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Time Course of H2 Production Following Oral Lactose Load in Children with and without Lactose Intolerance

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Abstract: *Aims*: To evaluate whether orocoecal transit time (OCTT) might improve the correct allocation (lactose absorbers *vs* malabsorbers) of subjects with "borderline" H2 Breath test (H2BT) values ranging 10-20 ppm, and to determine among malabsorbers if OCTT can aid to discern lactose intolerant from tolerant individuals.

Patients and Methods: OCTT and increment of H2 levels in breath following a dose of lactose were assessed in 49 children (mean age 3.3 years; range 0.6-11.0) suspected of lactose malabsorption. A rise > 20 ppm was used as the criterion to separate malabsorbers from absorbers.

Results: OCTT averaged 177 ± 40 minutes (mean ± SD) in 14 H2 producing lactose absorbers and 78 ± 39 minutes in 22 lactose malabsorbers (p< 0.0001). Among lactose malabsorbers, OCTT was more accelerated in intolerant *vs* tolerant subjects (42 ± 16 *vs* 131 ± 23 minutes, p< 0.0001). No lactose intolerant subject had an OCTT > 75 minutes and no lactose tolerant subject had an OCTT < 75 minutes (sensitivity and specificity 100%; PPV and NPV 100%).

Values between 105 and 175 minutes represented a gray area including both absorbers (21%) and all tolerant malabsorbers (100%). OCTT longer than 175 minutes excluded lactose malabsorption (sensitivity 100%; specificity 69%; PPV 84%; NPV 100%). In 6 out of 8 cases with borderline H2BT results, OCTT clear cut values were useful to reach the correct diagnostic allocation.

Conclusions: OCTT evaluation in addition to considering only H2 concentration is a methodological improvement of H2BT procedure. Although it does not represent an absolute gold standard, OCTT testing may aid in reaching a diagnostic conclusion in some patients where clinical and laboratory features after lactose ingestion remains unclear.

Keywords: Lactose malabsorption, lactose intolerance, H2 breath test (H2BT), orocoecal transit time (OCTT), cut-off criterion.

INTRODUCTION

Lactose malabsorption may result in chronic diarrhea, recurrent abdominal pain, and flatulence; these symptoms are entirely non-specific and are associated with a large spectrum of gastrointestinal diagnoses. A good clinical history revealing a relationship between lactose ingestion and symptoms may be helpful, but it is not always reliable [1, 2]. Accordingly, tests which objectively identify lactose malabsorbers are needed.

Hydrogen breath test (H2 BT) is the most widely non invasive technique used for diagnosing lactose malabsorption. The test is considered positive on the basis of the H2 concentration rise above the basal value following administration of a standardized amount of lactose. In children a cut off value of 20 ppm H2 above baseline following a load of 2 g lactose/kg of body weight up to a maximum of either 25 [1] or 50 g [2] is usually recommended. However data about the best H2 concentration threshold (10 *vs* 20 ppm) have been object of investigation and discussion, and suggest caution in results interpretation when dealing with patients having increases ranging in the interval between 10 and 20 ppm [3-5].

A limit of H2 BT is the failure to establish whether the symptoms are related or not to lactose malabsorption. Some individuals, classified as lactose malabsorbers on the basis of a breath test H2 rise, could be tolerant to lactose and their symptoms could be related to other–gastrointestinal disorders.

In adult lactose malabsorbers it has been demonstrated that a shorter orocecal transit time (OCTT) occurs in intolerant subjects compared to tolerant ones [6, 7], but in children data are not conclusive [8,9]. In lactose absorbers, OCTT has been studied only in adults where it is more prolonged than in malabsorbers [10].

The aim of this pilot prospective study was to evaluate whether the measurement of OCTT might improve the cor-

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rect allocation (absorbers *vs* non absorbers) of subjects with "borderline" H2 BT values ranging between 10 and 20 ppm. In addition we determined among lactose malabsorbers if OCTT can be helpful to discern lactose intolerant from tolerant individuals.

PATIENTS AND METHODS

We studied forty nine children with chronic diarrhea (> 2 weeks duration) and/or chronic abdominal pain (three or more episodes for more than 3 months and severe enough to interfere with daily activity). Patients characteristics including ages and types of presenting clinical features are shown in Table **1**.

Table 1. Patients Characteristics

Number	49
Age (mean ± SD; range; median) (years)	3.3 ± 1.0; 0.6-11.0; 2.9
Gender	F = 26; M = 23
Clinical symptoms and signs at time of presentation*	- Chronic diarrhea (n = 23); - Chronic abdominal pain (n = 29); - Growth retardation (n = 18); - Flatulence (n = 20); - Irregular bowel (n = 36)

*Individual patients had one or more clinical symptoms and signs.

