Chapter 8

Lactase activity in small bowel biopsy specimen is not an accurate reflection of overall intestinal lactose digestion capacity


Beatrix Children’s Hospital, University Hospital Groningen, Groningen, The Netherlands
(H.A.Koetse MD, B.C.Gonera-de Jong MD, P.J.J.Sauer MD, PhD, R.J.Vonk PhD, M.G.Priebe, F.Stellaard PhD)
Danone Vitapole, Le Plessis-Robinson, France
J. -M.Antoine
Grants: Financial support of Danone Vitapole, Le Plessis, France, is greatly acknowledged

Submitted
SUMMARY

Background
Lactase activity (LA) in a small intestinal bowel biopsy (SBB) specimen is frequently used as the indicator for the intestinal capacity to digest lactose, but its relation with the digestive capacity has not been established. We compared lactase activity in SBB with the results of a quantitative lactose digestion test.

Method
We compared LA in 18 children aged 0.8-10.9 yr (mean 3.9, SD 2.4) suspected of small bowel mucosal damage to the lactose digestion index (LDI), the lactose H₂ breath test and the histology in the biopsy.

Findings
In 5/6 patients with normal histology a normal LA was shown, in 1/6 the LA was low. In all 6 the Lactose Digestion Index (LDI) was normal (> 0.45) and the H₂ test was negative. In 3 of 5 patients with minor histological changes LA and LDI were both normal, with a negative H₂ test. One patient with medium histological changes had a low LA with a normal LDI and a negative H₂ result. In 6 patients with severe mucosal damage LA was low in 5/6, but 3/5 demonstrated a normal LDI while the H₂ test was negative in 3/6. One patient with severe mucosal damage showed a normal LA with a negative H₂ breath test and a low LDI.

Interpretation
Our results indicate that the total lactose digestive capacity can remain adequate despite low Lactase activity in a SBB. Extrapolation of the results of measurement of low LA in SBB specimens to the overall digestive gut function may not be reliable.
INTRODUCTION
Lactose (Galactose-β (1-4)-glucose) is digested in the small bowel into glucose and galactose by the brush border enzyme lactase. A decrease in the activity of this enzyme is considered an indicator of low lactose digestive capacity. Measurement of lactase activity (LA) in a small bowel biopsy (SBB) specimen is considered the “gold standard” for determination of lactose digestive capacity. All other diagnostic tests are validated against this standard\(^1\)\(^\text{-13}\), their sensitivity and selectivity have been disappointing. Recent data from Maiuri et al\(^1\)\(^\text{4}\) indicate that lactase activity in SBB’s from patients with mucosal damage actually has a patchy distribution, low and normal lactase activity can be demonstrated in different biopsies from the same patient. It can therefore be questioned if the lactase activity in small bowel biopsy material is the true reflection of overall lactose digestion capacity. We recently developed a lactose digestion test, using \(^{13}\text{C}\)-labelled lactose as a test substrate to measure lactase activity in vivo. We added \(^2\text{H}\)-glucose as a reference substrate to correct for individual variations in gastric emptying time, absorption and post-absorptive metabolism\(^1\). We validated this test by comparing healthy Chinese subjects who genetically exhibit low lactase activity with subjects of Dutch origin who are known to persist in high lactase activity\(^1\). The ratio between the \(^{13}\text{C}\)-glucose and \(^2\text{H}\)-glucose concentration in plasma was shown to be different between the Chinese and the Dutch subjects, indicating the difference in intestinal lactase activity. This ratio reflects the degree of digestion of the consumed dose of \(^{13}\text{C}\)-lactose and is referred to as the Lactose Digestive Index (LDI).

Here we compare the in vivo test for measuring lactose digestive capacity (LDI) with lactase enzyme activity measured in small intestinal biopsies, taken for different clinical reasons, as well as the Lactose \(\text{H}_2\) breath test, currently the most frequently used non-invasive lactose digestion test.

MATERIAL AND METHODS
Patients
18 consecutive patients (7 girls and 11 boys) who were scheduled in our hospital in 2001 for a small bowel biopsy for clinical reasons were included in this study. Their clinical details, including nutritional status and indications for the biopsy are shown in Table 1. All individuals originated from a Caucasian population with a high prevalence of genetically determined lactase persistence.

