Alimentary Pharmacology & Therapeutics

Methodology and indications of H_2-breath testing in gastrointestinal diseases: the Rome Consensus Conference

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SUMMARY

Background

Breath tests represent a valid and non-invasive diagnostic tool in many gastroenterological conditions. The rationale of hydrogen-breath tests is based on the concept that part of the gas produced by colonic bacterial fermentation diffuses into the blood and is excreted by breath, where it can be quantified easily. There are many differences in the methodology, and the tests are increasingly popular.

Aim

The Rome Consensus Conference was convened to offer recommendations for clinical practice about the indications and methods of H_2-breath testing in gastrointestinal diseases.

Methods

Experts were selected on the basis of a proven knowledge/expertise in H_2-breath testing and divided into Working Groups (methodology; sugar malabsorption; small intestine bacterial overgrowth; oro-coecal transit time and other gas-related syndromes). They performed a systematic review of the literature, and then formulated statements on the basis of the scientific evidence, which were debated and voted by a multidisciplinary Jury. Recommendations were then modified on the basis of the decisions of the Jury by the members of the Expert Group.

Results and conclusions

The final statements, graded according to the level of evidence and strength of recommendation, are presented in this document; they identify the indications for the use of H_2-breath testing in the clinical practice and methods to be used for performing the tests.

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INTRODUCTION

Breath tests represent a valid and non-invasive diagnostic tool in many gastroenterological conditions. In particular, the rationale of hydrogen-breath tests is based on the concept that part of the gas produced by colonic bacteria fermentation diffuse into the blood and is rapidly excreted by breath, where it can be easily quantified. Nevertheless, as no sufficient and univocally scientific data are still available, there are many differences in methodology among the various centres. However, in the mean time, this diagnostic instrument is experiencing a rapid widespread diffusion, maybe sometimes improper regarding, above all, the indications and methodology. This Consensus Conference was convened to offer recommendations for clinical practice about indications and methods of H_2-breath testing in gastrointestinal diseases.

METHODOLOGY

To implement this Consensus Conference, held on 10 and 11 December 2007 in Rome, Italy, guidelines derived from Methodological Manual – National Program for Guidelines of Italian Superior Institute of Health, published in 2002 and updated in 2004, were adopted (http://www.pnlg.it).[1]

Conference structure

The main steps in the implementation process were the following:

The promoters of the Consensus (A. Gasbarrini, G.R. Corazza, G. Gasbarrini) selected a panel of experts in the breath test field who, in a preliminary period of about 1 year, performed a systematic review of the literature, then formulated statements on the basis of scientific evidences;

Debate and vote of the preliminary phase statements by a multidisciplinary Jury, during a plenary session lasting 2 days with definitive drafting of written recommendations;

Revision of the recommendations, modified on the basis of Jury indications and final approval by the members of the Expert Group. The promoters of the Consensus did not interfere with the decisions of the Experts.

A more detailed description of each of these steps is reported in this document.

Selection of consensus groups

Members of the Expert Group were selected on the basis of a proven knowledge/expertise in H_2-breath testing by means of publication/research in this field.

The Expert Group was composed of 25 members, divided into five Working Groups, as follows:

(i) Methodology of H_2-breath testing;
(ii) Sugar malabsorption;
(iii) Small intestine bacterial overgrowth;
(iv) Oro-coecal transit time;
(v) Other gas-related syndromes.

(Experts: Caterina Anania, Marco Astegiano, Giovanni Barbara, Guido Basilisco, Luigi Benini, Patrizia Bonazzi, Gabriele Capurso, Maria Certo, Antonio Colecchia, Lucio Cuoco, Antonio Di Sario, Michele Di Stefano, Davide Festi, Cristiano Lauritano, Emanuela Miceli, Massimo Montalto, Gerardo Nardone, Andrea Parodi, Francesco Perri, Piero Portincasa, Roberto Risicato, Margherita Sorge, Antonio Tursi, Paolo Usai Satta, Piero Vernia).

Jury Members belonging to different fields of medical science linked with the Consensus topic. In particular, the Jury was composed of 26 members from the following areas: Gastroenterology, Internal Medicine, Nutrition, Pediatry, Allergology, Pharmacology, Microbiology and General Medicine. The group was coordinated by a President (G. Budillon), purposely not an expert of the subject, and who did not take part in the literature revision. During the 'Plenary Session', Jury Members listened to the Experts' reports about the controversial aspects of the topic under discussion, compared evidence derived from a critical revision of the literature with the Experts' opinions and expressed his judgment on the proposed statements by vote.

(Jury: Maria Teresa Bardella, Osvaldo Borrelli, Patrizia Brigidi, Gabriele Budillon (President), Giovanni Cammarota, Tino Casetti, Mario Cottone, Diego Currò, Antonino De Lorenzo, Camillo Del Vecchio Blanco, Renata D'Incà, Giuseppe Fedeli, Antonio Francavilla, Giovanni Gasbarrini, Maria Gabriella Gentile, Antonio Grieco, Alfredo Guarino, Paola Mastrantonio, Geltrude Mingrone, Sergio Morini, Gianna Moscato, Gian Ludovico Rapaccini, Vincenzo Savarino, Domenico Schiavino, Vincenzo Stanghellini, Enzo Ubaldi).

Systematic search

Systematic literature reviews, with defined inclusion and exclusion criteria, were conducted to identify and grade available evidence in support of each statement. Literature reviews were conducted of English language publications retrieved on MEDLINE, concerning human subjects. The search of review articles, and monographs was limited to the preceding 5 years. A number of search strings were used that are too numerous to be listed in this article. A complete list of the search strings can be obtained by communicating with the lead author of this article.

The literature review was qualitative and a score was assigned as previously described; only works with a ≥3 score were considered.[2] Quantitative meta-analyses were not performed. References cited in this article are a fraction of the articles reviewed in each area and were selected to stress the statements and the discussion in the Working Group.

Grades of evidence

Assignment of the grade of evidence for each statement employed the American Hearth Association (AHA) system which takes into account the level of evidence and the strength of recommendation.[3]

In particular, evidence was graded as indicated:

Class I	Conditions with evidence or general accord that a particular procedure or treatment is useful or effective
Class II	Conditions with conflicting evidence or discordant opinions that a particular procedure or treatment is useful or effective
IIa	The weight of evidence/opinion is in favour of utility/efficacy
IIb	Utility/efficacy is less well defined by evidence/opinions
Class III	Conditions with evidence or general accord that a particular procedure or treatment is not useful or effective while sometimes it can be dangerous

The strength of recommendations was graded as indicated:

A	Data derived from multiple large and intermediate size RCT
B	Data derived from a few, small-size RCT, from a careful analysis of nonrandomized studied or observational registers
C	Recommendations based on Experts' consensus

Voting

The entire process lasted 18 months and the Consensus Jury voted on two iterations of the statements. Between two votes, statements were revised by the Expert Group based on feedback from the Consensus Jury and additional literature reviews.

The first vote of the statements was performed during the plenary session after the debate following each Expert's report. On the basis of changes suggested during the plenary session discussion and after the first vote, the Experts drew a second version of the statements. Subsequently, a second vote was made by e-mail.

For the two votes, a simple two-point scale (agree/disagree) was used to rapidly identify areas where consensus/lack of consensus existed. Consensus was considered to have been reached if 90% or more of the Jury members supported the recommendations.

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Introductory remarks to intestinal gas metabolism

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SUMMARY

Different complex and strictly regulated processes are involved in intestinal gas metabolism, and, still now, a full understanding of these mechanisms is lacking.

Different techniques have shown that the volume of human intestinal gas is less than 200 mL. The composition of intraluminal gas varies along the entire gastrointestinal tract. More than 99% of the gas is composed of five non-odorous gases (N_2, O_2, CO_2, H_2 and CH_4). Various other odoriferous gases are present in trace quantities and account for <1% of flatus. Intestinal gas derives from three sources: swallowed air, intraluminal production (chemical reactions and bacterial metabolism, the latter characterized by both gas production and consumption) and diffusion of gas into the lumen from bloodstream. Stimulating and inhibitory reflexes strictly control gas transit. Gas removal from the intestinal tract occurs by eructation, absorption, bacterial consumption and anal evacuation.

A more complete understanding of these physiological mechanisms is required, mainly in order to fully grasp the pathological gas-related conditions.

INTRODUCTION

Intestinal gas metabolism involves a series of physiological processes, concerning gas production, consumption, excretion and disposal in different gut compartments.

This report intends to summarize the actual knowledge about intestinal gas physiology, from details on volume and composition to a description of mechanisms involved in its homeostasis.

The volume of human intestinal gas has been measured by different techniques.[1–3] By using a modification of the body pletismograph technique used to determine lung volume, in 1956, Bedell *et al.*[1] found a mean of 115 mL of gastrointestinal gas in normal subjects. Greenwald *et al.*[2] measured the increase in abdominal volume after exposure to hypobaric pressures, reporting that normal subjects contain about 111 mL of intestinal gas at atmospheric pressure. Later, Levitt[3] confirmed these results by measuring the volume of endogenous intraluminal gas by an 'intestinal washout technique', based on the infusion of argon into the upper jejunum of healthy subjects, and by measuring the amounts of different gases washed out at the rectal level. They found that the mean volume of intestinal gas was about 100 mL, but single patient's values ranged from 31 to 200 mL. Therefore, even in healthy subjects, a wide variability was evident.

The composition of intraluminal gas varies along the entire gastrointestinal tract:[4] stomach gas has a composition quite similar to that of atmospheric air,[3] while flatus composition shows an enormous interindividual variability, representing the net result of different metabolic processes occurring within the gastrointestinal tract.[4] More than 99% of intestinal gas is composed of five non-odorous gases (N_2, O_2, CO_2, H_2 and CH_4) and, in particular, N_2 ranges from 11% to 92%; O_2, 0% to 11%; CO_2, 3% to 54%; H_2, 0 to >86%; and CH_4 0% to 56%.[3] Various other odoriferous gases, such as NH_3, hydrogen sulphide, indole, skatole, volatile amines and short-chain fatty acids (SCFA), are present in trace quantities and account for less than 1% of flatus.[3, 4]

For many years, aromatic breakdown products of amino acids such as indole and skatole were believed to be the primary malodorous compounds in flatus. On the contrary, more recent studies have shown that sulphur-containing compounds, like hydrogen sulphide, dimethyl sulphide and methanethiol, are mainly responsible for the unpleasant smell of human faeces.[5, 6] In particular, hydrogen sulphide was the predominant sulphur gas in 78% of samples and the concentration of this gas has the strongest correlation

with odour.[6] Sulphur-containing compounds were not detected in breath samples. Hence, we have no information on the putative role of these compounds in the pathophysiology of abdominal symptoms.

Intestinal gas derives from three sources: swallowed air, intraluminal production (chemical reactions and bacterial metabolism) and diffusion of the gases into the lumen from the bloodstream.[7]

Small amounts of air reach the stomach by swallowing, both in combination with foods and separately; a sizable fraction is eliminated by belching, or may be propelled into the small intestine, in particular in supine position, when eructation is more difficult.[7]

Regarding the intraluminal production, it has been reported that CO_2, H_2 and CH_4 represent the predominant gases generated in the entire gastrointestinal tract. In the small bowel, enormous quantities of carbon dioxide (CO_2) are produced by the interaction of hydrogen ion and bicarbonate, in the presence of carbonic anhydrase. CO_2 is rapidly absorbed in the upper gut, thus it seems to contribute minimally to total flatus volume.[7] However, higher concentration of CO_2 passing per rectum can be found, usually when H_2 concentration in flatus is also high due to bacterial fermentation reactions.[8] It is possible that, like H_2 and CH_4, part of flatus CO_2 can also derive from fermentation reactions.[7, 8] Unlike CO_2, bacterial metabolic processes represent the only source of H_2 and CH_4 in the bowel, as neither germ-free rats nor newborn infants during the first 12 h of life[3, 9] can produce H_2 and CH_4. In fasting subjects, H_2 production is normally low, but, after ingestion of fermentable and undigested substrates, primarily carbohydrates, intraluminal bacteria release appreciable amounts of H_2. In healthy subjects some fruits and vegetables (particularly legumes and beans), or flour made from wheat, oats, potatoes and corn can contain oligosaccharides, which escape digestion by upper gastrointestinal tract enzymes, thus becoming available in the large intestine as substrates for bacterial fermentation.[10] However, when small bowel diseases occur and carbohydrate absorption is impaired, high amounts of substrate reach the colon and become available for bacterial fermentation, an anaerobic process producing gas (CO_2, H_2, CH_4) and organic acids, as lactic acid and SCFA. If gases are not utilized by bacteria, they are absorbed and then excreted in breath, or stools. In particular, H_2 can be rapidly absorbed into the blood and excreted by the lungs, the rationale behind H_2-breath test, widely used to detect carbohydrate malabsorption.[11] Absorbed H_2 is almost completely cleared from blood in a single passage through the lungs, thus the rate of H_2-breath excretion should equal that of its absorption into the bowel.[9] About 14–20% of H_2 released in the colon is excreted by the lungs:[12] accordingly, the measurement of H_2-breath concentration may be considered an expression of intestinal H_2 production.[11] Also poorly absorbed proteins can represent substrates for bacterial fermentation, although amino acids generate far less gas release than carbohydrates.[13] It has been reported that, in healthy subjects, high-caloric duodenal lipid loads and amino acids produce bowel gas retention. On the contrary, glucose does not cause gas retention and marked hyperglicaemia may accelerate postprandial gas clearance. Moreover, Harder *et al.* found that a low-caloric meal caused neither gas retention nor girth changes. In contrast, the high-caloric meal led to significant gas retention, while the lack of changes in abdominal and rectal perception led to suppose that, in normal conditions, intestinal mechanisms prevent relevant gastrointestinal symptoms, even under local stimuli, as trapped intestinal gas.[14, 15]

Different factors may influence the amount of gas released in the bowel. Faecal pH influences H_2 production: gas production progressively declines as colonic pH is lowered.[16] It has been shown that acidification of stool pH by ingestion of a nondigestible sugar, such as lactulose, decreases H_2 production, this condition being overcome by $MgSO_4$ pre-treatment and consequent increase in colonic pH.[17] Within the colon, the two main alternative pathways for H_2 disposal are methane production by methanogenic bacteria and hydrogen sulphide production from reduction of sulphate to sulphide by sulphate-reducing bacteria. Other mechanisms, such as acetic acid production through carbon dioxide reduction by acetogenic bacteria are thought to be less relevant.[18] About 30% of adult population (the so-called 'CH_4 producers') harbour high concentrations of methanogenic flora, normally present in the left colon, able to consume large quantities of hydrogen to produce methane.[4, 19–21] In particular, methanogenesis consumes 4 moles of H_2 to reduce 1 mole of CO_2 to CH_4. In the nineties Strocchi *et al.*[22] found that in human faeces, methane-producing bacteria outcompete other H_2-consuming bacteria for common H_2 substrate; moreover, in CH_4 producers sulphate-reducing bacteria, normally present throughout the colon, seem to be limited to the right colon.[4] In the same years, Christl *et al.* showed that

methanogenesis is regulated by dietary sulphate when sulphate-reducing bacteria are present in the colon. In fact, dietary sulphate intake may allow the growth of sulphate-reducing bacteria, inhibiting the growth of methanogens. When both types of bacteria are present in the large intestine, hydrogen metabolism may be regulated by the availability of sulphate in the colon. Some dietary components such as bread, food preserved with sulphur dioxide and alcoholic beverages such as beer, wine and cider are particularly rich in sulphate.[23]

However, the relative efficacy of each hydrogen consuming process may vary,[18] for example, in case of reduction of intraluminal substrates, such as mucine, cystine, or in case of modification of local conditions, in particular pH: it is well known that the optimum pH for sulphate-reducing bacteria is 7.5, while it is 7.0 for methanogens, and 6.5 for acetogens.

