Interval sampling of end-expiratory hydrogen (H₂) concentrations to quantify carbohydrate malabsorption by means of lactulose standards

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Abstract

Lactulose H₂ breath tests are widely used for quantifying carbohydrate malabsorption, but the validity of the commonly used technique (interval sampling of H₂ concentrations) has not been systematically investigated. In eight healthy adults we studied the reproducibility of the technique and the accuracy with which 5 g and 20 g doses of lactulose could be calculated from the H₂ excretion after their ingestion by means of a 10 g lactulose standard. The influence of different lengths of the test period, different definitions of the baseline and the significance of standard meals and peak H₂ concentrations was also studied. Regardless of baseline definition, estimates of malabsorption were most precise, if areas under the H₂ concentration *v* time curves for four hours or more from the start of the excess H₂ excretion were used. The median deviations from the expected values were 20–30% (5–60%, interquartile range). This corresponded to the deviation in reproducibility of the standard dose. We suggest that individual estimates of carbohydrate malabsorption by means of H₂ breath tests should be interpreted with caution if tests of reproducibility are not incorporated. Both areas under curves and peak H₂ concentrations seem valid for comparison of groups.

For more than two decades hydrogen (H₂) breath tests have been used to detect carbohydrate malabsorption.^{1–3} Bond *et al*⁴ used continuous sampling of total expired air to determine the H₂ excretion rate, and it was shown that the H₂ excretion increased linearly after graded doses of the synthetic non-absorbable disaccharide lactulose. It was also shown *in vitro*⁴ that the rate of H₂ formation from lactulose was similar to the H₂ formation from normally ingested mono- or disaccharides. Lactulose could therefore serve as a standard to estimate the malabsorbed amount of other simple sugars. After the introduction of the more simple interval sampling of end expiratory air with chromatographic or electrochemical detection of H₂ concentrations,^{5,6} the findings of Bond *et al*⁴ have been widely applied to these methods.

Carbohydrate malabsorption is most commonly quantified by comparing the areas under the breath H₂ excretion curves with the areas obtained after a reference dose of lactulose.^{7–12} The validity of this procedure is supported by scanty evidence only¹³ and has been questioned.^{14,15}

We therefore felt that a systematic evaluation was needed. We studied the H₂ response to

graded doses of lactulose in order to evaluate whether the findings of Bond *et al*⁴ were also applicable to methods using interval sampling of end expiratory H₂ concentrations.

Methods

SUBJECTS

Eight healthy adults (seven men and one woman, aged 21–33 years) were studied. None of the subjects were taking antibiotics, laxatives or salicylates, and they had no history of past or present chronic gastrointestinal or pulmonary disease. Blood screening tests were normal.

BREATH TESTS

We used interval sampling of end expiratory H₂ concentrations which were collected in duplicate from the mouth in 20 ml plastic syringes (Once®) fitted with a T-piece. Samples were taken before lactulose ingestion and every 15 minutes for seven hours and immediately analysed. The total study period was 12 hours and the last five hours samples were taken every 30 minutes in Plasticpak® (Becton-Dickinson) plastic syringes. The latter samples were stored overnight at 5°C for analysis the next morning.¹⁶ All H₂ concentrations were measured on a GMI (Renfrew, Scotland) H₂ monitor.¹⁷ The subjects fasted at least 12 hours overnight. Before breath collection all had a thorough mouth rinse with a 0.1% chlorhexidine solution.^{18,19} Smoking and sleeping was not allowed, and the subjects were non-ambulant.

DESIGN OF THE STUDY

To avoid colonic bacterial metabolic changes induced by lactulose^{20,21} a minimum of six days elapsed between the different challenges. All tests were carried out within approximately two months. There was a median time interval between the 5 g and 20 g lactulose tests and the 10 g standards of 32 days. The study was coordinated with a separate study of the influence of transit times on H₂ responses.²²

All subjects initially received 10 g lactulose in 100 ml tap water, and thus it was assured that they were all H₂ producers (≥10 ppm sustained rise of H₂ concentration). The lactulose solution contained 667 mg lactulose, 60 mg lactose and 110 mg galactose per ml (according to the supplier). Subsequently, the following tests were performed in random order (Table I): (a) 5 g lactulose in 50 ml tap water; (b) 10 g lactulose in 100 ml tap water; (c) 20 g lactulose in 200 ml tap