Test protocol
The \(^{13}\text{C}\)-lactose/\(^2\text{H}\)-glucose test was performed directly before the SBB procedure. Patients refrained from consumption of \(^{13}\text{C}\)-enriched foods like cane sugar, pineapple and corn derived products during the three days preceding the test. The subjects fasted for at least 8h prior to the test whereby only consumption of water was allowed.
Substrate
We used naturally labelled $^{13}$C-lactose ($^{13}$C-abundance 1.096%, δ$^{13}$CPDB – 13.7‰) derived from milk of cows fed cattle fodder corn for 5 weeks, produced by the Netherlands Institute for Dairy Research, Ede, The Netherlands. (Kindly provided by dr. R. van der Meer)\textsuperscript{2} as the test substrate. $^{13}$C-lactose (2g/kg body weight) was consumed in a 20% aqueous solution. As a reference substrate 6,6-$^2$H-glucose (98% $^2$H) was used (6,6-$^2$H-glucose was added to the substrate in a dose of 0.04 g/kg body weight). This was purchased from Isotec Inc, Miamisburg, OH, U.S.A.

An intravenous catheter (Becton Dickinson GMBH, Heidelberg, Germany) was put in place to facilitate repetitive blood sampling and to administer anaesthetics during the biopsy procedure. Blood samples (1 mL) were taken before substrate consumption and 15, 30, 45, 60, 75, 90, 105 and 120 minutes afterwards, and were injected into blood-sampling tubes (Vacutainer ®, Becton Dickinson GMBH, Heidelberg, Germany), which contained sodium fluoride and potassium oxalate. Post sampling handling was identical to the procedure described before\textsuperscript{1}.

Breath samples were collected in 20 ml syringes before substrate consumption and half hourly 2 hours afterwards to measure H$_2$ concentration as described previously\textsuperscript{2}. None of the study subjects had clinical symptoms of lactose intolerance like diarrhoea or vomiting during the test.

Biopsy
The biopsy was performed under general anaesthesia using an Olympus GIF 160 endoscope. Biopsies were taken from the distal duodenal mucosa.

Ethical considerations
The Medical Ethics Committee of the Groningen University Hospital approved this protocol. Informed consent was obtained from the patients and the parents in accordance with the principles expressed in the Declaration of Helsinki.

MEASUREMENTS
Lactase activity
The lactase activity was measured with a modified Dahlqvist method and expressed in units per gram protein\textsuperscript{15}. Normal values are above 10U/g protein, as described before\textsuperscript{2}.

Breath hydrogen concentration
3 ml of the 20 mL collected air was injected to a 3 mL Vacutainer tube \textsuperscript{6} (Terumo Europe NV, Leuven, Belgium). The tubes were stored at room temperature and analysed within the first week after the test. Under these storage conditions the quality of breath samples has been proven to remain unimpaired\textsuperscript{16,17}. The breath samples were analysed on a HP 5880 gas chromatograph for H$_2$ concentration\textsuperscript{18}. A positive test result was defined as an increase in concentration above basal H$_2$ values (delta H$_2$) of more than 20 ppm at any time point during the test period\textsuperscript{2}. 
**Lactase activity in small bowel biopsy specimen**

**13C-glucose and 2H-glucose concentrations in serum**
The analyses were performed as described previously. Briefly, serum was deproteinised and glucose was derivatised to the penta-acetate derivative. 13C-glucose enrichment was measured with GC/combustion/IRMS (Delta S/GC, ThermoFinnigan, Bremen, Germany) and 2H-glucose enrichment was measured with GC/MS, using a SSQ7000 quadrupole instrument (ThermoFinnigan, San Jose, CA, U.S.A.)

The mean ratio between the serum 13C-glucose and 2H-glucose concentrations at 45, 60 and 75 minutes after substrate consumption were used to calculate the Lactose Digestion Index, which is the ratio of the concentrations of both glucose markers in serum expressed as a percentage of the consumed dose of tracer. Multiplication of the LDI with the consumed dose of lactose results in the digested amount of lactose as shown in table 1.

**Histology**
The histopathological examination was done by one pathologist who had no prior knowledge of clinical or laboratory data. The biopsies were graded 0 (normal) to IIIC (total villous atrophy) according to the modified Marsh classification.

**The Lactose Digestion Index**
In a previous study we showed that a LDI of >0.45, indicating that more than 45% of ingested lactose is digested in the small intestine, discriminated between a lactase deficient and a lactase persistent population. A LDI cut off of 0.45 therefore was used in this study.

**RESULTS**
In 6 of the 18 individual biopsy specimens a normal mucosal architecture was shown (grade 0), 5 of them had a normal lactase activity (mean 33.5 U/g protein, SD 13.6) in their specimens. In one of the patients the measured LA was low (2.8 U/g protein), despite normal histology. However, in all 6 subjects the LDI was higher than 0.45 (mean 0.69, SD 0.07). The results of the H2 breath test were also normal in these individuals (Table 1).