Some individuals, the so-called 'low H_2 excretors',[24] fail to elevate their breath H_2 excretion following ingestion of non-absorbable sugars. Several authors suggested that it derives mainly from enhanced H_2 consumption, rather than from reduced absolute H_2 production.[22, 24–26] In particular, Strocchi *et al.*,[21] demonstrated that both increase in number or activity of methane-producing bacteria, and high faecal H_2 tension can be responsible for the rise in H_2 consumption. The composition of the colonic microflora can be determined by early environmental factors.[7] However, adaptive phenomena to alimentary habits occurring later in life may also influence colonic flora composition.[24, 25] Gibson *et al.* found that the addition of oligofructose and inuline, two indigestible carbohydrates, results in Bifidobacteria becoming the predominant bacterial species in faeces. Their ingestion also increased breath H_2, faecal wet, nitrogen, energy excretion.[26]

The rate of H_2 consumption also depends on H_2 tension in faeces, being more rapid at high H_2 faecal tension and negligible at low H_2 tension.[20] Moreover, because faecal mixing allows H_2 movement from faeces to the surrounding gas space with consequent reduction of H_2 faecal tension, in case of impaired colonic motility with consequent poor luminal stirring, H_2 faecal tension is higher and H_2 consumption increases.[20] However, antibiotics which may profoundly modify colonic flora, or laxatives which reduce fermentation or acidification time of colonic pH, may be responsible for low H_2 excretion.[27, 28] In normal conditions, H_2 production occurs in the colon by resident microflora, but in patients with predisposing conditions leading to small bowel bacterial overgrowth, H_2 production may also occur in other gut segments.[12]

As far as diffusion mechanisms are concerned, it is known that intraluminal gases passively diffuse into the blood, depending upon gas partial pressure gradient between lumen and blood, gas diffusibility and time of exposure to the gut–blood barrier.[29] H_2 and CH_4 always diffuse from lumen to blood, as their partial pressure is greater in the gut lumen.[7] Therefore, total H_2 volume in the intestinal lumen results from balanced absolute H_2 production, H_2 consumption and H_2 diffusion from lumen to blood.

Direction of movement of CO_2, N_2 and O_2 is quite variable. In particular, oxygen, from swallowed air, is absorbed from the stomach into the blood, atmospheric pO_2 being greater than blood pO_2, while luminal colonic pO_2 falls below that of venous blood, leading to O_2 diffusion from blood into the colonic lumen. pCO_2 is very low in the swallowed air but it markedly rises in duodenum, because of neutralization reactions of acid by bicarbonate, and CO_2, a highly diffusible gas, rapidly passes from bowel lumen to blood stream. Finally, N_2, a far less diffusible gas, is slowly absorbed into the stomach, as blood pN_2 is lower than pN_2 in swallowed air. On the contrary, N_2 usually diffuses from blood to bowel lumen down a partial pressure gradient created by CO_2 duodenal production, and by CO_2, H_2 and CH_4 release by colonic bacteria.[7]

Gas transit down the gastrointestinal tract does not seem to represent a passive process, it is rather thought that gut actively propels gas in a caudal direction. Stimulating and inhibitory reflexes stricly control gas transit;[30] for example supine position or the presence of intraluminal nutrients (particularly lipids) can delay it, while mechanical stimulations, in both the stomach and the bowel (for example focal gut distension), induce a prokinetic effect.[31]

Eructation, absorption, bacterial consumption and anal evacuation are responsible for gas removal from the intestinal tract. In a study by Tomlin *et al.*, total volume of gas expelled ranged from 476 to 1491 mL/24 h in 10 healthy volunteers on normal diet, with large interindividual variability. A major contribution to total daily flatus volume, on a normal diet, was made by fermentation gases, mainly hydrogen and carbon dioxide. Interestingly, ingestion of a 'fibre-free diet', not containing complex polysaccharides, thus depriving bacteria of exogenous substrates, markedly reduced the release of fermentation gases in flatus.[32]

In conclusion, intestinal gas metabolism represents a complex and very interesting step of intestinal physiology and further investigations are necessary to better understand its mechanisms and the actual relationships with 'gas-related' clinical syndromes.

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H_2-breath tests: methodological audits in adults and children

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SUMMARY

The hydrogen-breath test represents a simple and non-invasive tool, widely used mainly to diagnose carbohydrate malabsorption and bacterial overgrowth in the small intestine. Its diffusion, due to the low cost, simplicity and reproducibility, has not always been accompanied by a parallel awareness of its limits. Moreover, methodological issues have sometimes been disregarded and several methodological aspects are not yet standardized. The aim of the 'Methodology' audit was, therefore, to evaluate data supporting the different test protocols, taking into account both technical and practical aspects. These points should not be considered as minor details as the accuracy of the test is strictly related to a correct methodology.

INTRODUCTION

The hydrogen-breath test represents a simple and non-invasive tool, currently used in gastroenterology to diagnose some clinical conditions, thus avoiding invasive tests. Its wide diffusion, due to the low cost, simplicity and reproducibility, has not always been accompanied by a parallel awareness of its limits, and methodological issues have sometimes been disregarded. Moreover, several methodological aspects are not yet standardized. Consequently, differences in the adopted protocol make the results of various reports difficult to compare. The evaluation of protocols adopted in 15 gastroenterological centres in Italy revealed important differences in terms of carbohydrate load, test duration, sampling frequency and many other aspects, including positivity criteria.[1] Unfortunately, more than 10 years after the publication of this survey, the results are still unchanged. The aim of the 'Methodology' audit was, therefore, to evaluate data supporting the different procedures of performing the test, considering both technical and practical aspects, such as the instruments for hydrogen measurement, the accuracy of devices for breath sampling, and the suitability of some procedures before testing, like the diet or the use of drugs. These points should not be considered as minor details as the accuracy of the test is strictly related to a correct protocol.

Instruments for hydrogen measurement in breath

Hydrogen measurement in breath may be performed by two main types of gaschromatograph: dedicated and nondedicated. Standard gaschromatographs represent instruments not dedicated to the measurement of specific gases and use columns that can dose trace molecules, for example, for toxicology purposes. They are expensive, extremely versatile, but not designed to be used for a single gas. Accordingly, these instruments were selectively modified to allow for single gas determinations, i.e. hydrogen alone or in combination with methane and carbon dioxide, achieving a substantial cost reduction, though maintaining the original detector typology at a solid state, which measures modifications of thermal conductivity. These simpler, dedicated instruments can be stationary or portable. Stationary dedicated gaschromatographs represent the gold standard for hydrogen determinations in breath, as they were previously validated in comparison with nondedicated instruments, and tested in terms of linearity and reproducibility of results.[2–4] Portable instruments adopt a different technology, based on electrochemical cells first proposed by Bergman *et al.*,[5] then evaluated as prototype[6, 7] demonstrating good reproducibility.[7] However, on clinical grounds, the accuracy of only one portable instrument was assessed,[8] and an

overestimation of results against the stationary instrument was evident. Moreover, electrochemical cells are characterized by a predetermined life, suggested by the producer, and no scientific report on long-term stability of these cells is available in the literature.

As far as the instrument maintenance is concerned, stationary ones are particularly sensitive to the humidity transferred with breath sample during the dosing stage. This problem is effectively prevented by the periodical replacement of a column of drierite, which is a calcium-sulphate compound acting as a filter, which absorbs water up to 14% of its weight. First of all, portable instruments should be periodically tested for cell stability; second, during the calibration phase, particular attention should be paid to prevent excessive pressure of the standard gas damaging the electrochemical cell structure.

Breath sampling

Breath sampling represents a pivotal phase of the test: gas measurement must be performed on alveolar air; therefore, the procedure of breath sampling should avoid the interference represented by respiratory dead space air. A variable volume of the first part of exhaled air is simply a wash-out of the airways filled with the last portion of room air inhaled with the preceding breath. This volume is equal to approximately 2 mL/kg of body weight and with a normal tidal volume of about 500 mL/breath, the first one-third volume is represented by dead space air. Because of the laminar pattern of air flow through the major airways, roughly twice that volume should be exhaled before all of the dead space air is washed out. The problem is even greater with neonates, in whom dead space volume is represented by up to 50% of the tidal volume. Three collecting systems are currently available: the modified Haldane-Priestley tube, the Y-piece device and the two-bag system. Their comparison did not show any significant difference in terms of accuracy.[9–11]

In cooperating paediatric patients, the same systems used for adults can be used. On the contrary, in non-cooperating patients, breath samples can be collected invasively with nasal probes or non-invasively using facial masks with detectors of respiratory phases.

CO_2 levels in alveolar air are stable around 5%. Accordingly, this parameter can be considered a marker of correct sampling[12] and the normalization of breath hydrogen values to an alveolar concentration using the observed carbon dioxide concentrations reduces the range and variance of hydrogen concentrations, increasing the reliability of measurements.[12] Variability of duplicate measurements of hydrogen, methane, carbon monoxide and carbon dioxide was assessed comparing 4 different respiratory techniques: a) simply to expire into the apparatus with no instructions; b) to expire at the end of a normal inspiration and attempt to avoid hyperventilation or deep inspiration before expiration; c) to inhale maximally and exhale immediately into the collection apparatus and d) to inhale maximally, hold the inhalation for 15 s and then expire into the apparatus. It has been shown that the last method proved to be the only one able to produce an appreciable reduction in the variability of duplicates, as the 15-s period of breath holding guarantees complete respiratory exchange.[13, 14]

Storage of breath samples

Another definitely nonnegligible source of variability of gas measurement is represented by the technique of breath sample storage. Even if characterized by an appreciable stability, gas bags of Mylar-impregnated foil[15] and gas-tight syringes[2] are unsuitable when many samples have to be tested and also, they are too expensive. Hydrogen and methane may be present in vacutainer tubes as gaseous contaminants from either the silicone tube coating or the organic additives after sterilization by ionizing radiations.[16] Breath samples are currently stored in plastic syringes, an inexpensive method allowing the analysis of gases with no further handling. Unfortunately, an appreciable leakage of gas is present, but simple refrigeration of plastic syringes is sufficient to ensure the stability of hydrogen concentrations for a long time. At room temperature, after 5 days, the hydrogen concentration is reduced up to 30%, while at −20 °C the reduction is equal to 5% and only 7% after 15 days. Moreover, at −20 °C no hydrogen loss is detectable for 2 days.[11, 17] However, different brands show differences in the ability to maintain a constant H_2 level: these differences may be related to the tightness of the rubber stopper.[18] It is also possible that the low temperature modifies the permeability of plastic syringes to gases, and is not able to reduce their diffusibility.[17]

Hydrogen concentration in breath samples must therefore be determined within 6 h of sampling.[11, 17, 19]

Breath test and antibiotics

The accuracy of the test is strictly related to the ability of the colonic flora to produce hydrogen when malabsorbed carbohydrates are present. The use of antibiotics modifies the composition of colonic flora and may therefore be a cause of interference with the test results. Many antibiotic regimens have been tested, which significantly reduced breath hydrogen excretion[20] but an increase was also detected,[21] thus suggesting that the relationship between metabolic activity of colonic flora and antibiotic use is still not completely clarified. In particular, hydrogen consumption by faecal bacteria is an important determinant of hydrogen available for excretion[22–24] and the enhanced hydrogen excretion of subjects taking antibiotics may reflect the inhibition of this bacterial hydrogen consumption.

We have no data about the time needed for recovery of metabolic activity and composition of colonic flora after antibiotic administration. Hence, the exact period that must elapse between antibiotic assumption and hydrogen-breath test is unknown. Based on the experience in clinical practice, an interval of 4 weeks can be suggested. This interval will clearly change if relevant data become available.

Breath test and laxatives

Laxatives and electrolyte solutions administered for colonic cleansing before radiologic, endoscopic or surgical procedures, like antibiotics, could be responsible for alterations to the stability of colonic flora. It has been demonstrated that a combination of laxatives and enemas depresses hydrogen production very markedly. This is probably a quantitative effect, reflecting the reduced number of bacteria remaining in the colon, thus suggesting that the elimination of colonic flora with profuse diarrhoea could be also responsible for a false negative result of breath test.[20, 25] Like antibiotic assumption, we have no data about the time needed for recovery of flora after colonic cleansing; in this case also, a 4-week interval can be suggested.

Besides antibiotics and laxatives, other drugs could theoretically be responsible for modification of colonic flora stability: probiotics and prokinetics can definitely alter the composition of colonic flora and, consequently, its global metabolic activity through bowel colonization and possible elimination of an increased amount of bacteria in stools, respectively. However, while some inconclusive data are available for probiotics,[26–28] no data are available for prokinetics. Till date, similar role of antibiotics and laxatives is conceivable and a 4-week interval between the administration of these drugs and the hydrogen-breath test is suggested.

