Alimentary Tract

Agreement between indirect calorimetry and traditional tests of lactose malabsorption

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\textbf{A B S T R A C T}

\textit{Background:} Lactose malabsorption occurs frequently and the variable consequent intolerance may seriously impair quality of life. No reliable and convenient test method is in routine clinical practice. A recent animal study showed that the respiratory quotient changed significantly after ingestion of sucrose and lactose in naturally lactase-deficient rats.

\textit{Aims:} This exploratory study evaluated the relevance of monitoring the respiratory quotient after lactose ingestion to detect malabsorption.

\textit{Methods:} Healthy volunteers were identified and classified lactose absorbers and malabsorbers by a lactose tolerance test (25 g). After an overnight fast, a second lactose challenge was performed to monitor hydrogen excretion and respiratory quotient kinetics over 4 h. Participants also completed questionnaires to score and localise their gastrointestinal symptoms.

\textit{Results:} 20 subjects were enrolled (10 per group, 60% males, mean age 34 ± 4 years). Respiratory quotient kinetics were different between absorbers and malabsorbers during the first 100 min after lactose ingestion (p < 0.01) and during the initial 30–50 min period. Respiratory quotient was significantly, positively correlated to peak glycaemia (R = 0.74) and negatively correlated to hydrogen excretion (R = −0.51) and symptoms score (R = −0.46).

\textit{Conclusions:} Indirect calorimetry could improve the reliability of lactose malabsorption diagnosis. Studies on larger populations are needed to confirm the validity of this test and propose a simplified measurement.

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1. Introduction

Hypolactasia is a widespread intestinal problem that affects almost 75% of the population worldwide, with large variations depending on ethnic background. Despite the recent identification of the genetic locus, lactose intolerance remains poorly diagnosed by the medical community, mainly due to the heterogeneity of terminologies and the questionable reliability of the diagnostic tools.

Hypolactasia, which refers to the deficiency or absence of lactase secretion, may be detected by duodenal or jejunal biopsies and by genetic tests. A biopsy is the only direct method of detection, but is an invasive technique. The genetic test, identifying some mutations in the gene encoding lactase, has a reported sensitivity of 93–100% and a specificity of 95–100%, but remains poorly available.

Several other techniques are available to detect malabsorption, but they have some limitations. These tests require the ingestion of a lactose load and include the dynamic study of glycaemia, the analysis of stool pH, urine galactose, the hydrogen breath test (HBT) or the measurement of $^{13}$C-glucose in serum or $^{13}$CO$_2$ in exhaled air. Glycaemia analysis presents sensitivity and specificity both reported to be between 70% and 95% [1], with no reliability in diabetics or in patients with bacterial overgrowth. The stool pH is easily analysed but its reliability may be disturbed by intestinal motility and water reabsorption. Urine galactose analysis presents variable sensitivity (77–96%) and specificity (88–100%) [2]. The HBT, quantifying the amount of hydrogen created from the colonic fermentation of undigested carbohydrates, has been considered a gold standard for over 30 years. Its sensitivity and specificity are approximately 80–100% and 70–100%, respectively [3]. However, around 20% of patients present an intestinal bacterial overgrowth,
leading to false positive results, or a methanol excretion, leading to false negative results [4]. Since 2008 it has been suggested to detect both hydrogen and methane in the expired air to reduce the number of false negatives [5]. The monitoring of $^{13}$C-glucose in serum or $^{13}$CO$_2$ in exhaled air following ingestion of labelled $^{13}$C-lactose was developed in 2000. According to these studies, the proportion of lactose malabsorbers is almost 50% higher than described in the literature [6–8]. However, this technique remains rarely used.

Subjective techniques, related to the manifestation of symptoms, can highlight lactose intolerance. The available tests are usually self-completed questionnaires to score the main gastrointestinal symptoms, such as rumbling, bloating, cramping, nausea and diarrhoea. The sensitivity and specificity of these questionnaires are only 75% and 67%, respectively, due to the variability in evaluating one’s own symptoms [9], and present an overestimation observed in 30% of the subjects [10].

Thus, none of the available techniques allows a precise and absolute diagnosis. In this context, it is useful to evaluate new tools or new combinations of tests to optimise the diagnosis of lactose malabsorption.