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TABLE I Composition and order of administration of the different test substances

Test substance	Concentration (%)	Duration of test (h)	Order of administration
10 g lactulose + SM*	10	12	Initial in all
5 g lactulose + SM	10	12	
10 g lactulose + SM†	10	12	
20 g lactulose + SM	10	12	In random order
Total fasting	—	7	
Fasting + standard meal (SM)*	—	12	
10 g lactulose + SM	10	12	Final in all‡

*Standard meal (100 g minced meat and 50 g boiled rice) at time four hours; †Standard 10 g lactulose load; ‡Five to seven months from the initial lactulose load.

water; (d) Total fasting except for tap water ad libitum (study period only seven hours); (e) Fasting (except for tap water) together with a standard meal at time four hours. This meal consisted of 100 g minced meat stew together with 50 g boiled rice. The meal was consumed within a period of 15 minutes with 150 ml tap water; (f) After five to seven months from the start of the study a 10 g lactulose challenge identical to the initial challenge and challenge b was given.

In studies (a), (b), (c), and (f) a similar meal as (e) was given at time four hours and never before the oro-caecal transit time was determined (Table I).

CALCULATIONS AND STATISTICS

The following variables were determined and compared for different doses of lactulose or for fasting *versus* fasting plus standard meal: (1) Oro-caecal transit times as the interval between ingestion and the initial sustained rise in breath H_2 concentration of 10 ppm or more.²³ (2) Maximal rise of H_2 concentration from lowest previous values (H_2 -peak). (3) Time to H_2 -peak from oro-caecal transit time (t -peak). (4) The triangulated areas under the H_2 concentration *versus* time curves. These were calculated as²⁴:

Area under curve =

$$\left(\frac{c_1 - c_2}{2}\right) \times t_1 + \left(\frac{c_2 - c_b}{2}\right) \times t_2 + \dots + \left(\frac{c_{n-1} - c_b}{2}\right) \times t_{n-1}$$

Which, if the intervals are of equal length ($t_1 = t_2 = \dots = t_{n-1} = \Delta t$) can be reduced to:

Area under curve =

$$\left(\frac{1}{2} \times c_1 + c_2 + \dots + c_{n-1} + \frac{1}{2} \times c_n - (n-1) \times c_b\right) \times \Delta t \text{ (ppm} \times \text{min)}$$

where: $c_1, c_2, \dots, c_{n-1}, c_n = H_2$ concentrations in ppm at the beginning and ends of each interval, $n-1$ = number of intervals, n = total number of H_2 concentration measurements, c_b = basal value of H_2 concentration.

All areas were calculated for the first two, four, and six hours (area under curve (nh)) of the sustained increase in H_2 concentration (excess H_2 excretion) as well as for the whole period from the oro-caecal transit time to 12 hours (area under curve (total)). In two subjects the H_2 concentration did not reach baseline after 20 g lactulose

and the small additional areas were determined by extrapolation to base line. As basal H_2 levels value we used the lowest H_2 concentration previous to the oro-caecal transit time. The areas were further calculated by subtraction of the area under each fasting curve from the total area or from the area for the first four hours after oro-caecal transit time.

The accuracy of the technique with which the 5 g and 20 g dose of lactulose could be calculated from the H_2 excretion following their ingestion was assessed by using 10 g lactulose as standard by the following equation:

$$\frac{\text{Area under curve (5 g or 20 g lactulose)}}{\text{Area under curve (10 g lactulose)}} \times 10 \text{ (g)}$$

The deviation from the expected value was calculated as the numeric value of:

$$\frac{\text{Expected value (5 g or 20 g)} - \text{calculated value (g)}}{\text{expected value (5 g or 20 g)}} \times 100\%$$

The same calculations were carried out by the use of H_2 -peaks instead of areas under curves. The reproducibility was expressed as the deviation in percent of the standard and final 10 g load from the initial 10 g load as the numerical value of:

$$\frac{\text{Expected value} - \text{observed value}}{\text{expected value}} \times 100\%$$

Non-parametric statistical tests were used throughout (Pratt's test, Spearman's rank correlation analysis (one-tailed), Friedmans two-way analysis of variance in combination with multiple comparison procedures²⁵). All results were expressed as medians and interquartile ranges, $p < 0.05$ was considered significant.