None of the patients had received antibiotics from a minimum of two months prior to the study period.

A detailed medical history and a complete physical examination were performed in all patients. Supplementary investigations included routine blood tests, stool examination for common bacterial pathogens, and abdominal ultrasound.

After an overnight fast the lactose-breath-hydrogen test was performed, and breath samples were obtained every 10 minutes for 180 to 240 minutes after ingestion of oral lactose load (2 g/kg up to a maximum of 50 g as a 20% aqueous solution). The mean H2 concentration of two breath samples before lactose ingestion was taken as the basal value [11, 12]. The minimal concentration of detectable H2 was 0.5 ppm. A rise of H2 in expiring air of more than 20 ppm above baseline at any time of the course of the test was considered diagnostic of lactose malabsorption as previously described [12]; rises in H2 concentration ranging from 10 to 20 ppm above baseline were regarded as "borderline". OCTT was calculated as the time elapsed from the start of ingestion of lactose solution to the appearance of the first of three consecutively increasing H2 values in expired air [11, 13, 14]. This was possible in lactose malabsorbers and in those lactose absorbers producing low amounts of H2 derived from the small fraction of physiologically unabsorbed lactose.

Subjects not producing H2 following administration of lactose load were further investigated using an oral lactulose H2BT. A dose of 250 mg/kg lactulose in a 10% aqueous solution was administered the day after lactose H2BT to determine whether H2 producing flora was present. These absorber children were therefore excluded from further analysis since their lactose OCTT could not be calculated. Number and severity of symptoms were recorded by parents on a personal diary.

Patients who within 12 hours following lactose ingestion demonstrated abdominal pain, flatulence, and/or diarrhea were classified as lactose intolerant. Patients, who did not develop these symptoms, were classified as lactose tolerant.

Fecal reducing substances were measured by Clinitest tablets (Ames Laboratories) [15] in all children with watery diarrhea following the oral lactose load.

Results are expressed as mean ± Standard Deviation (SD). Student's *t* test and Pearson correlation test were used in statistical data evaluation. A Receiver Operating Characteristic curve (ROC curve) analysis was performed to find the OCTT value corresponding with the highest average of sensitivity and specificity for the allocation of absorbers *vs* non absorbers.

A $p < 0.05$ was considered statistically significant.

A verbal informed consent was obtained by all participants' parents.

RESULTS

Twenty seven (55%) of the 49 children produced H2 peak less than 20 ppm above baseline and were included into the lactose absorbers group (mean age ± SD: 3.0 ± 1.1; range: 0.8-7.0; median: 2.8 years). This group comprised children with Irritable Bowel syndrome (n = 15), Alimentary allergy (n = 3), Celiac disease (n = 3), Urinary Tract Infection (n = 2). No known causes were found in 4 patients.

Thirteen of the 27 absorbers who did not produce H2 following administration of lactose load, produced H2 after the lactulose load confirming that they were true absorbers. Among the remaining 14 patients producing H2, 8 had borderline H2 peak values ranging between 10 and 20 ppm above baseline (Table **2**). One of these eight patients (case No.1, Table **2**) developed characteristic clinical symptoms of intolerance following lactose ingestion (diarrhea and flatulence), with traces of reducing substances in the stools. Another borderline patient (case No. 8, Table **2**) had equivocal symptoms (soft, almost watery stools) with traces of positive reducing substances.

Twenty two (45%) of the 49 children produced H2 concentrations > 20 ppm above baseline, and were classified as malabsorbers (mean age ± SD: 3.7 ±2.4; range: 0.6-11.0; median: 2.9 years). In this group hypolactasia was primary (adult type) in 4 children (aged 7.6 ± 2.3 years; range 5.5-11; median 7.0 years) and secondary to biopsy proven intestinal mucosal damage in 18 children affected by celiac disease (n = 15), giardiasis (n = 3) (aged 2.8 ± 1,4 years; range 0.8-6; median 2.7 years).

Among these 22 malabsorbers, 14 had symptoms of intolerance following lactose ingestion (watery diarrhea, abdominal pain, and flatulence), so they were included into an intolerant group. The remaining 8 malabsorbers children had not symptoms after lactose ingestion and were classified as tolerant subjects.

Differences between mean values of peak H2 concentrations above baseline obtained in intolerant and tolerant group, respectively, were not statistically significant (54.3 ± 24.6 *vs* 38.6 ± 15.6 ppm).