A recent animal study showed that the kinetics of respiratory quotient (RQ), carbohydrate oxidation (Cox) and lipid oxidation (Lox), after ingestion of sucrose and lactose, depended on the capacity of the host to absorb sugars [11]. Therefore the aim of our study was to monitor the RQ, Cox and Lox during and after a lactose load (25 g) in selected absorbers and malabsorbers, and to evaluate the efficacy of these tests in diagnosing lactose malabsorption. Concomitantly, blood sugar levels were measured using a portable glycaemia reader, hydrogen breath excretion was quantified using a portable hydrogen detector, and RQ, Cox and Lox kinetics were monitored using a ventilated-hood system. Symptoms were also evaluated before ingestion of lactose and after 4 h, by subjectively scoring the 5 main gastrointestinal symptoms (diarrhoea, pain, nausea, rumbling and bloating).

2. Materials and methods

2.1. Participants

The study was carried out in healthy volunteers (lactose absorbers and lactose malabsorbers) identified by the 1-h glycaemia after lactose ingestion test. No participant had gastrointestinal or pulmonary diseases, had taken antibiotics or other drugs affecting intestinal function for 8 days prior to the study, or had practiced intense physical activity for 2 days prior to the study. Additionally, participants had followed the nutritional recommendations for meals during the 2 days before the study [12,13].

2.2. Experimental protocol

The study took place at the Gastroenterology Department of the Avicenne Hospital (Bobigny, France). After being identified as lactose absorbers or malabsorbers, the participants ingested 25 g of lactose diluted in 250 mL of water after an overnight fast. Their respiratory parameters were measured using a ventilated-hood system (canopy) for 4 h and their hydrogen excretions were recorded over 3 h. At the end of the test the participants also completed a symptoms questionnaire to score and localise their gastrointestinal symptoms.

2.3. Lactose tolerance test

Samples of capillary blood to test glucose concentration were taken at 0, 15, 30, 45 and 60 min, using a Precision XceedPro glycaemia reader (Abbott, Rungis, France). For each time point, measurements were taken twice on the same sample and the mean value was recorded. A glycaemia rise equal to or greater than 1.5 mmol/L was classified as “lactose absorption”; a plasma glucose rise equal to or less than 1.0 mmol/L was classified as “lactose malabsorption” [1]. All the included participants presented glycaemia peaks greater than or equal to 1.5 mmol/L for lactose absorbers and less than or equal to 1.0 mmol/L for lactose malabsorbers, confirming their classifications [2,14].

2.4. Hydrogen breath test (HBT)

The exhaled hydrogen was measured in parts per million (ppm) using a GastroLyzer (Bedfont Scientific Ltd, Maidstone, Kent, England). A hydrogen excretion 20 ppm higher than baseline in at least 2 subsequent measurements was associated with lactose malabsorption [15,16].

2.5. RQ, Cox and Lox kinetics

RQ, Cox and Lox were computed from VCO$_2$ exhaled and VO$_2$ consumed, recorded each minute, using a Deltatrac II canopy (Datrex Ohmeda, Limonest, France). In this system, the subject inhales atmospheric air through a hole in the capsule and exhales via a non-return valve into a measurement unit [17–19]. During these measurements participants were placed in the supine position with the canopy overhead; they had at least 30 min to familiarise with the system. Once parameters were stable, a 10-min baseline was recorded. Then the participants ingested lactose and measurements were taken over the following 4 h.

2.6. Symptom evaluation

Each participant rated the intensity of the 5 main gastrointestinal symptoms (diarrhoea, pain, nausea, rumbling and bloating), before and after the lactose challenge, on a 10-cm visual analogue scale ranging from 0 (no symptoms) to 10 (maximum symptoms) [9]. Symptoms were associated with lactose malabsorption for a mean delta above or equal to 7.5/10 [9].

Pain topology was also evaluated. Participants reported on a diagram the pain intensity that they experienced after the lactose challenge, using a 10 point Likert scale ranging from 0 (none) to 9 (extreme): right hypochondrium (segment 1), epigastrium (segment 2), left hypochondrium (segment 3), right lumbar (segment 4), periumbilical (segment 5), left lumbar (segment 6), right iliac (segment 7), hypogastrium (segment 8) and left iliac (segment 9) (Fig. 4).

2.7. Ethics

The study was conducted according to the Declaration of Helsinki, and approved by the Ethical Committee of Saint-Germain-en-Laye (Paris XI). Written informed consent was given by all participants before inclusion.