ETHICS

The study was carried out in accordance with the Helsinki Declaration II, and the study protocol was approved by the Copenhagen County medical ethics Committee.

Results

GENERAL APPEARANCE OF H_2 EXCRETION CURVES

Fasting levels were not significantly different in the various test groups ($p > 0.80$, Friedmans test). Initial peaks^{17,19} were only very occasionally identified. Oro-caecal transit times were significantly shorter when 20 g lactulose was given ($p < 0.01$, Friedmans test) (Table II). The duration of the lactulose induced H_2 excretion showed great interindividual variation, but increased significantly with increasing dose of lactulose ($p < 0.01$, Friedmans test) (Fig 1, Table II). The median duration of H_2 excretion was five, and seven hours, and more than 10 hours for 5, 10, and 20 g lactulose respectively (Table II). A large interindividual variation was also noted for H_2 peaks and areas under curves, both increased significantly with increasing dose of lactulose ($p < 0.01$, Friedmans test) (Fig 1, Table II). H_2 peaks were generally reached within two to three hours after oro-caecal transit time (Table II), and all but one within four hours. The time to reach peak H_2 concentration

TABLE II Principal features of the H_2 -excretion curves following different loads of lactulose (L). Basal levels were lowest for 5 g L. H_2 -peaks, areas under curves and duration of excess H_2 -excretion increased, and oro-caecal transit times decreased with increasing dose of L

	5 g L	10 g L	20 g L
Basal levels (ppm)	3 (2-9)	7 (5-13)	10 (3-14)
Orocaecal transit time (min)	135 (94-180)	90 (68-150)	60 (15-75)
t-Peak (min)*	120 (83-210)	75 (45-90)	150 (90-206)
H_2 -peaks (ppm)	34 (24-41)	60 (41-68)	78 (56-126)
Area under curve (ppm min 10^{-3})†	56 (29-66)	125 (91-164)	243 (182-334)
Duration of excess H_2 -excretion (min)	300 (270-390)	428 (394-473)	623 (548-657)

*Time from oro-caecal transit time to H_2 -peak; †Area under the H_2 -excretion curves from oro-caecal transit time to 12 hours with subtraction of area under the lowest previous value of H_2 -concentration. Results are given as medians and interquartile ranges.

Figure 1: Shape of H_2 excretion curves. Different curves of H_2 excretion v time from one subject challenged with 5, 10, and 20 g lactulose (L) as well as during fasting (F).

from the oro-caecal transit time (t-peak) was significantly shorter for 10 g lactulose compared with both 5 g and 20 g doses ($p < 0.01$, Friedman's test).

The magnitude of the H_2 peaks were for all lactulose doses closely correlated to the magnitude of the areas under curves (area under curve (total): $p < 0.001$, $p < 0.025$, $p < 0.005$ for 5, 10, and 20 g and area under curve (four hours): $p < 0.0025$, $p < 0.0025$, $p < 0.0005$ for 5, 10, and 20 g).

FASTING CURVES

There were no apparent differences between the shapes of the curves during total fasting (extrapolated from seven to 12 hours) and the curves obtained during fasting plus standard meal. In both series single deflections of the baseline of 2-10 ppm were observed, but there was no relation to meals and no sustained increases were

seen. The magnitude of the baseline deflections were not different in the two series ($p > 0.10$) and the total areas under the curves were similar ($p > 0.10$).

ESTIMATION OF CARBOHYDRATE MALABSORPTION

Irrespective of method of calculation the areas under curves increased with increasing dose of lactulose and generally in proportion to the dose given (Fig 1, Table II). The basal levels (ppm) of the different lactulose doses were not different for 10 g and 20 g lactulose but the basal levels of 5 g lactulose were lower compared with 10 g and 20 g ($p < 0.01$, Friedman), probably because of the longer transit times (Table II).