Breath test and diet

The accuracy of the test requires that colonic bacteria hydrogen production from malabsorbed carbohydrate in the test meal results in a clearly distinguishable increase in breath hydrogen signal. Accordingly, a low level of fasting breath hydrogen excretion facilitates the discrimination of peak breath excretion caused by fermentation of malabsorbed carbohydrate. The main source of substrates responsible for increased fasting breath hydrogen excretion is represented by persisting non-absorbable, fermentable carbohydrates at the colonic level.[29, 30] Accordingly, a diet reducing this interfering factor is needed. Two old studies reported prolonged monitoring of hydrogen excretion after ingestion of different carbohydrates; the ingestion of beans, potatoes, oats, white wheat and corn was shown to induce a significant increase in hydrogen-breath excretion.[31, 32] On the contrary, the ingestion of rice flour and meat is not accompanied by a detectable increase in breath hydrogen excretion.[10, 31–33] Accordingly, it is conceivable that the evening before hydrogen-breath test patients had been instructed to follow a restricted diet avoiding nonabsorbable substrates and, in short, containing rice and meat.

On the basis of the same studies, it is commonly recommended that subjects should fast overnight before the test. However, no reports evaluated the effect of different breakfasts on breath hydrogen excretion.

Breath test and cigarette smoking

Many gases are produced by tobacco combustion. In particular, besides methane and carbon monoxide, hydrogen levels may reach up to 2%.[34] Cigarette smoking, therefore, represents an important interference with breath hydrogen measurement and should be avoided before and throughout the test. This interference was first suggested in the late 1970s[35] but two recent studies have unequivocally confirmed it.[36, 37] Both studies have shown that breath hydrogen excretion markedly increases during smoking and rapidly

decreases thereafter, but it does not revert to basal values, remaining higher than 100% 15 min after the cigarette has burnt. Cigarette smoking should therefore be avoided but we have no data on the time needed for complete normalization of breath excretion after cigarette smoking.

Breath test and mouthwashing

Hydrogen may also be produced by the oropharyngeal flora and it is conceivable that fermentation of the test solution may occur, thus interfering with the result. Two studies have reported this problem; in the first one, the increase in breath hydrogen excretion after mouthwashing with a 20% sucrose solution was prevented by a mouthwash with 1% chlorhexidine solution.[36] Similarly, a significant increase in breath hydrogen excretion was shown to occur after sham lactose feeding, but no increase was detectable after sham saccharine feeding, intragastric or intraduodenal lactulose administration.[38] Oral cavity cleansing aimed at the inactivation of bacterial flora thus prevents the increase in an early peak of breath hydrogen excretion, which may cause false positive results.

Breath test, hyperventilation and physical exercise

Breath hydrogen concentrations vary with ventilation rate. In particular, an inverse correlation between hydrogen levels and ventilation rate is evident.[39] There is reduced breath hydrogen excretion during sleep[40] when hypoventilation is present. On the contrary, the disappearance of breath hydrogen excretion immediately after exercise[41] should be attributed to exercise-induced hyperventilation. Nocturnal hypoventilation was suggested as being responsible for elevated fasting concentrations of breath H_2 [40, 42] but fermentable substrates persisting at the colonic level are the actual cause of this phenomenon.[10, 29–33] Therefore, physical exercise before and during the hydrogen-breath test should be correctly avoided.

The clinical value of methane measurement

Methane production is critical for intraluminal hydrogen consumption.[22] A molecule of methane contains four atoms of hydrogen; therefore, it could be possible to reduce the volume of intraluminal gas by reducing the total number of molecules.

In the late 1970s, breath methane excretion was considered a putative marker of the presence of colonic cancer. In fact, it was suggested that the prevalence of colonic cancer was significantly higher in methane producers than in non-producers.[43] Later, these results were confirmed and even extended: the prevalence of methane excretion was shown to be significantly higher in patients with colonic cancer than in healthy volunteers, and after colonic resection, the prevalence decreased to values not significantly different from those found in healthy volunteers and patients with functional bowel disorders.[44] However, more recent studies have shown that breath methane excretion is present in patients affected by many clinical conditions, such as colonic diverticulosis, irritable bowel syndrome, functional bloating and even in healthy volunteers,[45] thus limiting the clinical value of this parameter in the screening of colon cancer, due to its very low specificity.

The usefulness to measure methane excretion also during hydrogen-breath test derives from the consideration that hydrogen consumption reduces the amount of intraluminal hydrogen available for excretion, thus reducing the breath hydrogen peak, in turn causing even false negative results. If we consider hydrogen excretion as the marker of ongoing fermentation, all mechanisms which reduce hydrogen excretion may make the test less accurate. Consequently, taking this reasoning to extremes, breath methane excretion represents an important alternative target for intestinal gas breath excretion measurement in the subgroup of hydrogen nonproducers, thus improving the test accuracy, as recently shown in a group of hydrogen nonproducer lactose intolerants.[46]

The reported prevalence of such H_2 nonproducing subjects harbouring colonic flora unable to produce hydrogen during fermentation varies widely from 2% to 43%.[20, 47–54] However, It should be considered that the prevalence of hydrogen nonproducers is inversely correlated to the test duration: it was shown that the prolongation of breath hydrogen measurement from 2 to 4 h induces a significant reduction of this prevalence.[55] A more recent report extended this result by prolonging the test up to 7 h:[21] it is therefore conceivable that a variable gastrointestinal transit of the substrate may be responsible for false negative results.

On the contrary, in subjects who are definitely hydrogen nonproducers, breath methane could represent an alternative marker. Unfortunately, up to now we have no conclusive data as few paediatric papers

are available[56–58] and studies in adults show contradictory results.[45, 47, 48, 59–65] The reason for this discrepancy lies mainly in methodological differences, the most important being inconsistent criteria for considering a subject as a methane producer.

In conclusion, we think that future studies on measurement of volatile organic compounds and other gases (mainly methane) further than hydrogen, should be encouraged, as they may add new insights on intestinal pathophysiology and improve clinical approaches.

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H_2-breath testing for carbohydrate malabsorption

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SUMMARY

Carbohydrate malabsorption is a frequent clinical condition often associated with abdominal disorders. Hydrogen-breath tests are considered practical and safe compared with more invasive methods in the diagnosis of these disorders.

Our aim was to review the impact of carbohydrate breath tests in clinical practice and to propose a more standardized technique. Although particular emphasis has been placed on lactose breath test, fructose and sorbitol have also been considered. Original articles and reviews were searched in PubMed and the most relevant articles concerning clinical and methodological aspects of breath test were selected according to criteria of evidence based medicine.

The search shows that breath testing can now be recommended in clinical practice to diagnose lactose malabsorption and intolerance in both adults and children. Criteria to perform this test and to record possible abdominal symptoms have been suggested. On the contrary, criteria of clinical utility for sorbitol and fructose breath tests are not yet acknowledged.

In conclusion, lactose breath tests may be suggested in clinical practice following a standardized technique, while fructose and sorbitol tests should be proposed only for research studies.

INTRODUCTION

Unabsorbed carbohydrates reaching the colon are fermented by bacteria and may be responsible for symptoms such as bloating, borborygmi, abdominal pain and diarrhoea.

Breath testing (BT) relies on the ability of intestinal bacteria to metabolize various carbohydrate substrates producing hydrogen and/or methane and leading to the release of measurable levels of these gases in air exhaled from the lungs. In particular, lactose, fructose and sorbitol BTs have been studied in patients with gastrointestinal symptoms.

A large part of this review will be dedicated to lactose BT, in consideration of its clinical impact and number of papers available in the literature. A short paragraph concerning fructose and sorbitol breath testing is also provided. Particular attention has been paid to paediatric clinical implications of BT.

BREATH TESTING FOR LACTOSE MALABSORPTION AND INTOLERANCE

Indications and diagnostic utility

Lactose, a disaccharide composed of glucose and galactose bound in a ß-glycosidic linkage, is the primary carbohydrate exclusively found in the mammalian milk. Absorption of lactose requires lactase-phlorizin hydrolase (LPH) activity in the small intestinal brush border to break the linkage between the two monosaccharides, a step preceding the transport of glucose and/or galactose across the brush border membrane.[1]

Primary adult-type hypolactasia, an autosomal recessive condition resulting from the physiological decline of LPH enzyme activity in the intestinal cells, occurs in a large proportion of individuals. A single nucleotide polymorphism, C/T-13910, 14 kb upstream the lactase gene, has recently been correlated with lactase persistence/nonpersistence in several populations.[2]

Secondary causes of hypolactasia, such as coeliac disease, gastroenteritis and Crohn's disease, may lead to transient lactase deficiency and appearance of

abdominal symptoms similar to those of primary lactose malabsorption.

A correct distinction between primary and secondary causes of lactose malabsorption is particularly important in children. The onset of adult-type hypolactasia is correlated to age and depends on the ethnicity.[3] In adult populations where up to 80–90% of subjects are primary lactose malabsorbers, the investigation of a secondary hypolactasia could be unnecessary, while it can be a crucial diagnostic objective in populations with a low prevalence of primary malabsorption.[4]

Lactose malabsorption represents a well-known cause of abdominal disorders, like diarrhoea, bloating, excessive flatus and abdominal pain. Lactose malabsorption testing should be recommended in subjects complaining of these symptoms after lactose ingestion. However, sugar malabsorption does not necessarily result in the development of intolerance symptoms; in fact, only about one-third to half of lactose maldigesters are also intolerants.[5, 6]

Different methods have been used to perform the diagnosis of lactose malabsorption. Lactose activity assay by jejunal biopsy has been proposed as the 'gold standard'.[7, 8] However, it seems too invasive for the diagnosis of such a mild condition and its results may be influenced by the irregular dissemination of lactase activity throughout the small intestine mucosa.[9] Lactose BT represents an indirect test for lactose malabsorption, and it is commonly considered the most reliable, non-invasive and inexpensive technique.[10] However, it is possible to find false negative breath tests, due to the inability of colonic flora to produce H_2 after ingestion of non-absorbable carbohydrates, or after a recent administration of antibiotics. False positive breath tests are less frequent and are mainly produced because of small bowel bacterial overgrowth.[11, 12] On the basis of reviewing different studies, lactose BT shows good sensitivity (mean value of 77.5%) and excellent specificity (mean value of 97.6%).[7, 8, 13–15] To evaluate critically the usefulness of BT in patients with malabsorption and intolerance symptoms, it is necessary to ascertain if the latter represents the clinical consequence of malabsorption. Is it sufficient to ask the patient about the correlation between symptoms and lactose ingestion? Probably not; indeed a series of studies have shown that patients with confirmed malabsorption and intolerance were unaware that their symptoms depended on the ingestion of this sugar.[16] On the other hand, it is possible that patients who believed to be severely intolerant even to small doses of milk were not lactose malabsorbers.[17] Mainly in these subjects, BT represents a valid diagnostic tool in clinical practice, showing that abdominal symptoms were erroneously related to lactose ingestion and therapeutic measures were unnecessary and not beneficial. For these reasons, after about 30 years from its initial utilization, the lactose BT must be considered more reliable than the clinical history and can be recommended in both adults and children with suspected lactose malabsorption and intolerance.

A different evaluation concerns the usefulness of investigating lactose malabsorption in IBS subjects. The relationship between these two conditions has probably been overestimated during the past and now it is thought that dietary lactose exclusion rarely ameliorates symptoms in these patients.[1, 18] Therefore, a lactose BT in IBS patients should not be recommended.

Methodological aspects in adults

No agreement on how to perform BT, lactose dosage and set cut-off level, sample intervals and duration of testing has been established. Considering the large utilization of BTs, a better standardization of this methodology must be desired.

Many published reports as well as some milestone studies of BT validation have utilized the dosage of 50 g lactose.[7, 8, 13, 14] However, the usage of 50 g of lactose (approximately corresponding to the amount of lactose contained in one litre of milk) has been criticized because it represents an amount far more elevated than that usually ingested at any one time. Besides, patients with lactose intolerance may experience a considerable discomfort when lactose BT is performed with this dosage, mainly abdominal pain and diarrhoea.[19]

Especially in the last 10–15 years, the need of using a more physiological dose of lactose has been emphasized in several reports.[15] As for more physiological amounts, the widely utilized dosages of 20 and 25 g of lactose (corresponding to the amount found in 400–500 mL of milk) produce an almost equivalent level of breath-H_2 peak response as that produced by a dose of 50 g of lactose. So far, a direct comparison between 20 g and 25 g has never been performed; based on standardized score[20] of the methodological quality of published studies, we found that the majority of recent

studies employed 25 g, and three recent reviews on BT suggested the use of 25 g.[1, 21, 22] As 400–500 mL of milk also often exceeds the common daily ingestion of dairy products, further studies are needed to clarify the utility of BT with fewer quantities of lactose, that is, less than 20 g, that are closer to physiological habits.

As far as the type of substrate is concerned, validation studies have generally preferred lactose in water solution.[7, 8, 13, 14] Milk represents a more physiological substrate than lactose; it delays gastric emptying, probably improving lactose absorption by a longer contact between substrate and residual intestinal enzymes. However, up to now, milk has not been sufficiently standardized, and about 1-3% of the general population, in particular children, is affected by milk protein allergy.[23] Therefore, further studies are necessary to investigate usefulness and applicability of milk such as substrate in clinical practice.

Variable criteria regarding sampling time intervals (15–30–60 min) and test duration (2–5 h) have been described.[24–26] Positivity criteria have also varied; 10 or 20 ppm over basal values have been considered indicative of lactose malabsorption.[27] A positivity cut-off value of 10 ppm probably increases BT sensitivity, while its specificity might worsen.[13, 15] A duration of the test lower than 4 h was shown to reduce BT sensitivity.[25, 26] New criteria based on breath hydrogen excretion greater than 6 ppm at the 6th hour and the sum of breath hydrogen values obtained at the 5th, 6th and 7th hours greater than 15 ppm were proposed, showing better sensitivity than conventional criteria with no impact on specificity.[28] However, few data are available regarding their adoption both in the literature and in clinical practice.

In conclusion, based on a systematic review of the literature, the following recommendations are suggested: (i) a test duration of 4 h, (ii) sample intervals of 30 min and (iii) a cut-off value of 20 ppm above the baseline.