2.8. Statistical analysis

Results are presented as means ± SEM. Analyses were performed with either an ANOVA for repeated measures or a Student’s t-test for unpaired data. The areas under the curves for RQ, glycaemia, HBT and symptoms were correlated by computing the Pearson’s coefficients. The software used was SAS (version 9.1). The significance level of all statistical analyses was set at $p < 0.05$. 
Table 1
Baseline characteristics of the two study groups based on lactose malabsorption.

<table>
<thead>
<tr>
<th></th>
<th>Absorbers</th>
<th>Malabsorbers</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
<td>0.09</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29±4</td>
<td>39±4</td>
<td>0.36</td>
</tr>
<tr>
<td>Male gender</td>
<td>4(40%)</td>
<td>2(20%)</td>
<td>0.6</td>
</tr>
<tr>
<td>BMI</td>
<td>22.3±0.9</td>
<td>24.4±1.7</td>
<td>0.29</td>
</tr>
<tr>
<td>RQ baseline</td>
<td>0.84±0.01</td>
<td>0.85±0.01</td>
<td>0.54</td>
</tr>
<tr>
<td>Cox baseline (W)</td>
<td>48.3±3.7</td>
<td>46.9±3.4</td>
<td>0.78</td>
</tr>
<tr>
<td>Lox baseline (W)</td>
<td>36.6±4.1</td>
<td>34.9±2.9</td>
<td>0.73</td>
</tr>
<tr>
<td>BMR</td>
<td>83.9±2.6</td>
<td>81.8±2.1</td>
<td>0.47</td>
</tr>
<tr>
<td>HBT baseline (ppm)</td>
<td>10±1.9</td>
<td>8±1.3</td>
<td>0.62</td>
</tr>
<tr>
<td>Glycaemia baseline (mmol glucose/L blood)</td>
<td>4.65±0.23</td>
<td>4.74±0.2</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Data are presented as means±SEM and are compared by Student’s t-test for unpaired data. BMI: body mass index; RQ: respiratory quotient; Cox: carbohydrate oxidation; Lox: lipid oxidation; BMR: basal metabolism rate; HBT: hydrogen breath test.

3. Results

3.1. Participants’ characteristics

During the study period 10 lactose absorbers (40% males, mean age: 29±4 years) and 10 lactose malabsorbers (20% males, mean age: 39±4 years) were enrolled. No statistical difference was observed baseline characteristics between the two groups, despite the lactose malabsorbers were slightly older than the absorbers (Table 1).

3.2. Glycaemia

Changes in glycaemia over 1 h and peak after lactose ingestion are reported in Fig. 1. Time and “time*status” had significant effects on glycaemia (p<0.001 for both) and differences between the 2 groups became significant after the first 45 min of observation. In serum, the average of the peak was 1.9±0.2 mmol/L in the absorbers’ group vs. 0.6±0.1 mmol/L in the malabsorbers’ group (p<0.001).

3.3. Hydrogen breath test

Changes in exhaled hydrogen over 3 h and peak after lactose ingestion are reported in Fig. 2. Time and “time*status” had significant effects on glycaemia (p<0.001 for both) and differences between the 2 groups became significant after 135 min. An 8-ppm increase was observed in the absorbers’ group while a significantly higher 40-ppm increase was seen in the malabsorbers’ group (p=0.0002). A peak was reached 150 min after the lactose ingestion. However, 1 lactose absorber (A6) presented a hydrogen peak of 23 ppm whereas 1 lactose malabsorber (M1) presented a hydrogen peak of 10 ppm.

3.4. RQ, Cox and Lox monitoring

The kinetics of RQ, Cox and Lox during the 4 h after ingestion of lactose are reported in Fig. 3. Time, status and “time*status” variables had significant effects on RQ (p<0.001, p<0.001, p<0.001, respectively), Cox (p<0.001, p<0.001, p=0.004, respectively) and Lox (p<0.001, p=0.002, p<0.001, respectively). RQ and Lox increased over time, reaching a maximum approximately 60 min post-ingestion in both groups, but showed lower increases in the malabsorbers’ group at 100 min post-ingestion (p=0.002 and p=0.009, respectively). Lox decreased over time, reaching a minimum approximately 60 min post-ingestion in both groups, but showed a lower inhibition in the malabsorbers’ group at 100 min post-ingestion (p=0.006). The differences between the 2 groups were also significant during the short period of 30–50 min after lactose load (p=0.001 for RQ, p=0.002 for Cox, p=0.001 for Lox).