In 12 biopsies mucosal damage was demonstrated, ranging from the modified Marsh grades II to IIIC. In 3/5 patients with minor histological changes (grade II) both the lactase activity and the LDI were normal together with a normal H2 breath test result. In 2 cases of grade II damage the LA was low (3.6 and 5.6 U/g), both had an abnormal H2 breath test result, while the LDI was low in one of them (0.31) and normal in the other one (0.50).

In the patients with grade IIIA-IIIC mucosal damage (n =7) only one patient demonstrated a normal LA (13.4U/g protein). This patient had a normal H2 test and a low LDI (0.30).

In the other 6 the LA was low (mean 4.7 U/g protein, SD 3.9). 2 of them had a normal H2 breath test result. In 3 of them the low LA was associated with a normal LDI and in the other 3 the LDI was low.
<table>
<thead>
<tr>
<th>Pat</th>
<th>Indication</th>
<th>Weight/I # (SD)</th>
<th>Length/a¶ (SD)</th>
<th>Histology (grade)</th>
<th>L A**</th>
<th>H₂BT††</th>
<th>LDI‡‡</th>
<th>Digested actose dose (g/kg bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CD*, no diet</td>
<td>-1</td>
<td>-2</td>
<td>IIIC</td>
<td>0</td>
<td>neg</td>
<td>0.62</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>CD*, gluten provocation</td>
<td>0</td>
<td>0</td>
<td>IIIC</td>
<td>0.7</td>
<td>pos</td>
<td>0.23</td>
<td>0.48</td>
</tr>
<tr>
<td>3</td>
<td>CD*, no diet</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td>2.8</td>
<td>neg</td>
<td>0.68</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>CD*, gluten provocation</td>
<td>0</td>
<td>0</td>
<td>IIIC</td>
<td>3</td>
<td>neg</td>
<td>0.75</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>CD*, diet</td>
<td>0</td>
<td>+1</td>
<td>II</td>
<td>3.6</td>
<td>pos</td>
<td>0.31</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>CD*, no diet</td>
<td>-2</td>
<td>0</td>
<td>IIIC</td>
<td>0.7</td>
<td>pos</td>
<td>0.23</td>
<td>0.48</td>
</tr>
<tr>
<td>7</td>
<td>CD*, diet</td>
<td>0</td>
<td>-1</td>
<td>II</td>
<td>5.6</td>
<td>pos</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>CD*, no diet</td>
<td>-2</td>
<td>0</td>
<td>IIIA</td>
<td>9.4</td>
<td>neg</td>
<td>0.59</td>
<td>1.26</td>
</tr>
<tr>
<td>9</td>
<td>CD*, no diet</td>
<td>-1</td>
<td>-2</td>
<td>IIIB</td>
<td>9.9</td>
<td>pos</td>
<td>0.57</td>
<td>1.1</td>
</tr>
<tr>
<td>10</td>
<td>CND†</td>
<td>-2</td>
<td>-2</td>
<td>IIIB</td>
<td>13.4</td>
<td>neg</td>
<td>0.3</td>
<td>0.59</td>
</tr>
<tr>
<td>11</td>
<td>Suspected Lactose intolerance</td>
<td>+1</td>
<td>-1</td>
<td>II</td>
<td>15.3</td>
<td>neg</td>
<td>0.71</td>
<td>1.42</td>
</tr>
<tr>
<td>12</td>
<td>CD*, diet</td>
<td>-1</td>
<td>0</td>
<td>II</td>
<td>15.5</td>
<td>neg</td>
<td>0.62</td>
<td>1.24</td>
</tr>
<tr>
<td>13</td>
<td>Suspected Lactose intolerance</td>
<td>+2</td>
<td>0</td>
<td>0</td>
<td>19.3</td>
<td>neg</td>
<td>0.64</td>
<td>1.27</td>
</tr>
<tr>
<td>14</td>
<td>RAP‡</td>
<td>0</td>
<td>-2</td>
<td>II</td>
<td>19.9</td>
<td>neg</td>
<td>0.56</td>
<td>1.09</td>
</tr>
<tr>
<td>15</td>
<td>RAP‡</td>
<td>-1</td>
<td>0</td>
<td>II</td>
<td>21.1</td>
<td>neg</td>
<td>0.73</td>
<td>1.46</td>
</tr>
<tr>
<td>16</td>
<td>FTT§</td>
<td>-2</td>
<td>-2</td>
<td>0</td>
<td>29.8</td>
<td>neg</td>
<td>0.74</td>
<td>1.49</td>
</tr>
<tr>
<td>17</td>
<td>CD*, gluten provocation</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td>45.3</td>
<td>neg</td>
<td>0.75</td>
<td>1.52</td>
</tr>
<tr>
<td>18</td>
<td>CND†</td>
<td>-2</td>
<td>-1</td>
<td>0</td>
<td>53.0</td>
<td>neg</td>
<td>0.74</td>
<td>1.57</td>
</tr>
</tbody>
</table>