Methodological aspects in children

In paediatric field there is also no agreement on the methodological aspects of BT. Studies in children have included lactose doses ranging from 0.5 to 2.0 g/kg at 10% or 20% concentrations.[27, 29, 30] The recommendation of a physiological dose of lactose is valid also in children.[31] According to published evidences, the test should be performed with the administration of a standardized amount of lactose corresponding to 1 g/kg, up to a maximum of 25 g. The considerations mentioned previously concerning the lack of standardization of milk as a substrate compared with lactose solution are even more crucial for children. To improve BT standardization and reach a diagnosis of lactose malabsorption, a test duration of 3 h, sample intervals of 30 min and a cut-off value of 20 ppm should be recommended in clinical practice. The duration of 3 h has been suggested because children do not tolerate prolonged periods of fasting and because of a shorter gut transit time at this age. In paediatric studies, no alternative models have been proposed.

Symptom recording during the test

Lactose malabsorption is not always associated with intolerance symptoms.[18] Only about one-third of lactose malabsorbers complain of symptoms during BT with the generally used doses of lactose. The factors responsible for symptom triggering are not yet completely understood, although a visceral hypersensitivity might play a role in the genesis of functional symptoms.[32] Despite these considerations, recording of abdominal symptoms related to lactose consumption during BT is important. On the basis of clinical response during BT, it may be possible to distinguish among malabsorbers and patients who can tolerate variable dosages of lactose. Variability among scores of severity and duration of symptoms is found in the literature as well as a poor agreement on the quality and quantity of symptoms to be considered.[13, 33, 34] Therefore, in both adults and children we propose the recording and scoring of the following four symptoms during the test and 8 h after: abdominal pain, bloating, flatulence and diarrhoea, by a visual-analogue scale (VAS).

BREATH TESTING FOR MALABSORPTION OF OTHER CARBOHYDRATES

Sorbitol is a sugar alcohol widespread in plants, particularly in fruits and juices. Fructose is a monosaccharide naturally present either in its free form or as sucrose in fruits, and widely used as a sweetener in different foods. Sorbitol is normally only partially absorbed, while fructose is absorbed by carrier-mediated facilitated diffusion.[1] Simultaneous ingestion of sorbitol and fructose seems to increase malabsorption of the latter.[1] The presence of fructose or sorbitol malabsorption, even in a patient with abdominal disease, cannot be considered pathological *per se* and certainly

Table 2. Recommended diagnostic parameters of lactose breath test and period of symptom observation after lactose consumption

H_2 BT	Lactose dose	Samples	Duration	Cut-off	Symptoms
Adults	25 g	30 min	4 h	20 ppm	12 h
Children	1 g/kg (up to 25 g)	30 min	3 h	20 ppm	12 h

cannot be related to symptoms. The optimal dosage of these sugars in fructose and sorbitol BT, to detect malabsorption, is yet unclear.[1, 35, 36] Another unresolved issue is how symptoms should be assessed during testing. No gold standard is available for these BTs and no significant validation studies have been published. Although several studies have found sorbitol BT effective in detecting small bowel damage,[35, 37] sorbitol BT as well as fructose BT should not be recommended in clinical practice in both adults and children while their use may be indicated for research purposes.

CONCLUSION

Hydrogen-breath tests are simple, safe and useful to diagnose carbohydrate malabsorption. Lactose BT can be performed with a more physiological lactose dose in standardized conditions; it is useful in clinical practice and at all ages. Stimulating questions, such as a more sensitive and simplified BT procedure, need additional polycentric studies. The clinical importance of sorbitol and fructose malabsorption remains to be evaluated.

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H_2-breath testing for small-intestinal bacterial overgrowth

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SUMMARY

Small intestinal bacterial overgrowth (SIBO) is an intestinal microflora unbalance, which can show a wide clinical spectrum ranging from a mild and unspecific intestinal symptoms to a severe malabsorption syndrome. The culture of jejunal aspirate is considered the gold standard diagnostic test for SIBO, however, glucose and lactulose breath tests (GBT and LBT) are currently used in clinical practice. Among them, GBT seems to have a higher diagnostic accuracy in studies comparing breath tests versus culture. Some conditions, such as hypo-aclorhydria, anatomical abnormalities or gastrointestinal motility failure, may cause SIBO and related malabsorption. In these cases, GBT may be useful in order to establish whether malabsorption is due to SIBO or to the underlying disease. Data about the role played by SIBO in irritable bowel syndrome are still inconclusive, and its search by breath test is not recommended in these patients.

BACKGROUND

The intestinal microflora consists of about 10^{14} microrganisms belonging to more than 500 different bacterial species. Its composition is influenced by several factors such as age, susceptibility to infections, dietary habits, immunological factors, intraluminal pH, interaction among components of intestinal flora and availability of fermentable substrates.[1–3]

Bacterial species distribution is not homogeneous along the digestive tract. Indeed, microflora is poorly

represented in the stomach, where gastric acidity maintains quite a sterile environment. Bacterial concentration gradually increases in the small intestine, with the appearance of anaerobic bacteria. The ileum is a transition area towards a colonic type flora, which is characterized by a relative increase in aerobic species and a consistent increase in anaerobic ones.[3]

The balance between bacterial flora and host is maintained by many factors. The most important control mechanisms are gastric acid secretion, anatomical integrity of the digestive tract, peristaltic activity, IgA secretive immunoglobulins and, to a lesser extent, other secretions such as saliva, bile and pancreatic juice.[4, 5] Failure of these mechanisms can be responsible for the development of intestinal microbial imbalance such as small intestinal bacterial overgrowth (SIBO).

Hypo-aclorhydria, commonly determined by chronic autoimmune atrophic gastritis, vagotomy, prolonged use of proton pump inhibitors and total gastrectomy, increases the risk of bacterial contamination of the small intestine by bacteria from the upper respiratory tract.[6–9]

Congenital anatomical disorders, such as intestinal duplication, partial atresia, stenosis and diverticula of the small intestine, or acquired abnormalities such as entero-colic fistulas, adhesions, resection of the ileum-cecal valve and Billroth type II partial gastrectomy with Roux-en-Y anastomosis, may favour the development of bacterial overgrowth, because all these conditions determine a lack of intestinal clearance and faecal stasis.[5, 10]

Alteration of intestinal motility due to small bowel diseases (e.g. Crohn's disease), neurological diseases (e.g. muscular dystrophy, myotonia), endocrine diseases (e.g. diabetes mellitus and hypothyroidism), iatrogenic disorders (e.g. post-operative blind loop, radiation enteritis), chronic renal failure and connective tissue diseases (e.g. scleroderma) can frequently determine SIBO.[5, 11–15]

Finally, bacterial contamination of the small intestine has been described even in patients with primary (e.g. selective IgA deficiency) or secondary immunodeficiency (e.g. lymphomas, chronic lymphatic leucaemia).[5, 16]

DEFINITION OF SIBO AND CLINICAL FINDINGS

In many studies, SIBO is defined as the microbiological presence of at least 10^5 colony-forming units (CFU) per millilitre (mL) of jejunal aspirate.[5, 17–20] However, the qualitative microbiological composition of contaminating flora is extremely important. In fact, two types of SIBO are identified on the basis of pathophysiological mechanisms.

The first type is predominantly supported by Gram-positive bacteria from the upper respiratory tract, and is secondary to a deficiency in gastric acid barrier.

The second type is characterized by an increase in colonic bacteria, and may occur in individuals with altered intestinal clearance or with abnormal communication between the large and the small bowel.[5]

In clinical practice, SIBO is characterized by a wide spectrum of manifestations, ranging from unspecific abdominal symptoms (e.g. bloating, abdominal discomfort, flatulence) to less frequent severe generalized malabsorption and nutrient deficiency (diarrhoea, steatorrhoea, weight loss).[5, 17–19] Malabsorption can be attributed in part to the effects of intraluminal bacterial replication and fermentation and partly to impaired enterocytes.[21–28]

DIAGNOSIS OF SIBO: THE ROLE OF JEJUNAL ASPIRATE CULTURE

Traditionally, diagnostic tests for the detection of SIBO are divided into invasive tests (which require patient intubation and enteric juice aspiration from the small intestine) and non-invasive tests (which measure the concentrations of bacterial metabolism products in plasma, urine or expired air). Invasive tests are the microbiological culture tests and SCFA dosage in the small intestine aspirate. Non-invasive tests are H_2- and $^{14/13}$C-breath tests (BTs), assay of serum unconjugated bile acids, dosage of urinary para-aminobenzoic acid (PABA) after administration of colil-PABA, and dosage of 24 h urinary indican.[29]

Jejunal aspirate culture is considered as the gold standard method for SIBO diagnosis. It is obtained by means of patient intubation and aspiration at multiple intestinal sites, more rarely during enteroscopy. The amount of liquid, the site of collection (traditionally beyond the ligament of Treitz) and the technical details of the microbiological tests (for both aerobic and anaerobic bacteria), as well as the cut-off value for definition of SIBO are not yet standardized, although many studies use a value of $>10^5$ CFU/mL.[30–43]

Besides these methodological problems of standardization, a still unsolved issue concerns the diagnostic accuracy of the culture in case of 'distal' SIBO, that is,

bacterial overgrowth affecting mainly the ileum, which is not assessable by means of traditional enteroscopy. However, we must emphasize that a good correlation has been demonstrated between sampling carried out within 15 cm away from each other, focusing on the possibility of a 'confluence' of SIBO between different intestinal loops, at least within that distance.[37]

DIAGNOSIS OF SIBO: H_2-BTS

H_2-breath tests are based on the determination of hydrogen concentration in expired air. Hydrogen and methane are produced by fermentation of intraluminal substrates (carbohydrates) by bacteria contaminating the small bowel. The most frequently used substrates in H_2-BT for SIBO diagnosis are glucose and lactulose. The former is a monosaccharide which is completely absorbed in the proximal small intestine; the latter is a poorly absorbed disaccharide which reaches the cecum. Both substrates are fermented by the contaminating bacterial flora in the small intestine with hydrogen production. In individuals suffering from SIBO, glucose breath test (GBT) generally shows a single 'early' peak of hydrogen excretion, while lactulose breath test (LHBT) shows two distinct hydrogen excretion peaks: the first 'early' peak due to the small bowel microflora activity and the second 'late' peak due to the colonic bacterial metabolism.

False negative H_2-BT results may be due to either the absence of H_2-producing bacteria or a low increase in H_2 excretion. Moreover, in case of LHBT, the early 'small bowel' H_2 peak can merge with the late 'colonic' peak, while a quick glucose absorption into the proximal bowel may result in a false negative GBT. On the other hand, false positive H_2-BTs have also been reported in individuals with an accelerated intestinal transit time.

By evaluation of different cross-validation studies between BTs and jejunal aspirate culture, diagnostic accuracy of BTs for SIBO has been achieved. It was influenced by several factors, such as definition of a positive culture, dose and concentration of substrates, length of the test, sampling intervals, cut-off value of hydrogen peak over basal and the prevalence of SIBO in the study population.

Many of these parameters were not uniform in the 11 studies we examined.[33–43] As for the dose of substrate, some authors use 50 g glucose[33, 36, 38, 40, 41] while other authors use higher doses (from 75 to 100 g).[35, 42, 43] In almost all the studies employing lactulose as substrate, the administered dose was 10 g.[34, 35, 39, 43] BTs lasted from 120[33, 36, 38, 40–42] to 180–240 min.[34, 35, 37, 39, 43] Nearly all the studies showed good SP and low SE for both methods. Median values of SE, SP, PPV, NPV and DA of GBT and LHBT are shown in Table 1.

Table 1. Diagnostic accuracy of GBT and LHBT compared to jejunal aspirate culture

	SE	SP	PPV	NPV	*DA*
GBT	62,5%	81,8%	80,0%	65,5%	*71,7*
LHBT	52,4%	85,7%	61,5%	53,6%	*55,1*

GBT: Glucose Breath Test; LHBT: Lactulose Hydrogen Breath Test; SE: Sensitivity; SP: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; DA: Diagnostic Accuracy.

Overall, regardless of the substrate dose and the test duration, GBT has shown a greater diagnostic accuracy than LHBT. Besides being accurate and non-invasive, H_2-BTs have other advantages such as lack of toxicity, low cost of substrates and easy accessibility to clinical practice (for the wide diffusion of gas-chromatographic devices).

Glucose BT protocol

Glucose breath test validation studies are divided into two main groups based on the substrate dose administered and the test length:

Glucose 50 g/250 mL; 120 min.

Glucose 75–100 g (in different concentrations); 180 min.

The diagnostic accuracy is around 70% for both tests. Theoretically, the use of a glucose dose greater than 50 g and a longer test length can also explore the distal segments of the small intestine, thus increasing the diagnostic accuracy of 'distal' SIBO. However, 75–100 g GBT did not show a significant improvement in sensitivity as compared to 50 g GBT.[35, 42, 43]

The most frequently used cut-off value for test positivity is 10–12 ppm. Higher cut-off values seem to reduce the test sensitivity below 60%,[38, 41] although these data have not been confirmed by other authors.[33–35]

The samples of expired air were generally collected every 15–20 min. The range of sampling does not seem to have a clear influence on GBT diagnostic accuracy. This latter seems to be better in studies that have enrolled patients with a high risk of bacterial overgrowth, due to a definite disease predisposing to SIBO and symptoms compatible with a malabsorption syndrome.[33, 35–37, 41]

Other studies, conducted in elderly[38, 40] or cirrhotic patients,[42] reported lower sensitivity and specificity.

SIBO AND IRRITABLE BOWEL SYNDROME

The role played by intestinal bacterial flora (and eventually by SIBO) in the pathogenesis of irritable bowel syndrome (IBS) is controversial.[44–47] Pimentel *et al.*[48, 49] first stressed the role of SIBO in IBS. In two studies, the prevalence of SIBO in IBS patients – as assessed by LHBT – was estimated to be around 78–84%. Antibiotic therapy also led to a significant improvement in IBS symptoms in 48–75% of decontaminated patients compared to 37% of patients treated but not decontaminated, and 11% of patients treated with placebo. However, the results of these studies have been highly questioned, particularly for the methodological criteria used. Subsequent studies, based on GBT for SIBO diagnosis, confirmed a correlation between SIBO and IBS. In fact, the prevalence of SIBO in patients with IBS fluctuated between 30% and 46%, as compared to 4% in healthy controls.[50, 51] Also in these studies, the culture of duodenal juice to confirm the diagnosis of SIBO was not carried out.

