

## Urinary mannitol:lactulose excretion ratios and jejunal mucosal structure

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**SUMMARY** A dual sugar (mannitol, lactulose) absorption test was evaluated using an iso-osmolar oral dose in two groups of children: a study group of 43 children divided into five subgroups, based on severity of mucosal damage, and a control group of 53 children with histologically normal jejunal biopsy specimens. After an oral dose, the three hour urinary mannitol:lactulose ratios in the control group showed a highly significant positive correlation with body surface area. After correction for the body surface area relationship, a control lower limit was defined by the mean  $-2SD$  of the  $\log_{10}$  transformed control mannitol:lactulose ratios.

Specificity and sensitivity for severe villous atrophy was 98% and 95% respectively but the sensitivity declined rapidly with decreasing degrees of mucosal damage, and the test would not therefore be an adequate screening procedure for all enteropathies. In sequential studies in 18 children, the changes in the mannitol:lactulose ratio were consistent with the changes in mucosal structure induced by gluten challenge or gluten withdrawal. The test may therefore have a role in any sequential study of lesions of the mucosa of the small intestine.

The diagnosis of a small intestinal enteropathy can be made only by jejunal biopsy. Non-invasive tests, such as the xylose absorption test have been used as preliminary screening procedures<sup>1</sup> but they have limitations.<sup>2</sup> Although satisfactory in principle, the inherent variables of gastric emptying, intestinal transit and blood flow, and renal function reduce the correlation between xylose absorption or excretion and the degree of mucosal damage.<sup>2</sup> The search for improved screening tests has continued and most recently a dual sugar absorption test has been proposed.<sup>3,4</sup> Dual sugar absorption tests are non-invasive and have the additional advantage of eliminating many of the variables associated with the single sugar absorption tests. Dual sugar absorption tests have been shown to be effective as screening tests for coeliac disease in adults.<sup>5-7</sup> The test has been used in a number of studies in children,<sup>8-12</sup> but its role as a diagnostic procedure for mucosal damage in childhood has not been fully validated.<sup>13,14</sup>

We have therefore assessed the value of a dual sugar test (mannitol-lactulose), by determining its efficiency in three potential applications: as a screen for an enteropathy; for assessing dietary compliance during gluten withdrawal; and for determining an appropriate time for a post gluten challenge biopsy.

### Patients and methods

#### SCREENING STUDY

The study group comprised 43 children undergoing jejunal biopsy for investigation of gastrointestinal symptoms (median age 6.8 years, range 0.4–17.2 years). All had either villous atrophy, of varying severity, or histological abnormalities without villous atrophy. They were divided into five groups by severity of histological abnormality, in a single blind situation, by the histopathologist of the Children's Hospital, Birmingham, using the criteria of Whitehead.<sup>15</sup> The five groups comprised: subtotal villous atrophy (n=14); severe partial villous atrophy (n=3); partial villous atrophy (n=6); mild, patchy partial villous atrophy (n=8); and increased cellularity of the lamina propria but no villous atrophy (n=12).

The control group comprised a further 53 children (median age 4.6 years, range 0.8–17.9 years) with gastrointestinal symptoms but whose jejunal biopsy specimen was histologically normal. None had a history of atopy.

#### DIETARY COMPLIANCE STUDY

In this part of the study nine children from the screening investigation, who had subtotal villous

atrophy, started a gluten free diet. In addition, eight children who were maintaining a histologically normal jejunal mucosa, while on a gluten free diet, started gluten challenge (20 g gluten/day). Dietary compliance was assessed at intervals of four to six weeks by the dietetic department of the Children's Hospital, Birmingham. The dietary findings were not communicated to the clinical investigators until the end of the study.

#### JEJUNAL BIOPSY

All patients in the study had one or more peroral jejunal biopsies as part of their routine gastrointestinal investigations. Biopsy specimens were obtained just distal to the ligament of Treitz using a paediatric size Watson modification of the Crosby capsule, positioned with fluoroscopy. None of the patients had bacterial contamination of the small bowel at the time of biopsy.