* Celiac disease  
† Chronic Non-specific Diarrhea  
‡ Recurrent Abdominal Pain  
§ Failure to thrive  
#Weight related to body length (Standard Deviation)  
¶ Body Length related to age (Standard Deviation)  
** Lactase Activity (Units/g protein)  
†† Hydrogen Breath Test  
‡‡ Lactose Digestion Index

**Table 1:**  
Characteristics and test results of 18 pediatric patients undergoing Small Bowel Biopsy
Lactase activity in small bowel biopsy specimen

DISCUSSION
Impaired lactase activity leading to incomplete small intestinal lactose digestion can cause intolerance symptoms like bloating, flatulence, abdominal pain and diarrhoea. At present, impaired lactase activity is diagnosed by decreased lactase activity in a small bowel biopsy. This measurement determines the lactose hydrolysing capacity in a biopsy in vitro, but the relation between these results and the in vivo lactose digestive capacity of the whole small intestine is unclear, because lactase activity in a damaged intestinal epithelium could have a patchy distribution. In the past decades many techniques have been developed to diagnose diminished lactase activity: (Lactose Tolerance Test, Lactose Tolerance Test with ethanol addition, H₂ breath test, ¹⁴CO₂ and ¹³CO₂ breath test). However, none of these methods has been shown to reliably quantify total lactose digestive capacity. When intestinal lactose digestive capacity is underestimated, dietary lactose restriction as a consequence will be too rigid. Since lactose is an important component of dairy products, intake of other dairy food components as proteins, minerals and vitamins will be reduced also. As an alternative for dietary lactose restriction the consumption of lactase preparations in addition to consumption of lactose containing foods can be prescribed. This significantly increases health costs.

Our recently developed ¹³C-lactose/²H-glucose test is based on the relation between the absorption of lactose derived monosacharides (glucose and
galactose) and the absorption of a reference monosaccharide (\(^2H\)-glucose). This method enables to determine the percentage of the consumed lactose that is first hydrolysed and then absorbed. By adding the glucose tracer, a correction is made for glucose malabsorption. The only difference between the \(^13\)C and \(^2H\)-plasma glucose concentration is the lactase activity. When we evaluated our technique in individuals with genetically determined differences in lactase activity (lactase persisting and lactase non-persisting subjects), the cut off level for full discrimination between both groups was documented to be 45\% of dose (LDI 0.45) using a lactose dose of 25 gr. In groups with genetically determined differences in lactase activity we can expect a homogenous distribution of lactase activity over the small intestine and not a patchy distribution and thus a correlation between the lactase activity in SBB material and the LDI. For ethical reasons we could not confirm this with a biopsy in this healthy population.

Small intestinal mucosal damage, due to a variety of causes like celiac disease, radiation damage, gastroenteritis and cytostatic treatment, can also result in decreased lactase activity. Decreased lactase activity in SBB material does not necessarily represent the physiological digestive capacity of the small intestine, because of the patchy character of the lesions. Our results indicate that the physiological lactose digestive capacity can remain high despite measured low LA in a biopsy specimen. The \(H_2\) breath test results (\(H_2BT\)) in the patients with a low lactase activity in the biopsy (n=9) were non conclusive: In 4/9 there was a normal and in 5/9 an abnormal \(H_2BT\). All 4 patients wit a normal \(H_2BT\) had a normal LDI (>0.45). However, 2 of the 5 patients with an abnormal \(H_2BT\) also had a normal LDI (0.50 and 0.57). In all patients with a high SBB lactase activity the \(H_2\) breath test results were normal.

In only one of the patients with a lactase activity of more than 10U/g protein (patient number 10, Table 1) the lactose digestive capacity index was impaired (0.30). The \(H_2\) breath test result in this boy was normal. This patient has an unspecified cellular energy deficit disease as shown by mitochondrial respiratory chain measurements in a muscle biopsy. The intracellular processing of glucose is therefore suspected to be impaired and for that reason the results of the LDI in this patient have to be interpreted with caution.

We believe that the discrepancy in the results of the LDI and the LA is due to the patchy distribution of the small intestinal mucosal damage. This means that the site where the SBB is taken is crucial for the diagnosis of a low lactase activity. Only when there is extensive mucosal damage, a low biopsy lactase activity may be considered representative for reduced overall intestinal lactose digestive capacity.

We recommend using a lactose digestive capacity test and not an intestinal biopsy to detect a reduced lactose digesting capacity. Negative effects of restrictive dietary regimens (costs, nutritional status) may be avoided.
Lactase activity in small bowel biopsy specimen

References:


