaccharidase activity caused by α-glucosidase inhibition, primary hypolactasia and coeliac disease. Gut 1996; 39:374–81.
20. Meddings JB, Gibbons I. Discrimination of site-specific alterations in gastrointestinal permeability in the rat. Gastroenterology 1998; 114:83–92.
21. Manning AP, Thompson WG, Heaton KW, Morris AF. Towards positive diagnosis of the irritable bowel syndrome. Br Med J 1978; 2:653–4.
22. Uil JJ. Clinical implications of the sugar absorption test. Chapter 2.3. Sensitivity and specificity of a hyperosmolar and "iso"-osmolar test solution for sugar absorption in recognizing small intestinal mucosal damage in coeliac disease [dissertation]. Utrecht: University Utrecht, 1996.
23. Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiological approach to the spectrum of gluten sensitivity ('celiac sprue'). Gastroenterology 1992; 102:300–54.
24. Solberg HE. Approved recommendation on the theory of reference values. Part 5. Statistical treatment of collected reference limits. J Clin Chem Clin Biochem 1987; 25:645–56.
25. Juby LD, Dixon MF, Axon ATR. Abnormal intestinal permeability and jejunal morphometry. J Clin Pathol 1987; 40:714–8.
26. Sutherland RL, Verhoef M, Wallace JL, Van Rosendaal G, Crutcher R, Meddings JB. A simple, non-invasive marker of gastric damage: sucrose permeability. Lancet 1994; 343:998–1000.
27. Wyatt J, Oberhuber G, Pongratz S, Püspök A, Moser G, Novacek G, *et al.* Increased gastric and intestinal permeability in patients with Crohn's disease. Am J Gastroenterol 1997; 92:1891–6.
28. Bode S, Gudmand-Høyer E. Incidence and clinical significance of lactose malabsorption in adult coeliac disease. Scand J Gastroenterol 1988; 23:484–8.
29. Duncan A, Park RP, Lee FD, Russell RI. A retrospective assessment of the clinical value of jejunal disaccharidase analysis. Scand J Gastroenterol 1994; 29:1111–6.
30. Young PG, Das L. Influence of duodenal secretions and its components on release and activities of human brush-border enzymes. Biochim Biophys Acta 1990; 1022:393–400.
31. Goda T, Yasutake H, Tanaka T, Takase S. Lactase-phlorizin hydrolase and sucrase-isomaltase genes are expressed differently along the villus-crypt axis of rat jejunum. J Nutr 1999; 129:1107–13.
32. Goda T. Regulation of the expression of carbohydrate digestion/absorption-related genes. Br J Nutr 2000; 84:S245–8.
33. Nichols BL, Dudley MA, Nichols VN, Putman M, Avery SE, Fraley JK, *et al.* Effects of malnutrition on expression and activity of lactase in children. Gastroenterology 1997; 112: 742–51.
34. Saltzman JR, Russell RM. The aging gut. Nutritional issues. Gastroenterol Clin North Am 1998; 27:309–24.
35. Lee MF, Krasinski SD. Human adult-onset lactase decline: an update. Nutr Rev 1998; 56:1–8.
36. Rijk MCM, Van Schaik A, Van Tongeren JHM. Disposition of mesalazine from mesalazine-delivering drugs in patients with inflammatory bowel disease, with and without diarrhoea. Scand J Gastroenterol 1992; 27:863–8.

Received 24 February 2003, revised 16 May 2003, accepted 23 May 2003

Corresponding author: Dr. Jan Hessels, Clinical Laboratory, Twenteborg Hospital, P.O. Box 7600, 7600 SZ Almelo, The Netherlands
Phone: +31 546833367, Fax: +31 546833371,
E-mail: famjhessels@hetnet.nl