Using the individual H_2 excretion after the standard 10 g dose of lactulose the amount malabsorbed was estimated from the H_2 excretion after ingestion of 5 g and 20 g lactulose, and the deviations from the actual values are shown in Table III for the different ways of defining area under curve. Two examples of the calculations are shown in Figure 2. There was no significant difference between the different methods of calculation for the 5 g lactulose dose ($p > 0.40$, Friedman's test). One subject (no 5, Fig 2) had disproportionately high H_2 production after 5 g lactulose. For the 20 g dose the analysis of variance (Friedman) showed a significant difference between the estimates based on the different ways of calculating area under curve or on H_2 peaks ($p < 0.02$). By multiple comparison procedures it was found that area under curve for two hours was significantly more imprecise compared with all other estimates of the 20 g dose. The difference between the estimates based on area under curve for two hours and area under curve for four hours was highly significant ($p < 0.01$, Pratt's test) (Table III). The most precise estimate of a 20 g dose was apparently obtained by using total H_2 excretion (Fig 2), although it was not significantly different from the four hour estimate ($p = 0.20$). The latter seemed to underestimate the 20 g dose (Fig 2).

Figure 2: Quantitation of carbohydrate malabsorption. Quantitative estimates of 5 g (hatched columns) and 20 g (open columns) doses of lactulose by means of a standard 10 g dose based on either total excess H_2 excretion (AUC (total)) (A) or H_2 excretion following four hours from oro-caecal transit times (AUC (4 h)) (B) in eight subjects.

TABLE III Calculations of malabsorbed amount (cma) of lactulose (L) by means of a 10 g L standard and the deviation (Dev) from the correct value. Results are given as medians and interquartile ranges

	5 g L testdose		20 g L testdose	
	cma (g)	Dev (%)	cma (g)	Dev (%)
Area under curve (total)*	3.9 (3.3-4.8)	29.5 (16-61)	19.8 (15-22.2)	15.5 (4-29)
Area under curve (6 h)	4.4 (3.8-5.2)	16.5 (11-56)	15.9 (12.8-18.5)	20.5 (9-36)
Area under curve (4 h)	5.1 (4.1-6.3)	19 (4-39)	15.5 (10.9-18.3)	22.5 (9-46)
Area under curve (2 h)	4.9 (3.0-5.3)	7 (3-49)	12.3 (9.5-13.9)	39 (30.3-53)
F-area under curve (total)*	3.7 (1.3-5.1)	34 (3-82)	19.2 (15.9-24.2)	19 (6-25)
F-area under curve (4 h)	4.5 (2.6-5.9)	22 (9-49)	14.6 (9.9-20.5)	27.5 (7-51)
H_2 -peaks	5.2 (3.4-7.1)	34 (4-63)	16.2 (11.6-20.4)	19 (5-42)

*Areas under the H_2 excretion curves from oro-caecal transit time to n hours, subtracting the area under the lowest previous H_2 -concentration (area under curve (n h)) or the area under the fasting curves (F-area under curve (n h)).

TABLE IV Reproducibility of the H₂ excretion after loads of 10 g lactulose.

	Initial load	Standard load		Final load	
		Abs val	Dev (%)	Abs val	Dev (%)
H ₂ -peak (ppm)	66 (39–83)	60 (41–68)	21 (8–43)	40 (34–61)	42 (27–54)
Area under curve (total) (ppm min 10 ⁻³)*	143 (104–163)	125 (91–164)	20 (11–27)	85 (67–178)	44 (28–64)
Area under curve (4 h) (ppm min 10 ⁻³)*	97 (54–115)	93 (67–112)	20 (11–41)	65 (54–94)	31 (24–48)
Time interval from initial load (days)		35 (10–39)		154 (134–228)	

*Areas under the H₂ excretion curves from oro-caecal transit time to 12 hours from the start of the study period (total) or for the first four hours after oro-caecal transit time. dev: deviation from the values of the initial load in per cent; abs val: absolute values. Results are given as medians and interquartile ranges.

The areas under curves for two hours were closely correlated to total area under curve for 10 g and 20 g lactulose ($p < 0.0025$, $r = 0.929$ and $p < 0.01$, $r = 0.845$) but not for 5 g ($p < 0.25$, $r = 0.476$). Areas under curves for four hours were uniformly very closely correlated to total areas under curves (5 g: $p < 0.0005$, $r = 0.976$; 10 g and 20 g: $p < 0.005$, $r = 0.881$). The median deviation from the estimates of a 5 g and 20 g lactulose load was about 20–30% with interindividual variations in the interquartile range of 5–60% (Table III). It should be noted that the two hour estimate of the 5 g dose was rather precise (Table III).

There was no detectable advantage of using subtraction of individual fasting curves compared with subtraction of basal values defined as the lowest value preceding the oro-caecal transit time. Calculation of lactulose malabsorption by means of H₂ peaks alone was also attempted (Table III). The precision of the quantitative estimates of both 5 g and 20 g by means of H₂ peaks alone compared with areas under curves were not significantly different ($p > 0.10$) (Table III).

REPRODUCIBILITY

The median deviation of the standard 10 g lactulose load from the initial load (Table I) was for total area under curve, area under curve over four hours and for H₂-peaks about 20% (Table IV). There was no significant difference between the areas under curves or H₂-peaks of the two 10 g challenges ($p > 0.10$). The areas under curves of the initial and the standard load were significantly correlated (area under curve (two hours) and area under curve (four hours): $p < 0.025$, $r = 0.779$) as were H₂-peaks ($p < 0.05$, $r = 0.714$).

The final 10 g lactulose load showed larger deviation from the initial 10 g load (Table I) compared with the standard load (44% *v* 20%, $p < 0.05$) (Table IV). The overall H₂ production was lower after the final loads (Table IV) and none of the areas under curves were significantly correlated to areas under curves of the initial loads ($p > 0.25$).

Discussion

The purpose of the present study was to validate the use of lactulose standards for quantitation of carbohydrate malabsorption by the use of interval sampling of end-expiratory breath H₂ concentrations. Bond *et al*⁴ used continuous

sampling of total expired air and measured expired H₂ volume directly. The relation between this direct assessment of expired H₂ volume and the method used in the present study can be expressed as follows:

$$V = \int_{t_1}^{t_2} C(t) dt \times RF \times T_v$$

where V = total expired H₂ volume, the integral is of the H₂ concentration (C) *v* time (t) in the interval $t_1 - t_2$, RF = frequency of respiration and T_v = tidal volume. If $RF \times T_v$ is considered an individual constant (in non-ambulatory subjects), the volume of H₂ excreted is proportional to $\int C(t) dt$. This integral is approximated by calculation of the area under the H₂ concentration *v* time curve from the oro-caecal transit time and a period ($t_1 - t_2$) of variable length. The validity of using area under curve is also dependent on the precision and reproducibility of the measurements of H₂ concentration in the end-expiratory air.^{6,17} If short-time variations of alveolar H₂ diffusion are significant the calculation is more precise if short sampling intervals are used.

Areas under curves are therefore relevant indices of total H₂ excretion with proper reservations only, and quantitative estimates of carbohydrate malabsorption by interval sampling methods could be less precise compared with total sampling techniques. Despite the wide application of H₂ breath test this aspect has not been systematically investigated.

The main conclusions of our present study are:

(1) The most precise estimates are obtained if areas under curves are based on the H₂ excretion during four hours or more after the oro-caecal transit time. With a 10 g lactulose standard, the estimates of a 5 and 20 g dose deviates from the expected values by a median of 20–30%, with interindividual variations in the interquartile range of about 5–60%.

(2) The precision of calculations based on areas under curves is not improved by using individual fasting curves compared with using the lowest value of H₂ concentration previous to oro-caecal transit time to reflect basal H₂ excretion. Incorporation of a standard meal as ours does not seem to influence the H₂ excretion.

(3) The magnitude of areas under curves and H₂-peaks are closely correlated and the use of H₂-peaks obtained within four hours after oro-caecal transit time will provide fairly good estimates of the magnitude of carbohydrate malabsorption.

(4) The reproducibility of H₂ excretion calculated as percent deviation from expected values, is in the same order of magnitude as the deviations of the calculated malabsorbed amounts of other doses (about 20%), and deteriorates with increasing time intervals between the tests.