In recent studies, the prevalence of SIBO in patients with IBS, tested with different diagnostic techniques, was shown to be rather low and not significantly different from that of healthy controls.[52, 53] In particular, SIBO diagnosis based on the culture of duodenal juice was performed in only 4% of IBS patients and BT positivity was similar in IBS patients and in healthy controls.[53] However, a marked increase in average bacterial counts in duodenal juice of patients with IBS was found, even if the cut-off value of 10^5 CFU/mL was not reached.[53]

In summary, although it is likely that the intestinal bacterial flora plays a role in the pathogenesis of IBS, the lack of data uniformity currently present in the literature makes it impossible to clearly identify a correlation between IBS and SIBO. Therefore, BTs for SIBO in patients with IBS are not recommended.

CONCLUSIONS

In clinical practice, some conditions, such as hypoaclorhydria, anatomical abnormalities or gastrointestinal motility failure, may cause SIBO and related malabsorption. In these cases, it could be difficult to establish whether malabsorption is due to SIBO or the underlying disease. GBT is a useful, non-invasive and inexpensive test to evaluate the role played by SIBO in these clinical conditions. The diagnostic accuracy of BTs is quite good, even if the results of these tests should be interpreted with caution, always taking into account the patient clinical history.

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H_2-breath testing for evaluation of oro-caecal transit time

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SUMMARY

The appearance in the expiratory breath of gases produced by colonic ferementation of an ingested organic compound may be used to measure oro-caecal transit. Both a liquid meal containing lactulose or a solid meal containing backed beans can be used in the assessment of oro-caecal transit. Ingestion of a meal is followed by a biphasic breath hydrogen profile in most subjects. The initial peak of hydrogen closely follows the meal whereas the second peak, associated with the head of the meal entering the caecum often occurs hours later. The H_2 breath test is safe and usually well tolerated. However, the wide variation in the measurement of oro-caecal transit in normal subjects and the poor reproducibility of the test, in particular with the liquid meal, have limited its applications to the clinical. The test performance is better when applied to clinical pharmacology studies and it has been used to demonstrate the drug effects on oro-caecal transit.

INTRODUCTION

The appearance in the expiratory breath of gases produced by colonic fermentation of an ingested organic compound may be used to measure orocaecal transit[1–5].

Several substrates can be used in the assessment of oro-caecal transit. A liquid meal containing 10 g of lactulose in 100 mL of water[6] or a solid meal containing baked beans as a source of non-absorbable carbohydrate (stachyose and raffinose)[7] is most often used, although no significant correlation has been shown between the two measurements. The outcome of reproducibility test with the liquid meal is poorer than with the solid meal.[6, 8, 9] The major factor determining the poor reproducibility of the liquid meal is the phase of the interdigestive migrating motor complex.[10] Transit time with the solid meal is significantly longer than with the liquid meal in the same individuals[7]. The addition of lactulose to a solid meal accelerates small bowel transit.[11]

End expiratory breath samples are collected before and at regular intervals after ingestion of the meal. Breath samples are analysed in duplicate at baseline and thereafter at every 10–15 min up to transit time assessment.[12, 13] Breath samples are analysed for H_2 content expressed in parts per million (ppm), corresponding to 0.045 μmol/liter.[14] The detector accuracy should be tested with a resolution of less than 2 ppm and a linear response in the range of 0–100 ppm.

Ingestion of a meal is followed by a biphasic breath hydrogen profile in most subjects. The initial peak of hydrogen closely follows the meal; whereas, the second peak, associated with the head of the meal entering the caecum, often occurs hours later. Carbohydrate fermentation by bacteria in the mouth may account for the initial peak and may be prevented by mouth wash.[5] Larger initial peaks are probably caused by residues entering the colon from a previous meal stored in the ileum and propelled by test meal ingestion, a manifestation of the gastro-ileal reflex.[15]

Increments of at least 3, 5 or 10 ppm of hydrogen above baseline (mean of the two pre-meal samples) maintained or increased in the two following determinations have been used to define the incoming of the head of the meal in the caecum.[6, 16] The oro-caecal transit time lengthens as values of hydrogen content above baseline increase (74 min at 5 ppm vs. 87 min at 10 ppm).[16]

The oro-caecal transit time in healthy subjects ranges between 40 and 170 min for lactulose meal[8, 16–23] and between 192 and 232 min for a solid meal.[7] Transit time shortens with increasing doses of lactulose.[8] The current protocol employing the liquid solution includes 10 g of lactulose in 100 mL of water, and a cut-off value of hydrogen ≥10 ppm (based on barium meal

studies[16]) followed by at least two other subsequent increments.[24]

About 5–27% of normal subjects fail to produce an increment of hydrogen breath concentration after the meal due to the absence of hydrogen-producing flora in the colon.[25, 26] Whether the menstrual cycle influences oro-caecal transit time[27, 28] remains controversial.

Recently, inulin, a naturally occurring polysaccharide, has been proposed as an ideal substrate to be added to a solid meal for hydrogen breath test and transit time assessment.[29, 30] Inulin has a higher degree of polymerisation than lactulose and, consequently, is less active osmotically. At variance with lactulose, inulin does not shorten oro-caecal transit time that ranged between 420 and 570 min after ingestion of 5 or 10 g inulin with the solid meal; the advantages of this solid meal in comparison to those used previously remain to be established.

SAFETY

The H_2-breath test is safe and usually well-tolerated. Bloating and abdominal distension may occur after colonic fermentation of lactulose.[31]

APPLICATIONS TO THE CLINICAL SETTING

Despite its non-invasiveness, safety and simplicity to be performed, the inherent limitation for the clinical application of the hydrogen-breath test to estimate oro-caecal transit is due to a wide variation of results in healthy people. Moreover, the test reproducibility, in particular with the liquid meal, is rather poor. A delayed oro-caecal transit assessed by hydrogen-breath test has been reported in various sub-groups of patients, including those with depression,[18] chronic alcoholism,[23] constipation,[22] acromegalics,[32] diabetics,[33] irritable bowel syndrome,[19] beta thalassemia major,[20] pregnancy,[34] cholecystectomy,[35] obesity,[17] cirrhotics,[36] scleroderma,[13] dyspeptic patients,[13] in the chronic phase of corrosive injury after acid or alkali ingestion,[37] and in constipated children.[38, 39] By contrast, fast oro-caecal transit has been reported in patients with the irritable bowel syndrome,[14] chonic alcoholics,[40] partial gastrectomy,[41] post-vagotomy diarrhoea[42] and hyperthyroidism.[43–45] Despite such wide applications to the research field, so far recent guidelines do not suggest a definite clinical indication for the test in the clinical setting.[24]

However, the test performance is better when applied to clinical pharmacology studies. Given its excellent safety, the test has been used to demonstrate the drug effects on oro-caecal transit. Transit was accelerated by misoprostol,[46] erythromycin,[47] metoclopramide[48] and paroxetine,[49] and it was delayed by loperamide,[50, 51] ritodrine,[52] codeine,[5] dopamine,[53] peppermint oil,[54] n-butylscopolamine[54] and imipramine,[49] In this context, a liquid meal containing 10 g of lactulose in 100 mL of water is expected to give a mean oro-caecal transit time of about 80 min with a 51 min reproducibility coefficient; in these experimental conditions, 34 subjects will be needed to assess a 50% difference with a 0.80 power and 44 subjects with a 0.90 power.

CONCLUSIONS AND FUTURE PERSPECTIVES

Breath testing in the assessment of oro-caecal transit time is a safe, well-tolerated and non-invasive technique as office- and field-based test. However, the wide variation in the measurement of orocaecal transit in normal subjects and the poor reproducibility of the test, in particular with the liquid meal, have limited its applications to the clinical setting.

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H_2-breath testing for other gas-related syndromes

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INTRODUCTION

The 'gas-related syndrome' is defined as the presence of nonspecific abdominal symptoms (bloating, flatulence, abdominal distension and discomfort) attributed to an excess of abdominal gas by the patient.[1] Abdominal bloating is the most common among these symptoms and its prevalence ranges between 16% and 30% in the general population and up to 90% in patients with irritable bowel syndrome (IBS).[1, 2] Two-thirds of patients sent to tertiary care centres rate bloating as the most bothersome symptom[3] and IBS patients refer that bloating is present roughly 30% of the times.[4] Bloating is more frequently associated with female gender and constipation-predominant IBS (IBS-C) as compared with diarrhoea-predominant IBS (IBS-D).[5] It often gets worst in the evening and is exacerbated by meals.[6] Although visible abdominal distension is linked to bloating in roughly 50% of instances, they are not invariably associated, supporting the view that they are sustained by different, although likely overlapping, pathophysiological mechanisms. The association between abdominal bloating and distension is more commonly observed in IBS-C (72%) than in IBS-D (30%) patients.[5]

The mechanisms underlying bloating and distension are not fully understood; however, the current view suggests that multiple factors, such as increased gas production or decreased intraluminal gas consumption, abnormal gastrointestinal (GI) motility and gas excretion, visceral hypersensitivity abnormal abdominal wall muscular activity and psychological factors, in

different combinations, are involved in the origin of these symptoms.

Visceral hypersensitivity

Visceral hypersensitivity is characterized by a reduced threshold of perception of intestinal stimuli.[7] This phenomenon has been described to occur in 40–60% of patients with functional GI disorders and is currently considered one of the main mechanisms underlying the perception of pain and discomfort.[7] Agrawal *et al*. have recently explored the role of visceral hypersensitivity in the pathophysiology of abdominal bloating and distention. Abdominal girth was measured using abdominal inductance plethysmography and intestinal sensitivity was assessed with the aid of a barostat. These authors demonstrated that patients with visceral hyposensitivity were more likely to show abdominal distension, while those with hypersensitivity more frequently experienced abdominal bloating.[8] Although visceral hypersensitivity is generally evoked by mechanical stimuli applied to the bowel wall, a recent interesting study by Di Stefano *et al*.[9] suggests that a subgroup of IBS patients with bloating shows a selective chemical hypersensitivity to fermentable colonic products, which could underlie symptom generation in at least a subset of patients.

Stress, anxiety and psychological factor

Psychological factors are associated in roughly half of patients with IBS and other functional bowel disorders.[10] However, the role played by stress, anxiety, depression and somatization in the pathogenesis of abdominal bloating and distension has been poorly investigated. In general, the available studies suggest that psychological factors represent a secondary factor. Accordingly, patients do not correlate their symptoms with stress and CT scan studies show that patients do not increase dorsal lordosis neither lower diaphragm muscle on purpose to accentuate abdominal distension.[6]

Abnormal abdominal wall muscular activity

Recent studies have also been directed to the identification of abnormal abdominal anterior wall muscular activity, particularly in those patients who show measurable abdominal distension. Electromyography of abdominal skeletal muscles has demonstrated that patients with abdominal distention show a paradoxical relaxation of anterior wall muscles, which probably contributes to abdominal distention in patients with bloating. Although interesting, these observations need further confirmation.[11]

Intestinal gas production, fermentation and gas consumption

The amount of gas present within the GI lumen results from a number of different processes, ranging from the amount of swallowed gas, intraluminal production and/or consumption of volatile compounds (as a consequence of chemical reaction and the metabolic activities of host and flora) and bidirectional fluxes of gas through the intestinal wall.

Swallowed air (mainly O_2 and N_2) and gas originating from the reaction between gastric acid and intraduodenal bicarbonate (mainly CO_2) account for most of the gas present in the upper GI tract.[12, 13]

Diet represents the primary source of fermentable substrate, primarily in relation to its fibre content. The term 'dietary fibre', however, applies to a variety of different complex carbohydrates, sharing the characteristic of escaping digestion by human gut enzymes. Some related compounds, like sugar-alcohols (i.e. sorbitol) and lignine, are also comprised within the general term of 'dietary fibre'. However, fibre fermentability varies in relation to its chemical structure. Soluble fibre (found in large amounts in oat bran, beans, peas and most fruits) is readily fermented, and favours the intraluminal production of volatile compounds. In contrast, insoluble fibre contained in wheat bran and some vegetables is less readily fermented.

Similarly, the proportion of carbohydrate escaping absorption varies significantly in relation to the chemical structure of ingested oligosaccharides. Fructose is more efficiently absorbed as compared with lactose, whereas over half sorbitol escapes absorption in normal subjects when ingested at the dose of 20 g.[14, 15] In most geographical areas, lactose, found in milk and dairy products, represents one of the most common sources of indigested carbohydrates.

The relationship between diet and gas production is complex and not completely understood, but some general suggestions may help in identifying the dietary products responsible for increased gas production, and possibly symptoms, in at least a subgroup of patients complaining of gas-related symptoms. Onions, artichokes, pears, wheat and some soft drinks contain

fructose; apples, pears, peaches, prunes contain the sugar-alcohol sorbitol; beans, cabbage, brussels sprouts, broccoli, asparagus and whole grains contain raffinose. Some sugar-free foods, chewing gums and candies contain artificial sweeteners such as sorbitol and mannitol. In some patients, a reduced consumption of these foods may prove effective in limiting gas-related symptoms.

The amount of gas generated from fermentation processes is surprisingly high: 1 g of fermented carbohydrate results in about 700 mL gas '*in vitro*'. '*In vivo*', the figure is reduced to a mean value of 100–110 mL, in healthy volunteers, in relation to the utilization of gas by the metabolic activities of intraluminal bacteria and lumen-to-wall fluxes. The main role of bacterial metabolism in producing and/or reducing the volume of intraluminal gas is demonstrated by classical studies in normal and germ-free rats.[16, 17] Bacterial metabolism may lead to consumption of intraluminal gas, mainly H_2, by the activities of methanogenic flora (present in about 30 of people in western countries), acetogenic and sulphate-reducing bacteria.[18–21]

Intraluminal gas content and gas-related symptoms

The use of argon washout techniques and indirect measurements has demonstrated that, irrespective of gas composition, about 200 mL of gas are present within the GI tract of normal subjects. This content is the result of a dynamic phenomenon resulting from about 700 mL (470–1500) of gas excretion every day in 10–20 bowel actions.[21–23] However, available data indicate a large variability within the normal population and at different times in the same individual, mainly in relation to diet.

The relationship between gas intestinal content and symptom generation is controversial. Only in a subset of patients, clear and measurable increased gas content is responsible for symptom generation. This applies particularly to those patients complaining of flatulence.[24] Whether excessive intestinal gas production (and/or content) is responsible for abdominal bloating in patients with functional bowel disorders remains a matter of debate. This uncertainty is linked to the limited number of available studies, their small size, heterogeneity of patient populations as well as methodological limitations. The wash-out technique showed that, on average, IBS patients have gas volumes similar to those of healthy controls.[22, 25, 26] In contrast, some studies based on intestinal gas quantification with plain radiography have evidenced increased gas content in IBS compared to controls,[27, 28] although this has not been invariably confirmed.[6] Also, the study by King *et al.*[29] showed, with calorimetry, highly increased H_2- and CH_4-breath excretion in IBS patients compared to that in controls. Unfortunately, the study was carried out in six IBS patients only and, consequently, it requires confirmation.

Using gas infusion techniques, the Barcelona group demonstrated that patients complaining of abdominal bloating are characterized by altered transit and retention of gas within the intestinal lumen.[25, 26, 30] Salvioli *et al.* showed that the small bowel is the main intestinal segment responsible for gas retention.[31] Furthermore, gas retention in patients with IBS can be exacerbated by intestinal lipid infusion, which correlates well with the recognized exacerbation of meal induced bloating.[32] However, the correlation between the volume of retained gas and the severity of abdominal bloating was poor, suggesting that other factors, such as visceral hypersensitivity, abnormal abdominal wall muscular activity and psychological factors, most likely contribute to symptom generation.

Carbohydrate malabsorption, gas production and gas-related symptoms in GI tract disease

Carbohydrate intolerance (e.g. lactose, fructose and sorbitol) is rather common in patients with functional bowel disorders but usually no more common than in the general population[33] (Table 1). In 1972, Bond and Levitt[34]suggested that measurement of the area under the H_2 excretion curve (AUC), minus the extrapolated baseline H_2 excretion, could adequately represent the cumulative amount of H_2 excreted with breath after oral lactulose administration. In Table 2 and 3, data on AUC in different diseases are reported, showing that in the majority of the available papers, AUC calculated in patients was significantly higher than in healthy volunteers.[16, 35–50] Although previous studies showed that the correlation between carbohydrate malabsorption and symptoms is poor,[51–54] in the only three papers[16, 49, 50] in which the relationship between increased breath hydrogen excretion and symptoms was evaluated, the correlation was present. In these studies, the administration of the potentially absorbable substrates sorbitol,[16, 39, 40] glucose[46–50] and xylose,[41–43] raises some questions on the interpretation of data, as the amount of substrate load actually reaching the colon remains unknown. Thus, the

Table 1. Gas production in irritable bowel syndrome

Author	Country	Nr pts	Positive HBT		P	Symptoms after challenge	P	Improvement after diet (%)
			IBS	Controls				
Bozzani 1986[34]	Italy	40	88%	62	<0.02	–	–	53
Tolliver 1994[35]	USA	196	26%	–	–	–	–	–
Bohmer 1996[36]	Netherlands	70	24%	6	<0.01	–	–	–
Tolliver 1996[37]	USA	161	29%	–	–	–	–	83
Vernia2001[38]	Italy	337	67%*	71%**	n.s.	–	–	–
Vernia 2004[39]	Italy	475	76%***	69%****	n.s.	43 / 41	n.s.	30/65
Hamm 1999[40]	USA	1452	23	––	–	–	–	–
Farup 2004[41]	Norway	82	4	4	n.s.	38 / 20	<0.01	–
Gupta 2007[42]	India	124	72	60	n.s.	55 / 34	<0.04	–

HBT, H2-breath test; IBS, irritable bowel syndrome.
*IBS, **Self-reported milk intolerance, *** milk intolerants, ****milk consumers
Nr pts: number of patents included in the study

Table 2. Intestinal gas production and celiac disease

	Duration (h)	Dose (g)	Parameter	↑ Gas	Symptoms
Fasting					
Nunes *et al.*[35]	–	–	Basal	Y	NA
Corazza *et al.*[36]	–	–	Basal	N	NA
Di Stefano *et al.*[37]	9	–	AUC	Y	NA
Lactulose					
Di Stefano *et al.*[38]	4	10	AUC	N	NA
Di Stefano *et al.*[37]	9	10	AUC	N	NA
Sorbitol					
Corazza *et al.*[16]	4	5	Peak	Y	Pos
Tursi *et al.*[39]	4	5	Peak	Y	NA
Tursi *et al.*[40]	4	5	Peak/AUC	Y	NA
Xylose					
Casellas *et al.*[41]	5	25	AUC	Y	NA
Casellas *et al.*[42]	5	25	AUC	N	NA
Casellas *et al.*[43]	5	25	AUC	Y	NA

N, not increased gas content; Y, increased gas content; Pos, positive correlation between gas and symptoms.

measured differences in breath H_2 excretion could result from differing amounts of the fermented substrate. Similarly, data from papers adopting protocols based on lactulose administration,[37, 38] in which the amount of fermented substrate is known, do not provide information on the relationship between gas excretion/production and symptoms.

Finally, the primary aim of all the papers reported in Tables 2 and 3 was not to quantify cumulative breath H_2 excretion after carbohydrate load, nor to

Table 3. Intestinal gas production and small bowel bacterial overgrowth

	Duration (h)	Dose (g)	Parameter	↑ Gas	Symptoms
Fasting					
Corazza *et al.*[44]	–	–	Basal	Y	NA
Riordan *et al.*[45]	–	–	Basal	N	NA
Lactulose					
No available study					
Glucose					
Yang *et al.*[46]	2	50	Fasting/peak	Y	NA
Attar *et al.*[47]	2	50	AUC	Y	NA
Stotzer *et al.*[48]	2	50	Peak	Y	NA
Di Stefano *et al.*[49]	2	50	AUC	Y	Pos
Di Stefano *et al.*[50]	2	50	AUC	Y	Pos

N, not increased gas content; Y, increased gas content; Pos, positive correlation between gas and symptoms.

investigate the relationship between gas production/excretion and abdominal symptoms. Thus, any attempt to derive reliable information on the accuracy of breath test in gas-related symptoms in GI tract diseases is at best debatable.

In conclusion, the role of intraluminal gas in the pathophysiology of functional symptoms is still a matter of debate. The precise measurement of intraluminal gas, both with direct and indirect techniques, represents a difficult task. Accordingly, more data are needed to draw definitive conclusions. As far as hydrogen breath tests are concerned, at present no test, and no substrate, proved effective for measuring intraluminal gas content. Future research should be aimed at providing more information on this topic.

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Methodology and indications of H_2-breath testing in gastrointestinal diseases: final statements from the Ist Rome Consensus Conference

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I. H_2-breath testing: methodology in adults and children

Improving the accuracy of the hydrogen breath test must be based on a correct test protocol. The following text reports the available evidence in terms of methodological aspects.

Instruments for hydrogen measurement in breath

Breath hydrogen measurement is performed with stationary or portable analysers. For stationary analysers, which represent the gold standard, validation data together with the analysis of linearity and reproducibility of results are available.[1–3] As far as portable instruments are concerned,[4–6] although acceptable linearity has been demonstrated[6, 7] no data are available on reproducibility or long-term stability of the electrochemical cell, which is characterized by a relatively short life.

STATEMENT

All stationary analysers are characterized by good accuracy

Level of evidence	I
Strength of recommendation	A

Comment

The lack of data on stability of electrochemical cells suggests the need for periodic evaluation of measurement accuracy by the owners.

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Devices for breath sampling

The aim of breath sampling is the collection of alveolar air. Hence, an efficient device must be able to exclude expired air from dead space. The Haldane-Priestley tube, the Y-piece device, and the two-bag system allow correct sampling and comparison of the efficacy of these three systems did not show any significant difference.[1–3]

For paediatric patients, if they are able to cooperate, the same systems as adults can be used. On the contrary, in noncooperating subjects, breath samples may be collected invasively with the help of nasal probes. A non-invasive alternative is represented by a mask with a respiratory detector.

STATEMENT

Hydrogen measurements on two-bag system, Y-piece device and modified Haldane-Priestley tube do not differ

Level of evidence	I
Strength of recommendation	B

STATEMENT

The most accurate system for breath sampling in noncooperating paediatric patients is represented by a facial mask with an automatic detector of end-expiratory phase

Level of evidence	I
Strength of recommendation	B

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Breath sampling: the role of patients

In alveolar air, CO_2 levels are very constant around 5%; this parameter may therefore be considered as a marker of correct sampling.[1] Comparison of CO_2, H_2 and CH_4 levels in breath samples obtained through four different ways of breathing showed that a maximal inspiration followed by a 15-s period of apnoea and a prolonged expiration is characterized by good reproducibility.[2, 3]

STATEMENT

The best method for correct breath sampling in a cooperating patient is represented by a maximal inspiration followed by a 15-s period of apnoea and a prolonged expiration

Level of evidence	I
Strength of recommendation	B

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Storage of breath samples

The storage of samples from collection to measurement may represent another cause of incorrect sampling management. Previously suggested systems may be too expensive or even inaccurate[1–3] and the adoption of 60-mL plastic syringes seems adequate. However, the long-term stability of the sample is a crucial point and only the storage at −20 °C does guarantee that the initial concentrations of hydrogen and methane persist beyond 6 h[4, 5].

STATEMENT

Breath sample is stable for 6 h at room temperature; hence, gas measurement must be performed within 6 h from collection. If measurement is delayed, storage at −20 °C is needed

Level of evidence	I
Strength of recommendation	B

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Antibiotics

The physiology of hydrogen breath test implies that colon harbours a normal flora. Previous antibiotic administration may alter colon flora and induce false results, being able to both reduce[1] and increase[2] breath hydrogen excretion. Hence a test delay is recommended.

STATEMENT

Antibiotics modify breath hydrogen excretion

Level of evidence	I
Strength of recommendation	B

Comment

No information is available as yet on the time needed for recovery of bacterial flora. It is conceivable to wait for a 4-week period. Similarly, but without any scientific proof, it is advisable to adopt the same approach for prokinetics and probiotics.

REFERENCES

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2 Strocchi A, Corazza GR, Ellis C, Gasbarrini G, Levitt MD. Detection of malabsorption of low doses of carbohydrate: accuracy of various breath H2 criteria. *Gastroenterology* 1993; **105**: 1404–1410.

Laxatives and colonic clearing

Laxatives and solutions for colonic clearing may interfere with the stability of colonic flora. In fact, it was shown that colonic clearing before colonoscopy modifies breath hydrogen excretion.[1] A delay is therefore useful after colonic clearing procedures.

STATEMENT

Colonic clearing before endoscopic and radiological tests or surgery modifies breath hydrogen excretion

Level of evidence	I
Strength of recommendation	B

Comment

No information is available as yet on the time needed for bacterial flora recovery. It is conceivable to wait for a 4-week period.

REFERENCE

1 Gilat T, BenHur H, Gelman Malachi E, Terdiman R, Peled Y. Alterations of the colonic flora and their effect on the hydrogen breath test. *Gut* 1978; **19**: 602–605.

Diet

Non-absorbed carbohydrates are fermented in the colon. The persistence at the colonic level of non-absorbed carbohydrates, previously ingested with the diet, represents a confusing factor. Hence, a restricted diet, containing only rice and meat is suggested.[1, 2] Such a diet has proved to be effective in maintaining low levels of fasting breath hydrogen excretion.[3]

STATEMENT

A restricted diet, free of non-absorbable carbohydrates, the evening before the test maintains low levels of fasting breath hydrogen excretion

Level of evidence	I
Strength of recommendation	A

Comment

On the basis of results of the same papers, prescription of an overnight fast seems appropriate. It is conceivable that coffee, tea, milk and jam modify colonic intraluminal microenvironment or motility. However, as yet we have no data on the effect of breakfast on breath hydrogen excretion.

REFERENCES

1 Anderson IH, Levine AS, Levitt MD. Incomplete absorption of the carbohydrate in all-purpose wheat flour. *NEJM* 1981; **304**: 891–892.

2 Levitt MD, Hirsh P, Fetzer CA, Sheahan M, Levine AS. H_2 excretion after ingestion of complex carbohydrates. *Gastroenterology* 1987; **92**: 383–389.

3 Di Stefano M, Miceli E, Missanelli A, Malservisi S, Strocchi A, Corazza GR. Fermentation of endogenous substrates is responsible for increased fasting breath hydrogen levels in celiac disease. *J Lab Clin Med* 2004; **143**: 163–168.

Cigarette smoking

Cigarette smoking interferes with breath hydrogen excretion.[1–3] Breath hydrogen shows a rapid increase during smoking and a similar rapid decline after the end of the cigarette, but a recovery of basal values is not evident, breath concentration still being 100% higher than basal values after 15 min. No data on the time needed for complete normalization of breath excretion after cigarette smoking are available.

STATEMENT

Cigarette smoking modifies breath hydrogen excretion and must be avoided before and during the test

Level of evidence	I
Strength of recommendation	B

REFERENCES

1 Tadesse K, Eastwood M. Breath hydrogen test and smoking. *Lancet* 1977; **ii**: 91.

2 Thompson DG, Binfield P, DeBelder A, O'Brien J, Warren S, Wilson M. Extraintestinal influences on exhaled breath hydrogen measurements during the investigation of gastrointestinal disease. *Gut* 1985; **26**: 1349–1352.

3 Rosenthal A, Solomons NW. Time-course of cigarette smoke contamination of clinical hydrogen breath-analysis tests. *Clin Chem* 1983; **29**: 1980–1981.

Mouthwashing

Bacterial flora of the oral cavity may ferment orally administered carbohydrate, interfering with measurement of colonic hydrogen production.[1, 2] Mouthwashing with a sucrose solution increases breath hydrogen excretion within 10 min[1] and mouthwashing with a chlorexidine solution prevents this increase.[2]

STATEMENT

Mouthwashing with chlorexidine solution before test substrate administration prevents oral fermentation of the substrate by bacterial flora of the oral cavity

Level of evidence	I
Strength of recommendation	B

REFERENCES

1 Thompson DG, Binfield P, DeBelder A, O'Brien J, Warren S, Wilson M. Extraintestinal influences on exhaled breath hydrogen measurements during the investigation of gastrointestinal disease. *Gut* 1985; **26**: 1349–1352.

2 Mastropaolo G, Rees WD. Evaluation of the hydrogen breath test in man: definition and elimination of the early hydrogen peak. *Gut* 1987; **28**: 721–725.

Hyperventilation and physical exercise

Breath hydrogen excretion is modified by respiratory frequency[1] and a reduction in breath hydrogen excretion during hyperventilation is evident. Accordingly, breath hydrogen excretion is reduced during physical exercise and increases during the recovery phase.[2]

STATEMENT

Hyperventilation interferes with breath hydrogen excretion. Accordingly, during the test, patients must be at rest

Level of evidence	I
Strength of recommendation	B

REFERENCES

1 Perman JA, Modler S, Engel RR, Heldt G. Effect of ventilation on breath hydrogen measurements. *J Lab Clin Med* 1985; **105**: 436–439.

2 Payne DL, Welsh JD, Claypool PL. Breath hydrogen response to carbohydrate malabsorption after exercise. *J Lab Clin Med* 1983; **102**: 147–150.

Breath methane excretion

Methane production represents the main intraluminal pathway for hydrogen consumption and it is detectable in patients affected by various conditions, both benign and malignant, and also in healthy volunteers.

Breath methane excretion might improve the diagnostic accuracy of breath test in hydrogen nonproducers, representing an alternative gaseous marker. Unfortunately, neither paediatric nor adult studies have produced conclusive suggestions.[1–7]

STATEMENT

Measurement of breath methane excretion is not currently recommended to improve the diagnostic accuracy of the hydrogen breath test

Level of evidence	IIa
Strength of recommendation	B

REFERENCES

1 Myo-Khin, Bolin TD, Khin-Mar-Oo, Tin-Oo, Kyaw-Hla S, Thein-Myint T. Ineffectiveness of breath methane excretion as a diagnostic test for lactose malabsorption. *J Pediatr Gastroenterol Nutr* 1999; **28**: 474–479.

2 Corazza GR, Benati G, Strocchi A, Malservisi S, Gasbarrini G. The possible role of breath methane measurement in detecting carbohydrate malabsorption. *J Lab Clin Med* 1994; **124**: 695–700.

3 Vernia P, Camillo MD, Marinaro V, Caprilli R. Effect of predominant methanogenic flora on the outcome of lactose breath test in irritable bowel syndrome patients. *Eur J Clin Nutr* 2003; **57**: 1116–1119.

4 Bjørneklett A, Jenssen E. Relationships between hydrogen (H2) and methane (CH4) production in man. *Scand J Gastroenterol* 1982; **17**: 985–992.

5 Cloarec D, Bornet F, Gouilloud S, Barry JL, Salim B, Galmiche JP. Breath hydrogen response to lactulose in healthy subjects: *relationship to methane producing status. Gut* 1990; **31**: 300–304.

6 Kajs TM, Fitzgerald JA, Buckner RY, *et al.* Influence of a methanogenic flora on the breath H_2 and symptom response to ingestion of sorbitol or oat fiber. *Am J Gastroenterol* 1997; **92**: 89–94.

7 Rumessen JJ, Nordgaard-Andersen I, Gudmand-Høyer E. Carbohydrate malabsorption: quantification by methane and hydrogen breath tests. *Scand J Gastroenterol* 1994; **29**: 826–32.

II. H_2-breath testing for sugar malabsorption

(A)
Lactose malabsorption: clinical indications of lactose hydrogen-breath test

The determination of lactase activity in jejunal biopsy is currently considered the gold standard for lactose malabsorption[1, 2]. However, its results can be influenced by the irregular dissemination of lactase activity throughout the small intestine mucosa[1]. On the basis of literature review, the lactose breath test is a reliable, non-invasive technique, which is provided with good sensitivity and optimal specificity[1–5].

STATEMENT

Although an unequivocal reference test for lactose malabsorption is not available, breath testing is recommended to evaluate this clinical condition in both adult and pediatric subjects

Level of evidence	I
Strength of recommendation	A

REFERENCES

1 Newcomer AD, McGill DB, Thomas PJ, *et al.* Prospective comparison of indirect methods for detecting lactase deficiency. *N Engl J Med* 1975; **293**: 1232–1235, 1975.

2 Hiele M, Ghoos Y, Rutgeerts P, *et al.* 13CO2 breath test using naturally 13C-enriched lactose for detection of lactase deficiency in patients with gastrointestinal symptoms. *J Lab Clin Med* 1988; **112**: 193–200.

3 Strocchi A, Corazza GR, Anania C. Quality control study of H2 breath testing for the diagnosis of carbohydrate malabsorption in Italy. *Ital J Gastroenterol Hepatol* 1997; **29**: 122–127.

4 Solomons NW, Barillas C, *et al.* The cut-off criterion for a positive hydrogen breath *test in children: a reappraisal. J Pediatr Gastroenterol Nutr* 1986; **5**: 920–5.

5 Koetse HA, Stellaard F, Bijleveld CM, *et al.* Non-invasive detection of low-intestinal lactase activity in children by use of a combined 13CO2/H2 breath test. *Scand J Gastroenterol* 1999; **34**: 35–40.

Methodological aspects in adult subjects

Most of the published reports on breath test (BT) validation have utilized the dosage of 50-g lactose[1–3]. Nevertheless, this dose has been criticized because it represents an amount far more elevated than that usually ingested at once. Twenty and twenty-five grams of lactose represent the most widely utilized dosages as they are closer to physiological habits. Comparative studies between 20 and 25 g were never performed. Based on a methodological assessment of quality of available studies and more recent reviews on BT, 25 g represent the most used dosage and now recommended dosage[4–8].

STATEMENTS

Fifty grams of lactose is the most standardized substrate

Level of evidence	I
Strength of recommendation	A

The most physiological dosage of 25 g of lactose in a 10% water solution, is recommended in clinical practice

Level of evidence	IIa
Strength of recommendation	B

REFERENCES

1 Metz G, Jenkins DT, Peters TJ, *et al.* Breath hydrogen as a diagnostic method for hypolactasia. *Lancet* 1975; **24**: 1155–7.
2 Bodlaj G, Stöcher M, Hufnagl P, *et al.* Genotyping of the lactase-phlorizin hydrolase – 13910 polymorphism by LightCycler PCR and implications for the diagnosis of lactose intolerance. *Clin Chem* 2006; **52**: 148–151.
3 Szilagyi A, Malolepszy P, Hamard E, *et al.* Comparison of a real-time polymerase chain reaction assay for lactase genetic polymorphism with standard indirect tests for lactose maldigestion. *Clin Gastroenterol Hepatol* 2007; **5**: 192–6.
4 Romagnuolo J, Shiller D, Bayley RJ. Using breath tests wisely in a gastroenterology practice: an evidence-based review of indications and pitfalls in interpretation. *Am J Gastroenterol* 2002; **97**: 1113–1126.
5 Simren M, Stotzer PO. Use and abuse of hydrogen breath tests. *Gut* 2006; **55**: 297–303.
6 Saad RJ, Chevy WD. Breath tests for gastrointestinal disease: the real deal or just a lot of hot air? *Gastroenterol* 2007; **133**: 1763–1766.
7 Carroccio A, Montalto G, Cavera G, *et al.* Lactose intolerance and self-reported milk intolerance: relationship with lactose maldigestion and nutrient intake. Lactase Deficiency Study Group. *J Am Coll Nutr* 1998; **17**: 631–6.
8 Buchowski MS, Semenya J, Johnson AO. Dietary calcium intake in lactose maldigesting intolerant and tolerant African-American women. *J Am Coll Nutr* 2002; **21**: 45–54.

As for the substrate, validation studies have generally preferred lactose solutions. Milk represents a more physiological substrate than lactose[1]; nevertheless, until, it has not been sufficiently standardized[2, 3].

STATEMENT

No evidence shows that milk represents a better substrate than lactose

Level of evidence	IIb
Strength of recommendation	C

REFERENCES

1 Arrigoni E, Rainnie DG, McCarley RW, *et al.* Tolerance and absorption of lactose from milk and yogurt during short-bowel syndrome in humans. *Am J Clin Nutr* 1994; **60**: 926–9.
2 Rao SS, Ozturk R, Laine R, *et al.* Prevalence of lactose maldigestion. Influence and interaction of age, race, and sex. *Dig Dis Sci* 1994; **39**: 1519–24.
3 Paige DM, Witter FR, Bronner YL, *et al.* Lactose digestion in pregnant African-Americans. *Public Health Nutr* 2003; **6**: 801–7.

To perform breath test (BT), the best evidence suggests a duration of 4 h, sampling every 30 min and a cut-off of 20 ppm[1–3]. A cut-off of 10 ppm probably increases the sensitivity, while the specificity can worsen[4]. A lower duration reduces BT sensitivity[5, 6].

STATEMENT

A cut-off of 20 ppm over the baseline, sampling every 30 min over a 4-h period are recommended for diagnosing lactose malabsorption

Level of evidence	I
Strength of recommendation	A

REFERENCES

1 Brummer RJ, *et al.* Lactose malabsorption. Optimalization of investigational methods. *Scand J Gastroenterol Suppl* 1993; **200**: 65–9.
2 Meloni GF, Colombo C, La Vecchia C, *et al.* High prevalence of lactose absorbers in Northern Sardinia patients with type 1 and type 2 diabetes mellitus. *Am J Clin Nutr* 2001; **73**: 582–5.
3 Vernia P, Di Camillo M, Marinaro V. Lactose malabsorption, irritable bowel syndrome and self-reported milk intolerance. *Dig Liv Dis* 2001; **330**: 234–9.
4 Strocchi A, *et al.* Quality control study of H2 breath testing for the diagnosis of carbohydrate malabsorption in Italy. *Ital J Gastroenterol Hepatol* 1997; **29**: 122–127.
5 Casellas F, *et al.* Applicability of short hydrogen breath test for screening of lactose malabsorption. *Dig Dis Sci* 2003; **48**: 1333–8.
6 Di Camillo M, Witter FR, Bronner Y, *et al.* Hydrogen breath test for diagnosis of lactose malabsorption: the importance of timing and the number of breath samples *Can J Gastroenterol* 2006; **20**: 265–8.

About 15 years ago, an alternative simplified method was proposed[1–4]. Although this procedure seems to show a better sensitivity, few data are available regarding the adoption of these new criteria in clinical practice.

STATEMENT

The determination of an absolute value of hydrogen excretion greater than 6 ppm at hour 6 is a diagnostic alternative with less scientific evidence

Level of evidence	IIb
Strength of recommendation	B

REFERENCES

1 Strocchi A, Corazza G, Ellis CJ, *et al.* Detection of malabsorption of low doses of carbohydrate: accuracy of various breath H2 criteria. *Gastroenterology* 1993; **105**: 1404–10.
2 Strocchi A, Corazza GR, Anania C, *et al.* Quality control study of H2 breath testing for the diagnosis of carbohydrate malabsorption in Italy. *Ital J Gastroenterol Hepatol* 1997; **29**: 122–127.
3 Di Stefano M, Missanelli A, Miceli E, *et al.* Hydrogen breath test in the diagnosis of lactose malabsorption: accuracy of new versus conventional criteria. *J Lab Clin Med* 2004; **144**: 313–8.
4 Di Stefano M, Veneto G, Malserviti S, *et al.* Lactose malabsorption and intolerance in the elderly. *Scan J Gastroent* 2001; **36**: 1274–1278.

Methodological aspects in paediatric subjects

With a lesser number of articles available, the dose of 1 g/kg is the mostly utilized in paediatric studies and it is confirmed to be closer to the physiological amount[1–3].

STATEMENT

The lactose dosage of 1 g/kg up to maximum 25 g in a 10% water solution is recommended in clinical practice

Level of evidence	IIa
Strength of recommendation	A

REFERENCES

1 Solomons NW, Barillas C. The cut-off criterion for a positive hydrogen breath test in children: a reappraisal. *J Pediatr Gastroenterol Nutr* 1986; **5**: 920–5.

2 Tadesse K, Leung DT, Yven RC. The status of lactose absorption in Hong Kong Chinese children. *Acta Paediatr* 1992; **81**: 598.

3 Webster RB, DiPalma JA, Gremse DA. Lactose maldigestion and recurrent abdominal pain in children. *Dig Dis Sci* 1995; **40**: 1506.

Regarding the substrate, like in adults, milk has not been sufficiently standardized[1, 2]; milk represents a more physiological substrate; nevertheless, it has been reported that about 2–3% of children suffer from allergy to milk proteins[2].

STATEMENT

The use of milk as a substrate is not recommended

Level of evidence	IIb
Strength of recommendation	C

REFERENCES

1 Høst A. Frequency of cow's milk allergy in childhood. *Ann Allergy Asthma Immunol* 2002; **89**: 33–7.

2 Heine RG, Elsayed S, Hosking CS. Cow's milk allergy in infancy. *Curr Opin Allergy Clin Immunol* 2002; **2**: 217–25.

The definition of a positive test in children is similar to that in adults. The duration is shorter in consideration of a different paediatric gut transit time[1–3].

STATEMENT

A cut-off of 20 ppm over the baseline, a sample every 30 min over a 3-h period are recommended for diagnosing lactose malabsorption

Level of evidence	I
Strength of recommendation	B

REFERENCES

1 Rosado JL, Solomons NW. Sensitivity and specificity of the hydrogen breath-analysis test for detecting malabsorption of physiological doses of lactose. *Clin Chem* 1983; **29**: 545–8.

2 Solomons NW, Barillas S. The cut-off criterion for a positive hydrogen breath test in children: a reappraisal. *J Pediatr Gastroenterol Nutr* 1986; **5**: 920–5.

3 Tadesse K, Leung DT, Yuen RC. The status of lactose absorption in Hong Kong Chinese children. *Acta Paediatr* 1992; **81**: 598–600.

Symptom indications

Different methodologies have been applied for symptoms evaluation in lactose intolerance; however, an univocal one is not available yet[1–3]. We propose a new method based on symptom evaluation symptoms in the 12 h after substrate ingestion, by a visuo-analogue scale.

STATEMENT

It is useful to evaluate the onset and severity of symptoms (abdominal pain, meteorism, flatulence and diarrhoea) during the test and 8 h after, to determine lactose intolerance both in adults and in children

Level of evidence	IIb
Strength of recommendation	B

REFERENCES

1 Suarez FL, Savaiano D, Arbisi P, *et al.*. Tolerance to the daily ingestion of two cups of milk by individuals claiming lactose intolerance. *Am J Clin Nutr* 1997; **65**: 1502–6.

2 Vernia P, Di Camillo M, Marinaro V, *et al.* Lactose malabsorption, irritable bowel syndrome and self-reported milk intolerance. *Dig Liver Dis* 2001; **33**: 234–9.

3 Di Stefano M, Veneto G, Malservisi S, *et al.* Lactose malabsorption and intolerance in the elderly. *Scand J Gastroenterol* 2001; **36**: 1274–8.