Table 2. Summary of Clinical and Laboratory Data in 8 Patients with "Borderline" H2BT Results (Ranging 10-20 ppm)

Patient No.	H2BT (ppm)*	Fecal Reducing Substances	Clinical Intolerance	OCTT (min)
1	18.5	Traces	Yes***	40
2	15	nd **	no	240
3	15	nd **	no	210
4	18	nd **	no	210
5	16	nd **	no	140
6	12	nd **	no	150
7	11	nd **	no	225
8	10	Traces	Equivocal****	245
Normal values	< 20	negative or traces	no	Normal > 175 Abnormal < 105 Gray area 105-175

* = Maximal H2 peak rise.
** = Not determined (due to formed stools).
*** = Watery diarrhea and flatulence.
****= Soft, almost watery stools.

As shown in Fig. (**1**), in the 22 malabsorbers and in the 14 H2 producing absorbers it was found a statistically significant inverse correlation between breath H2 peak after ingestion of lactose and OCTT (r-.63, $p < 0.0001$).

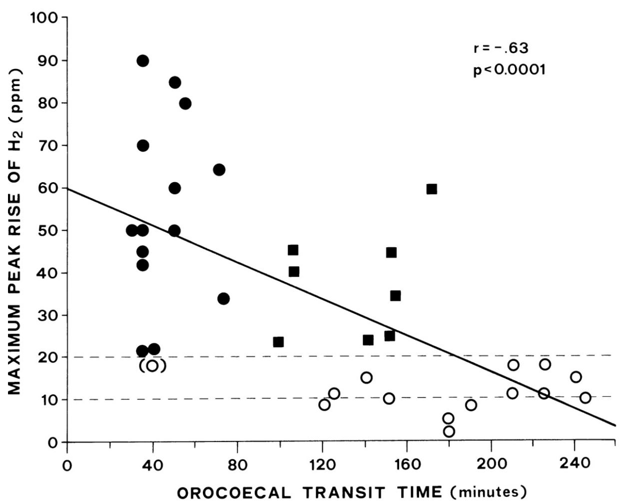
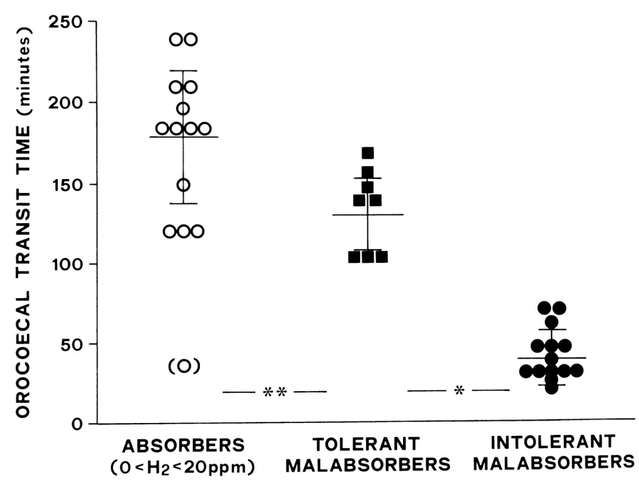


Fig. (1). Correlation between orocoecal transit time (minutes) and maximum peak rise of H2 (ppm) in lactose absorbers (0< H2 < 20 ppm; open dots), tolerant (full squares) and intolerant (full dots) lactose malabsorbers (H2 > 20 ppm). The dotted lines represent 10 and 20 ppm cut-off values. The open dot in brackets refers to a patient with a most probably false negative H2BT result (see text and Table **2**, case No 1).

As shown in Fig. (**2**), mean value of OCTT in absorbers was significantly longer than in malabsorbers (177 ± 40 *vs* 78 ± 39 minutes; $p < 0.0001$). Within the group of lactose malabsorbers, OCTT was significantly shorter in intolerant compared to tolerant subjects (42 ± 16 and 131 ± 23 minutes, respectively, $p < 0.0001$).

Using the ROC curve analysis, the criterion value of ≤ 105 minutes was found to be the value with the highest average of sensitivity and specificity (Sensitivity 78.26%, Specificity 100%, PPV 100%, NPV 72.2%) for the allocation of absorbers *vs* non absorbers. Values between 105 and 175 minutes represented a "gray area" including both absorbers (21%) and all tolerant malabsorbers (100%). OCTT longer than 175 minutes excluded lactose malabsorption (sensitivity 100%; specificity 69%; PPV 84%; NPV 100%) and included 71% of absorbers subjects. Among the malabsorber subjects, lactose intolerant OCTT values never exceeded 75 minutes, whereas tolerant ones always had OCTT greater than 75 minutes (sensitivity and specificity 100%; PPV and NPV 100%).