3.5. Symptom evaluation

Symptom scores and localisation are presented in Fig. 4. In the malabsorbers’ group, the total score of symptoms was significantly higher (p=0.04), with an increase in bloating (p<0.05) and hypogastric pain (segment B) (p=0.02). During the test, 5 participants experienced moderate to high intensity symptoms and 2 participants experienced low intensity symptoms. There were no other differences in symptoms or segments between the 2 groups.

3.6. Correlations between parameters

The correlations between parameters are presented in Table 2. The correlations between RQ and traditional techniques are good indicators of RQ reliability in diagnosing lactose malabsorption. One-hour RQ was positively correlated to glycaemia (R=0.74, p=0.0002) and negatively correlated to HBT (R=−0.51, p=0.03) and the total score of symptoms (R=−0.46, p<0.05). Other parameters were also significantly correlated: 1-h Cox with glycaemia (R=0.9, p=0.003) and HBT (R=−0.99, p=0.04), 3-h Cox with hypogastric pain (R=−0.48, p=0.04), and 4-h Cox with hypogastric pain (R=−0.49, p=0.03).

3.7. Individual analyses

Results of all outcomes for each participant are presented as supplementary data (S1). The following cut-offs were used according to the literature: a 1.5 mmol/L peak for glycaemia [14], a 20 ppm peak.
Table 2
Correlations between the evaluated parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glycaemia</th>
<th>HBT</th>
<th>Total symptoms score</th>
<th>Hypogastic pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>RQ (1 h)</td>
<td>0.74 (p = 0.0002)</td>
<td>-0.48 (p = 0.03)</td>
<td>-0.51 (p = 0.05)</td>
<td>-0.44 (p = 0.005)</td>
</tr>
<tr>
<td>RQ (3 h)</td>
<td>ND</td>
<td>-0.51 (p = 0.03)</td>
<td>-0.35 (p = 0.1)</td>
<td>-0.49 (p = 0.003)</td>
</tr>
<tr>
<td>RQ (4 h)</td>
<td>ND</td>
<td>-0.35 (p = 0.2)</td>
<td>-0.35 (p = 0.2)</td>
<td>-0.23 (p = 0.3)</td>
</tr>
<tr>
<td>Cox (1 h)</td>
<td>0.99 (p = 0.001)</td>
<td>-0.99 (p = 0.04)</td>
<td>-0.87 (p = 0.3)</td>
<td>-0.80 (p = 0.41)</td>
</tr>
<tr>
<td>Cox (3 h)</td>
<td>0.44 (p = 0.06)</td>
<td>-0.38 (p = 0.12)</td>
<td>-0.20 (p = 0.4)</td>
<td>-0.48 (p = 0.04)</td>
</tr>
<tr>
<td>Cox (4 h)</td>
<td>ND</td>
<td>ND</td>
<td>-0.30 (p = 0.2)</td>
<td>-0.49 (p = 0.03)</td>
</tr>
<tr>
<td>Lox (1 h)</td>
<td>-0.30 (p = 0.19)</td>
<td>0.28 (p = 0.22)</td>
<td>0.23 (p = 0.34)</td>
<td>0.23 (p = 0.33)</td>
</tr>
<tr>
<td>Lox (3 h)</td>
<td>-0.32 (p = 0.17)</td>
<td>0.32 (p = 0.18)</td>
<td>0.14 (p = 0.58)</td>
<td>0.21 (p = 0.37)</td>
</tr>
<tr>
<td>Lox (4 h)</td>
<td>ND</td>
<td>ND</td>
<td>0.11 (p = 0.66)</td>
<td>0.18 (p = 0.45)</td>
</tr>
</tbody>
</table>

AUC were computed for RQ, glycaemia, HBT and symptoms during the specified time windows (1, 3 and 4 h) and were correlated by Pearson’s coefficients. RQ: respiratory quotient; HBT: hydrogen breath test; ND: not determined.

for HBT [15] and a score of 8/10 for total symptoms [9]. A 0.05 peak was arbitrarily proposed for the 1-h RQ. In the absorbers’ group 1 participant (10%) presented divergent results, with RQ and glycaemia suggesting lactose absorption while HBT and score of total symptoms suggesting malabsorption. In the malabsorbers’ group 5 participants (50%) were asymptomatic, but in 4 (40%) HBT, RQ and glycaemia indicated malabsorption.