#### DUAL SUGAR (MANNITOL-LACTULOSE) ABSORPTION TEST

After an overnight fast (six to eight hours in infants) and after passing the first morning urine specimen (the 'fasting specimen'), the test sugar solution was ingested. After one hour, the test subjects were encouraged to drink water freely to increase urine flow but no other drinks or food were permitted for a further two hours when the test ended. The fasting specimen and all urine specimens passed during the test period (the 'test specimens') were saved separately in bottles containing 0.5 ml 10% w/v sodium azide. The time of each test specimen was recorded.

The test sugar solution contained 10 g/l mannitol and 75 g/l lactulose (274 mosm/l) and 0.1% Nipasept preservative. The dose was body surface area related (100 ml/square metre) with lower and upper dose limits of 20 and 100 ml.

The test was done within two days of the jejunal biopsy in 85% of the children and within five days in the remaining 15%. In the 17 children on gluten withdrawal or challenge, the tests were carried out at intervals of four to six weeks using the same sugar dose throughout for a given patient. None of the children was receiving drugs before or during the investigations. The test procedure was approved by the local ethical committee and informed verbal consent was obtained from parents of all the children before testing.

#### MANNITOL AND LACTULOSE ASSAY

All urine samples were stored at  $-20^{\circ}\text{C}$  until analysed. All test urines passed up to three hours after the sugar dose were pooled before analysis. Pooled samples were prepared for analysis by centrifuging cold to remove any deposit followed by desalting with Amberlite MB-1, acetate form. The

volume of desalted urine containing 2  $\mu\text{mol}$  creatinine was further purified by preparative thin layer chromatography on foil-backed plates.<sup>16</sup> Areas of the plates corresponding to mannitol and lactulose standards were excised and eluted with acetonitrile:water (72.5:27.5 v/v) and the eluant dried under vacuum. The residue was dissolved in 200  $\mu\text{l}$  acetonitrile:water just before chromatographic analysis. Mannitol and lactulose were measured by high performance liquid chromatography using a 250 $\times$ 5 mm Spherisorb-5 amino column (Phase Separations Ltd) or 250 $\times$ 4 mm LiChrosorb amino column (E Merck) eluted with acetonitrile:water (72.5:27.5 v/v) at 1.5 ml/minute. Eluted solutes were detected with a mass detector (Applied Chromatography Systems) and quantitated by comparison with standard mannitol-lactulose mixtures, peak areas being measured by a Milton-Roy CI-10 integrator. Test results were expressed as the mannitol:lactulose concentration ratio measured in the three hour post sugar ingestion urine pool.

Tests with fasting urines containing more than a trace of solutes with chromatographic retention times corresponding to mannitol or lactulose were discarded. All analyses were done in duplicate from the desalt stage and mean values taken. The coefficient of variation of differences between duplicate analyses was 2.5%. The recovery of mannitol and lactulose added to sugar free urine was 85% or greater.

The statistical analysis was non-parametric. Spearman's rank correlation coefficient was used to determine the degree and significance of association of two variables.

(Mannitol is a hexitol not a hexose but has been designated 'sugar' in this report for convenience).

## Results

#### SCREENING STUDY

Despite the use of a body surface area related sugar dose, mannitol:lactulose ratios showed a significant positive correlation with body surface area in the control children ( $r=0.646$ ,  $p<0.001$ ). The relationship was similar in children with villous atrophy but was less pronounced (fig 1). The ratios were therefore corrected for body surface area (ratio/BSA). The corrected data from the control group exhibited a positive skew and it was therefore  $\log_{10}$  transformed before determining population parameters. The lower limit of the control distribution shown in fig 2 is the mean  $-2\text{SD}$  of the  $\log_{10}$  transformed control data (mannitol:lactulose ratio of 3.09).

Fig 2 shows the corrected mannitol:lactulose ratios for the study and control groups. All but one

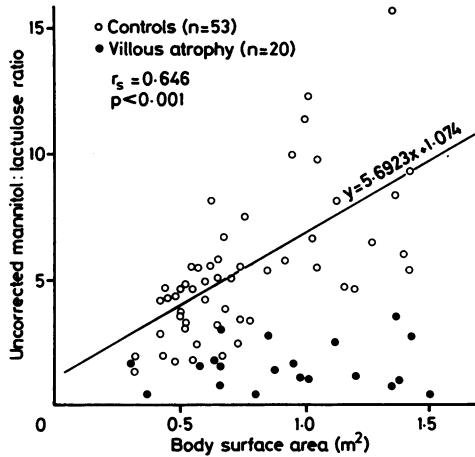


Fig 1 Three hour urinary mannitol:lactulose ratio—body surface area relationship. Children with villous atrophy comprised 14 with subtotal villous atrophy, three with severe partial villous atrophy, and three with partial villous atrophy. The body surface area correlation data applies only to the control group.

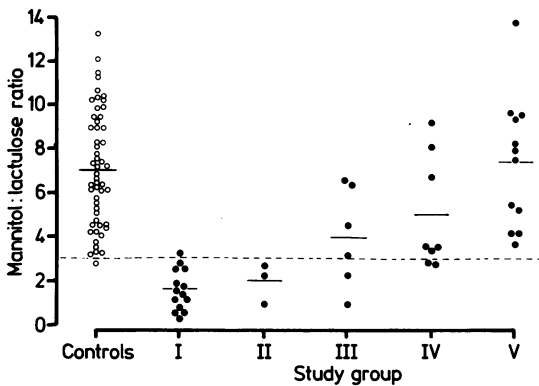


Fig 2 Body surface area corrected mannitol:lactulose ratios in control and study groups. The study group was divided into five subgroups based on severity of mucosal damage. I=subtotal villous atrophy (n=14); II=severe partial villous atrophy (n=3); III=partial villous atrophy (n=6); IV=mild, patchy partial villous atrophy (n=8); and V=increased cellularity of the lamina propria without villous atrophy (n=12). The horizontal bars show the mean value for each subgroup. The broken line denotes the lower limit of the control range defined by the mean -2SD of the log<sub>10</sub> transformed control mannitol:lactulose ratios.

of the 17 children with severe mucosal damage (subtotal villous atrophy or severe partial villous atrophy) had ratios below the lower limit of the control range. Lesser degrees of villous atrophy or histological abnormality were associated with ratios

within the control range. A clear trend in the ratios was seen, the ratios being abnormal with severe villous atrophy but reverting progressively to the control range with diminishing villous atrophy. Only two of six children with partial villous atrophy had ratios in the abnormal range.

DIETARY COMPLIANCE STUDY

Fig 3 shows the results in nine children, initially with subtotal villous atrophy, treated by gluten withdrawal. In the six children shown independently by dietary records to have complied with the diet, mannitol:lactulose ratios returned to normal. In the three children in whom gluten elimination was not complete, ratios remained in the abnormal range.

In five children being treated as 'coeliac' with a gluten free diet who had normal jejunal mucosae and mannitol:lactulose ratios, gluten challenge resulted in ratios falling below the lower limit for controls (fig 4). The ratios remained subnormal until the post gluten challenge biopsy specimen, which showed that subtotal villous atrophy had been caused in all five. On subsequent gluten withdrawal, ratios returned to the normal range in all five children. In three other 'coeliac' children challenged with gluten, both the ratio and mucosal structure remained normal throughout the whole of the challenge periods of 28–32 weeks (not shown).

Discussion

Mannitol and lactulose were selected as permeabil-

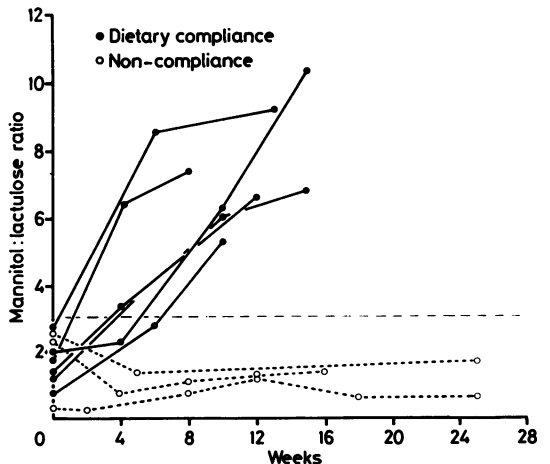


Fig 3 Mannitol:lactulose ratios during gluten withdrawal in nine children initially with subtotal villous atrophy. The broken line denotes the lower limit of the control range defined by the mean -2SD of the log<sub>10</sub> transformed control mannitol:lactulose ratios.

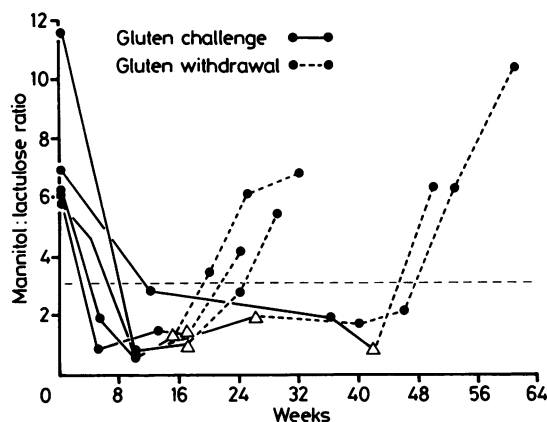


Fig 4 The change in mannitol:lactulose ratios during a gluten challenge and after subsequent withdrawal. All the children had a histologically normal mucosa on starting the challenge.  $\Delta$  denotes a post gluten challenge biopsy specimen showing subtotal villous atrophy. The broken line denotes the lower limit of the control range defined by the mean  $-2$  SD of the  $\log_{10}$  transformed control mannitol:lactulose ratios.

ity markers because they fulfill many of the criteria for a satisfactory test molecule.<sup>3,4</sup> They are constituents of normal infant diet, are present in proprietary infant milk formulas, and both have already been used in studies in preterm neonates<sup>8</sup> and infants.<sup>9,10</sup> They are not modified in the gut lumen<sup>17,18</sup> or in the body and although some metabolism of mannitol occurs in the liver, it is insignificant.<sup>19</sup> Both molecules are excreted by the kidney with clearances similar to creatinine.<sup>18</sup> Excretion is therefore directly proportional to the quantity crossing the mucosa of the small intestine.

The urine collection period of three hours was based on the results of a preliminary study (unpublished) which had shown that a three hour collection produced a better discrimination between normal and severely damaged mucosa patients than the more conventional five hours collection. The abbreviated test period and consequent reduction in the period of fasting is particularly advantageous in children.

The correlation of the mannitol:lactulose ratio with body surface area was an interesting finding. Previous workers have used a standard sugar dose for all body sizes<sup>10,13</sup> and it is possible that our body surface area related dose had the opposite effect to that intended—that is, to eliminate any body size effect. This observation suggests that the ratio of sugars ingested and the gut permeability to the sugars are not the only factors influencing the excreted sugar ratio. Gut permeability may change

with age but it seems more likely that the absorption kinetics of the two sugars changes differently with dose quantity or volume or size of subject. This feature warrants further investigation.

The sensitivity of the test for detecting an enteropathy was only acceptable when there was subtotal or severe partial villous atrophy (95%). Sensitivity fell to 33% for partial villous atrophy and to 25% for mild patchy villous atrophy. The test did not detect jejunal abnormalities characterised by increased cellularity only. In the control group, only one mannitol:lactulose ratio fell below the lower limit of the control range defined by the mean  $-2$ SD giving a specificity of 98%. Mannitol:lactulose ratios, however, remained in the control range in many children with an appreciable degree of villous atrophy or histological change. As a screening procedure therefore, the dual sugar absorption test, in the form that we have used, will only identify the most severe degrees of villous atrophy. In contrast, jejunal biopsy, with conventional histology, shows all degrees of structural damage and also provides information on the likely aetiology. Confirmation of a suspected enteropathy must still therefore depend on biopsy of the jejunum.

Many factors such as the ratio of the ingested sugars, the osmolarity of the test solution, size of the subject, and the duration of the urine collection alter the urinary ratio of the ingested sugars. Furthermore, the analytical methods and the individual sugars used have varied from one study to another. Few of the data already published therefore are directly comparable. The same applies to comparisons between published reports and our data. We are not aware, for example, of any other group that has used the advantage of the shorter, three hour urine collection that we report. Most other workers report ratios on five hour urine collections.<sup>6,7,10,11,13</sup> We have also chosen to use a mannitol:lactulose ratio rather than a lactulose:mannitol ratio. There is no theoretical basis for expressing the ratio with either sugar as numerator so for practical reasons we have expressed our results as mannitol:lactulose ratios. As the test is likely to be used in a routine clinical context, we consider it better to produce whole numbers that will be more readily appreciated in magnitude and discriminatory function. In addition, the fractional decimal numbers produced by a lactulose:mannitol ratio are more likely to cause transcription errors.

Other workers have expressed the excretion of test sugars as a percentage of the ingested dose before calculating the urinary sugar ratios. Expressed in this way, the overlap of mannitol:lactulose ratio data between our control and study groups was increased compared with the use

of simple urinary concentration ratios, so no advantage was obtained. The overlap also appeared to be greater than that found by other workers.<sup>6 7 13 14</sup> This may have been due to our use of iso-osmolar sugar solutions but, for reasons already discussed, such comparisons are difficult and may not be valid.

The permeability of the small intestine to lactulose is known to be increased in the presence of hyperosmolar solutions.<sup>20 21</sup> This phenomenon occurs both in the normal and in the severely damaged jejunal mucosa.<sup>20</sup> The increase in permeability in the damaged mucosa is greater than that of the normal mucosa, thus increasing the separation between the two groups. This has been the rationale for using hyperosmolar solutions in adults<sup>5-7</sup> and moderately hyperosmolar solutions in children.<sup>13 14</sup> Hyperosmolar solutions do, however, have the potential risk of inducing osmotic diarrhoea in children with an already damaged jejunal mucosa. We therefore considered their use unjustifiable in children. Others working with children have also chosen iso-osmolar test solutions.<sup>10 22</sup> The high degree of sensitivity and specificity that we report (fig 2) in the presence of severe villous atrophy did not produce complete separation of the two groups, and segregation was not improved when the results were expressed as percentage mannitol:lactulose ratios. We cannot therefore recommend the test in the present form as a screening procedure for enteropathies. Further studies evaluating the safety and usefulness of hyperosmolar solutions in children are required.

In the longitudinal studies the data from the children on gluten free or gluten challenge regimes suggested an important role for the test. The changes in the mannitol:lactulose ratio from normal to abnormal, and the reverse, were consistent throughout with the dietetic record and biopsy data. A change in bowel habit occurred in only one of the five children on gluten challenge that responded with mucosal relapse so that the change in ratio to subnormal levels was the sole indication of mucosal damage in the remainder before biopsy. The three children challenged with gluten, who did not respond in the challenge period with a change to subnormal ratios, did show a lesser initial decrease but remained within the control range. The importance of this small initial change is not known but may become apparent during the two year follow up on a normal diet necessary for exclusion of coeliac disease.

In the children with subtotal villous atrophy treated with a gluten free diet, only those not practising complete gluten exclusion failed to respond with mannitol:lactulose ratios returning to normal values within four to 12 weeks.

The value of the test therefore is much greater in longitudinal studies in an individual patient where the importance of the mannitol:lactulose ratio does not depend on its absolute value but on its relation to the results of earlier tests. A recent report has described the use of a mannitol-lactulose test, similar to ours, for following the improvement in ratio which accompanied the resolution of diarrhoea in children in the Gambia.<sup>22</sup>

The mannitol-lactulose dual sugar absorption test, using a sugar dose osmolarity appropriate for children with diarrhoeal disease, is not sufficiently sensitive to detect lesser degrees of villous atrophy and is not therefore a satisfactory screening procedure for an enteropathy. It may, however, have a useful role in the assessment of dietary compliance in children on a gluten free diet, and for determining the appropriate time for post gluten challenge biopsy. Its role in the sequential study of other lesions of the small intestinal mucosa deserves further study.

The authors wish to thank Sister Lynne Styles, dietetics department, department of medical illustration, and the medical consultant staff of the Children's Hospital, Birmingham.

Dr IW Booth made valuable comments during the preparation of the manuscript.

KA Nathavitharana was supported by the Association of Commonwealth Universities and the British Council.

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Accepted 8 March 1988