*** = p < 0.0001** = p < 0.01**

Fig. (2). Orocoecal transit time (individual values and mean ± SD) in lactose absorbers (0 < H2 < 20 ppm; n = 14) and malabsorbers (H2 > 20 ppm; tolerant n=8, and intolerant n = 14). Symbols as in Fig. (**1**). Horizontal lines show mean values ± SD. The open dot in brackets refers to a patient with a most probably false negative H2BT result (see text and Table **2**, case No 1).

Six of the eight patients with "borderline" H2 values (cases Nos. 2 to 7, Table **2**) were clinically tolerant, and showed OCTT values ranging from 140 to 225 minutes. A diagnosis of lactose absorption was feasible in those 4 (cases Nos. 2, 3, 4, 7) with OCTT values > 175 minutes (true H2BT negative) but not in other 2 (cases Nos. 5 and 6) with OCTT results in the overlap area. Cases Nos. 1 and 8 had some signs and symptoms of clinical intolerance. By using the H2 20 ppm cut off the first (breath H2 = 18.5 ppm) should have been classified as lactose absorber, but the very short OCTT below 75 minutes allowed to put a correct diagnosis of lactose malabsorption (false negative H2BT result). In the second (breath H2 = 10 ppm), OCTT clear-cut results (245 minutes) allowed to correctly allocate him in the absorber group (true negative H2BT result) and to consider his equivocal symptoms as unrelated to lactose intolerance.

DISCUSSION

H2 breath test is considered the gold standard technique to diagnose lactose malabsorber subjects [16]. Some aspects however are still not entirely defined. One regards the amounts of lactose in aqueous solution or as milk which have been proposed over the years depending on whether the goal was that of detecting the ability of absorbing the usual daily intakes of lactose as milk and dairy products or that of defining the condition found to correlate with lactase activity in duodenal biopsies [17]. Another one regards the cut off for H2 excretion in diagnosing lactose malabsorption. Although some studies had suggested advantages of a value of 10 ppm [4, 5], the original value of 20 ppm above baseline is nowadays recommended [1, 2]. Because H2 borderline values ranging from 10 to 20 ppm may cause problems in results interpretation, in the present study lactose breath test and OCTT were investigated to establish whether their combination might be helpful to improve the correct diagnosis in children with clinical suspect of lactose malabsorption and H2 BT borderline values.

In agreement with others [10], we found that in lactose malabsorbers OCTT are significantly shorter than in the absorbers. A statistically significant inverse correlation between breath H2 peak after ingestion of lactose and OCTT existed. The mechanism of accelerated OCTT in lactose malabsorbers probably reflects the great amounts of malabsorbed carbohydrates reaching the colon. Osmotic load and consequent fluid secretion induce a small intestinal dilatation, resulting in an accelerate motility and in a shorter OCTT. The grade of digestion of a lactose dose is determined by the residual activity of the small intestinal lactase and by the time available for lactose hydrolysis in the small intestine, as shown by the OCTT [11].

In our series, the 20 ppm cut off seems to be more reliable than the 10 ppm one, as suggested by at least 6 of the 8 cases with borderline values of H2 ranging 10 to 20 ppm (absorbers according to the 20 ppm cut off criterion). In fact, two patients had some clinical symptoms following lactose ingestion compatible with lactose malabsorption. One showed a short OCTT (< 75 minutes), another had a very long OCTT (> 240 minutes). According to OCTT criteria along with symptoms presence, their H2BT results were interpreted as false negative and true negative, respectively.

A correct diagnosis of lactose absorption was feasible in other 4 patients with OCTT > 175 minutes (true H2BT negative). However -due to the existence of overlapping areas of OCTT results regarding the final diagnostic groups- the OCTT test in these cases needs to be part of a thorough evaluation of all available features necessary to reach the correct diagnosis.

In our series the OCTT of intolerant subjects was significantly faster than that of tolerant ones confirming previous data [6, 7, 18], and shows that H2 production timing may be useful to detect lactose intolerant individuals among malabsorbers resulting from H2 BT (75 minutes OCTT 100% positive and negative predictive values).

In conclusion, the timing of H2 production after oral lactose load may be proposed as a relatively simple part of the H2BT procedure based on a 20 ppm cut-off criterion. Although it does not represent an absolute "gold standard", the good discriminating ability of OCTT evaluation found in our pilot study may prove helpful to reach a definitive diagnosis and dietary guidance in some patients with unclear aspects of clinical and/or laboratory picture. By a practical point of view, because of the added expense and inconvenience due to increasing the number of breath collections on H2 analyses, the evaluation of the H.2 production timing could be carried out as a repeated test in those few patients whose H2 production is lying in the uncertainty zone (10-20 ppm) and does not allow hitherto a precise diagnosis of lactose absorption or malabsorption and/or have equivocal symptoms after lactose ingestion.

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