4. Discussion
The objective of this exploratory study, performed in 10 lactose absorbers and 10 lactose malabsorbers, was to evaluate the reliability of monitoring RQ after lactose ingestion to highlight malabsorption.

![Fig. 2. Expired hydrogen evolution over 3 h (A) and peak (B) after lactose ingestion (N = 20).](image)

![Fig. 3. RQ (A), Cox (B) and Lox (C) kinetics over 4 h after lactose challenge (N = 20).](image)
glucose utilisation in the post-prandial state. Conversely, lactose malabsorption leads to a moderate switch or even no switch at all from lipid to carbohydrate utilisation. Furthermore, monitoring RQ over a short period after ingestion seems to be sufficient to detect malabsorbers. Indeed, differences in RQ changes were observed between absorbers and malabsorbers within 45 min after the lactose load (p < 0.01), implying a putative diagnostic usefulness.

RQ changes, in particular the 1-h RQ changes, were significantly correlated with the results of traditional techniques, such as glycaemia, hydrogen expiration and symptoms. Besides, similar correlations were found between RQ and symptoms, and between HBT and symptoms, suggesting that RQ could also be used to detect lactose intolerance. However, these last 2 correlations, RQ with HBT and RQ with total score of symptoms, are low and clinical relevance may be questioned despite the low p-values.

The total score of symptoms was significantly higher at the end of the test in the malabsorbers' group compared to the absorbers' group. The significantly different symptom reported by the malabsorbers was bloating, which confirms that bloating is the most sensitive symptom of lactose intolerance [20]. Besides, the main localisation of symptoms in the hypogastrum corresponded to the rectosigmoid and was linked to the significant increase in bloating.

The significant differences in hydrogen expiration confirmed the correct allocation of the participants in the 2 groups, but raised questions for participants A6 and M1. The status of participant A6 was confirmed by his glycaemia (1.8 mmol/L) and RQ (0.11) delta versus baseline. This analysis led us to believe that participant A6 may not have fully followed the recommended residue-free diet, or may have had an undiagnosed intestinal bacterial overgrowth. The status of participant M1 was more uncertain since different statuses could be determined according his glycaemia (0.3 mmol/L) and RQ (0.08) delta versus baseline. We also noticed that the RQ kinetics of this participant were not similar to those of other absorbers and presented abrupt peaks. Such irregular kinetics are more specific to hyperventilation than enhanced glucose oxidation [21,22].

Thus, considering that the RQ peaks of participant M1 were an artefact, the kinetics would fit with their low glycaemic peak. Additionally, methanol production, occurring in 20% of the population, would account for the absence of both a hydrogen peak and of gastrointestinal symptoms. A second battery of tests, including the evaluation of methane expiration, would be necessary to confirm or rebut our diagnosis.

In conclusion, while these 2 participants illustrated well the questionable reliability of HBT, our exploratory clinical study suggests that monitoring RQ may optimise the diagnosis of lactose malabsorption. However, we have to concede that the main limitation of RQ is the risk of hyperventilation, which disturbs kinetics. The baseline step should not be neglected and should last at least 30 min before the beginning of recording, which, however, raises the problem of duration. The initial selection of lactose absorber and malabsorber participants should also be more restrictive in order to avoid incorrect classification. To do so, an option is to combine the glycaemia test and the HBT as inclusion criteria.

Future work will involve confirming the reliability of RQ for detecting lactose malabsorption in larger and more variable populations, such as participants presenting a glycaemic peak in the twilight zone, diabetics, patients with intestinal resection, or short transit time. Even though the benefits of RQ are questionable, this diagnostic strategy could be used as an alternative, especially for these populations that are often misdiagnosed. Complementary studies would help determine standard responses and cut-offs in each population, identify the specific time of response to reduce the time window of RQ measurements, and define the degree of malabsorption depending on the amplitude of the RQ changes. Finally, complementary studies are essential to optimise protocol design by defining specific points of measurements which would not only reduce time under the canopy and patient discomfort, but would also allow simultaneous evaluation of 2 or 3 patients, as is currently possible with HBT.

Conflict of interest statement
No conflict of interest has been reported.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.dld.2013.03.015.

References