Our results regarding the precision of quantitative estimates and the reproducibility are not far from the findings of Bond *et al*,⁴ that the 95% confidence interval of the actual quantity of carbohydrate malabsorbed was about $\pm 50\%$ for continuous total sampling techniques. In the same study it was found that a two hour period after the oro-caecal transit time satisfactorily represented the H₂ excreted as compared to a

four hour period. In our study, four hour periods gave clearly more precise estimates of 20 g doses. Flourié *et al*¹⁵ also concluded that a two hour period from oro-caecal transit time was insufficient if lactulose H₂ breath tests were used as standards for quantification of starch malabsorption. Their results, however, are not fully comparable with the present findings because ingestion of lactulose was compared with infusion of starch. No data on reproducibility are available for comparison.

In our studies of fructose and sorbitol malabsorption^{11,26} one and a half hour periods of excessive H₂ production seemed valid for quantitation because the outcome of this procedure corresponded very well to the results obtained by determining the individual absorption capacity by graded doses of fructose or sorbitol.

It has been suggested that the ileal emptying rate of different carbohydrates may be an important determinant of the shape of the H₂ excretion curves, and thus affect the precision of procedures for quantitation.¹⁴ We did not find a significant effect of differences in the oro-caecal transit times on the magnitude of the H₂ excretion curves.²² The differences in oro-caecal transit time between 5, 10, and 20 g lactulose observed in the present study, is therefore not likely to influence the precision of the quantitative estimates.

The linearity of the H₂ response to larger amounts of malabsorbed carbohydrate may be compromised, when the capacity of the colonic flora to ferment the available substrate is overridden. It has been suggested that this capacity is variable in healthy adults,²⁷ and if it is exceeded, a blunted H₂ response should be expected. The lactulose doses used in the present study seems to be within a relevant physiological and clinical range.^{11,12,26}

The results of the present study may be applicable also to patients with chronic or intermittent carbohydrate malabsorption, although these patients could respond differently due to instability of the gut flora. Similar studies have not been carried out in patients with gastrointestinal disease. Our results may, however, be applied to routine clinical lactose or sucrose H₂ breath tests, in which quantitative information would often be helpful. The results of a H₂ breath test is rather difficult to evaluate if the capacity of the test subject to produce H₂ is not known,¹⁷ and comparison with a lactulose challenge would furthermore allow a comparison of the symptomatic response. We consider lactulose standards essential for the evaluation of fructose malabsorption.^{11,12,26} Lactulose standards seem necessary for evaluation of starch malabsorption in different disease entities,^{9,28} and they could prove valuable in studies of patients with inflammatory bowel disease,²⁹ or in studies of the assimilation of different food items.^{8,10,30}

The main factor governing the precision of the quantitative estimates of carbohydrate malabsorption by means of H₂ breath tests is apparently the reproducibility of the H₂ response, that is, the reproducibility of the fermenting processes of the colonic microflora. Our calculations were based on a median time

interval of about 30 days, and would probably be more precise with shorter intervals between standard load and test load. This, however, is not always practicable in larger, randomised study series, the conditions of which the present study was intended to mimic. Repeated doses of unabsorbable carbohydrates with short intervals may furthermore induce metabolic changes of the intestinal microflora,^{20,21} which was another reason for the spacing of the different tests in the present study. Our findings indicate that incorporation of a test of reproducibility may give a very good impression of the precision of the quantitative estimates.

The reproducibility might be improved by determination of other fermentation products than H₂. Adequate assessment of the carbohydrate fermentation of the colonic flora may require parallel measurement of breath H₂ and methane.³¹ In a study of a disaccharide mixture (Palatinin®)³² it was necessary to determine both breath H₂ concentrations and breath methane concentrations to achieve a linear dose-effect relation in methanogenic individuals. This aspect merits further investigations. Another end product of colonic carbohydrate fermentation is acetic acid, and venous blood acetate can be quantitatively related to lactulose or pectin ingestion and colonic carbohydrate fermentation.³³

We conclude that quantitative estimates of malabsorption of simple sugars by means of interval sampling of end-expiratory H₂ concentrations are comparable with the results of Bond *et al*⁴ based on more complicated techniques. Individual determinations should be interpreted with caution, but may be useful provided that the test periods are long enough to obtain a representative H₂ response. A more thorough evaluation is possible if tests of reproducibility are incorporated in larger series. Both areas under curves and H₂-peaks seem valid for comparison of groups.

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