(B) H_2-breath testing for other sugars malabsorption

No gold standard is available for diagnosis of fructose and sorbitol malabsorption. The diagnostic procedure of BTs is not standardized and the clinical impact remained unclear[1–4].

STATEMENT

Fructose and sorbitol breath tests are not recommended in clinical practice

Level of evidence	III
Strength of recommendation	C

REFERENCES

1 Corazza GR. Sorbitol malabsorption in normal volunteers and in patients with coeliac disease. *Gut* 1998; **29**: 44–8.

2 Tursi A. Sorbitol H2-breath test versus anti-endomysium antibodies to assess histological recovery after gluten-free diet in coeliac disease. *Dig Liv Dis* 2002; **34**: 846–50.

3 Choi YK. Fructose intolerance: an underrecognized problem. *Am J Gastroenterol* 2003; **98**: 1348–53.

4 Rao SS. Ability of the normal human small intestine to absorb fructose: evaluation by breath testing. *Clin Gastr Hepatol* 2007; **5**: 959–63.

III. H_2-Breath testing for small intestine bacterial overgrowth

Small intestine bacterial overgrowth (SIBO) is traditionally defined as the microbiological presence of at least 10^5 colony-forming units (CFU) per millilitre of jejunal aspirate. Consequently, patient intubation, aspiration and culture of enteric juice from the small intestine are required for SIBO diagnosis[1–3]. However, non-invasive tests measuring the concentrations of bacterial metabolism products in plasma, urine or expired air (breath tests) are now available[4, 5].

Diagnostic tests for SIBO

STATEMENT

The jejunal aspirate culture is traditionally considered the gold standard diagnostic test for SIBO, despite some serious methodological limitations and lack of accessibility to clinical practice.

Level of evidence	IIA
Strength of recommendation	B

REFERENCES

1 Bouhnik Y, Alain S, Attar A, *et al*. Bacterial populations contaminating the upper gut in patients with small intestinal bacterial overgrowth syndrome. *Am J Gastroenterol* 1999; **94**: 1327–31.
2 Bardhan PK, Gyr K, Beglinger C, *et al*. Diagnosis of bacterial overgrowth after culturing proximal small-bowel aspirate obtained during routine upper gastrointestinal endoscopy. *Scand J Gastroenterol* 1992; **27**: 253–6.
3 Husebye E. The pathogenesis of gastrointestinal bacterial overgrowth. *Chemotherapy* 2005; **51**(Suppl 1): 1–22.
4 Kerlin P, Wong L. Breath hydrogen testing in bacterial overgrowth of the small intestine. *Gastroenterology* 1988; **95**: 982–8.
5 Corazza GR, Menozzi MG, Strocchi A, *et al*. The diagnosis of small bowel bacterial overgrowth. Reliability of jejunal culture and inadequacy of breath hydrogen testing. *Gastroenterology* 1990; **98**: 302–9.

Role of glucose and lactulose breath tests for small intestine bacterial overgrowth

Glucose and lactulose are the most frequently used substrates in investigating SIBO[1–5]. Both sugars are fermented by intestinal bacteria contaminating small bowel, resulting in hydrogen production. Several studies have been assessed to establish the diagnostic accuracy for both breath tests vs. the culture of jejunal aspirate[2, 3]. It has been shown that H2 glucose breath test (GBT) has a greater diagnostic accuracy than H2 lactulose breath test (LBT)[2–5].

STATEMENT

Glucose Breath Test is the most accurate hydrogen breath test for non-invasive diagnosis of SIBO.

Level of evidence	IIA
Strength of recommendation	B

REFERENCES

1 King CE, Toskes PP. Comparison of the 1-gram [14C]xylose, 10-gram lactulose-H2, and 80-gram glucose-H2 breath tests in patients with small intestine bacterial overgrowth. *Gastroenterolog* 1986; **91**: 1447–51.
2 Kerlin P, Wong L. Breath hydrogen testing in bacterial overgrowth of the small intestine. *Gastroenterology* 1988; **95**: 982–8.
3 Corazza GR, Menozzi MG, Strocchi A, *et al*. The diagnosis of small bowel bacterial overgrowth. Reliability of jejunal culture and inadequacy of breath hydrogen testing. *Gastroenterology* 1990; **98**: 302–9.
4 Riordan SM, McIver CJ, Walker BM, *et al*. The lactulose breath hydrogen test and small intestinal bacterial overgrowth. *Am J Gastroenterol* 1996; **91**: 1795–803.
5 Stotzer PO, Kilander AF. Comparison of the 1-gram (14)C-D-xylose breath test and the 50-gram hydrogen glucose breath test for diagnosis of small intestinal bacterial overgrowth. *Digestion* 2000; **61**: 165–71.

Methodological aspects

The diagnostic accuracy of GBT is influenced by several factors including the dose and concentration of substrate, the length of the test, sampling intervals and positivity criteria[1–5]. As these parameters are heterogeneously set in most studies, there is a need to standardize the protocol for GBT performance.

STATEMENT

GBT protocol ensuring the best diagnostic accuracy in adults is:	
Dose:	*50g/250 mL*
Duration:	*120 minutes*
Sampling intervals:	*15 min*
Cut-off:	*12 ppm compared to baseline*
Level of evidence	IIA
Strength of recommendation	B

REFERENCES

1 King CE, Toskes PP. Comparison of the 1-gram [14C]xylose, 10-gram lactulose-H2, and 80-gram glucose-H2 breath tests in patients with small intestine bacterial overgrowth. *Gastroenterolog* 1986; **91**: 1447–51.

2 Kerlin P, Wong L. Breath hydrogen testing in bacterial overgrowth of the small intestine. *Gastroenterology* 1988; **95**: 982–8.

3 Corazza GR, Menozzi MG, Strocchi A, *et al.* The diagnosis of small bowel bacterial overgrowth. Reliability of jejunal culture and inadequacy of breath hydrogen testing. *Gastroenterology* 1990; **98**: 302–9.

4 Stotzer PO, Kilander AF. Comparison of the 1-gram (14)C-D-xylose breath test and the 50-gram hydrogen glucose breath test for diagnosis of small intestinal bacterial overgrowth. *Digestion* 2000; **61**: 165–71.

5 Bauer TM, Schwacha H, Steinbruckner B, *et al.* Diagnosis of small intestinal bacterial overgrowth in patients with cirrhosis of the liver: poor performance of the glucose breath hydrogen test. *J Hepatol* 2000; **33**: 382–6.

Clinical indications of H_2-breath test for SIBO diagnosis

Homeostasis between the intestinal flora and the host is continuously ensured by several factors including intestinal motility, gastric acid secretion and immune system modulation[1, 2]. Several gastrointestinal and systemic disorders altering these mechanisms can predispose patients to bacterial contamination of the small intestine[3–5].

STATEMENT

GBT is indicated in symptomatic patients with predisposing conditions to SIBO.	
Level of evidence	IIA
Strength of recommendation	B

REFERENCES

1 Husebye E. The pathogenesis of gastrointestinal bacterial overgrowth. *Chemotherapy* 2005; **51**(Suppl 1): 1–22.

2 Laine L, Ahnen D, McClain C, *et al.* Review article: potential gastrointestinal effects of long-term acid suppression with proton pump inhibitors. *Aliment Pharmacol Ther* 2000; **14**: 651–68.

3 Lock G, Holstege A, Lang B, Schölmerich J. Gastrointestinal manifestations of progressive systemic sclerosis. *Am J Gastroenterol* 1997; **92**: 763–71.

4 Cuoco L, Montalto M, Jorizzo RA, *et al.* Eradication of small intestinal bacterial overgrowth and oro-cecal transit in diabetics. *Hepatogastroenterology* 2002; **49**: 1582–6.

5 Pignata C, Budillon G, Monaco G, *et al.* Jejunal bacterial overgrowth and intestinal permeability in children with immunodeficiency syndromes. *Gut* 1990; **31**: 879–82.

Utility of H_2-Breath tests in IBS patients

The role played by intestinal bacterial flora and especially by SIBO in the pathogenesis of irritable bowel syndrome (IBS) is still controversial. Although it is likely that the intestinal bacterial flora can be involved[1–3], the lack of uniformity of available data makes it impossible to identify clearly a correlation between IBS and SIBO[4, 5].

STATEMENT

There is no conclusive evidence for the utility of breath tests for SIBO in IBS patients.

Level of evidence	IIA
Strength of recommendation	B

REFERENCES

1 Pimentel M, Chow E, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms in irritable bowel syndrome. *Am J Gastroenterol* 2000; **95**: 3503–3506.
2 Pimentel M, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome: a double blind, randomized controlled study. *Am J Gastroenterol* 2003; **98**: 412–419.
3 Lupascu A, Gabrielli M, Lauritano EC, *et al.* Hydrogen glucose breath test to detect small intestinal bacterial overgrowth: a prevalence case-control study in irritable bowel syndrome. *Aliment Pharmacol Ther* 2005; **22**: 1157–60.
4 Walters B, Vanner SJ. Detection of bacterial overgrowth in IBS using the lactulose H2 breath test: comparison with 14C-D-xylose and healthy controls. *Am J Gastroenterol* 2005; **100**: 1566–70.
5 Posserud I, Stotzer PO, Björnsson ES, *et al.* Small intestinal bacterial overgrowth in patients with irritable bowel syndrome. *Gut* 2007; **56**: 802–8.

IV. H_2-Breath testing for oro-caecal transit time

After the ingestion of a meal or drink containing a non-absorbable carbohydrate, a rise in breath hydrogen concentration signals that the meal or drink enters the caecum. This gives a measure of mouth to caecum transit time[1]. The test relies on the preferential localization of gut bacteria into the colon. Small intestine overgrowth by anaerobic colonic bacteria will result in an early and considerable increase in breath hydrogen concentration occurring well before the meal reaches the colon, thus hindering the assessment of oro-caecal transit time.

Applications in the clinical setting

Despite its non-invasiveness, safety and simplicity, the inherent limitation for clinical application of the hydrogen breath test to estimate oro-caecal transit is due to a wide variation of results in healthy people[2–11]. Moreover, the test reproducibility, in particular with the liquid meal, is rather poor. About 5–27% of normal subjects fail to produce an increment of hydrogen breath concentration after the meal due to the absence of a hydrogen producing flora in the colon[12, 13]. Accordingly, recent guidelines do not suggest a clear clinical indication for the test in the clinical setting[14].

STATEMENT

H_2 breath test to assess oro-caecal transit time has no definite clinical indications

Level of evidence	I
Strength of recommendation	C

REFERENCES

1 Bond JH, Levitt MD, Prentiss R. Investigation of small bowel transit time in man utilizing pulmonary hydrogen measurements. *J Lab Clin Med* 1975; **85**: 546–56.
2 Read NW, Miles CA, Fisher D, *et al.* Transit of a meal through the stomach, small intestine, and colon in normal subjects and its role in the pathogenesis of diarrhea. *Gastroenterology* 1980; **79**: 1276–82.
3 La Brooy SJ, Male PJ, Beavis AK, Misiewicz JJ. Assessment of the reproducibility

of the lactulose H2 breath test as a measure of mouth to caecum transit time. *Gut* 1983; **24**: 893–6.
4 Hirakawa M, Iida M, Kohrogi N, Fujishima M. Hydrogen breath test assessment of orocecal transit time: comparison with barium meal study. *Am J Gastroenterol* 1988; **83**: 1361–3.
5 Basilisco G, Camboni G, Bozzani A, Vita P, Doldi S, Bianchi PA. Orocecal transit delay in obese patients. *Dig Dis Sci* 1989; **34**: 509–12.
6 Gorard DA, Gomborone JE, Libby JW, Farthing MJ. Intestinal transit in anxiety and depression. *Gut* 1996; **39**: 551–5.
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Applications in clinical pharmacology

Given its excellent safety, the test has been used to demonstrate the drug effects on oro-caecal transit. The transit was accelerated by misoprostol[1], erythromycin[2], metoclopramide[3], and paroxetine[4], and it was delayed by loperamide[5, 6], ritodrine[7], codeine, dopamine[8, 9], peppermint oil[10], n-butylscopolamine[10] and imipramine[4]. In this contest, a liquid meal containing 10 g of lactulose in 100 mL of water is expected to give a mean oro-caecal transit time of about 80 min with a 51 min coefficient of repeatability; in these experimental conditions, 34 subjects with a power of 0.80 will be needed to assess a 50% difference and 44 subjects with a power of 0.90.

STATEMENT

H2 breath test is useful to assess oro-caecal transit time in clinical pharmacology.

Level of evidence	I
Strength of recommendation	B

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V. H_2-Breath testing for other 'gas-related' syndromes

The '*gas-related syndrome*' is defined as the presence of nonspecific abdominal symptoms (bloating, borborygmi, flatulence, abdominal distension and discomfort) attributed to an excess of abdominal gas by the patient.[1] Several factors concur to the genesis of 'gas-related' symptoms, the most important being increased intra-abdominal gas content, abnormal intestinal motility, abdominal wall muscles relaxation, visceral hypersensitivity, composition of diet and, to a minor extent, stress anxiety and depression.[2–4]

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Abdominal gas content in patients complaining of gas-related symptoms

A higher than normal gas production has been documented in a proportion of patients presenting with gas-related symptoms. This is particularly true in those complaining of flatulence.[5] However, available data are not really consistent with this finding and mainly refer to IBS patients. In fact, different studies, with gas perfusion techniques, calorimetry or imaging, led to conflicting results, showing that intestinal gas content proved in turn similar, non-significantly higher or significantly higher than in normal controls.[2, 6–10]

STATEMENT

Available data do not clearly prove that gas production and intestinal gas content in patients with 'gas-related symptoms' differ from controls.

Level of evidence	II A
Strength of recommendation	B

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Role of hydrogen breath tests in patients complaining for gas-related symptoms

Our findings derived from studies not specifically aimed at investigating the relationship among abdominal symptoms, intestinal gas production and breath hydrogen excretion. Therefore, caution in the interpretation of data is mandatory. Moreover, in most instances, they were carried out in IBS patients.

Data from studies on small intestinal bacterial overgrowth and sugar malabsorpion were mainly analysed. Some authors suggest that bacterial overgrowth diagnosed by means of a lactulose breath test may play a role in inducing symptoms in IBS. However, these studies are not flawless and the prevalence of bacterial overgrowth was probably overestimated.[11, 12] Similarly, conflicting results have been reported in studies using the glucose breath test.[13–15]

The prevalence of sugar malabsorption is shown to be similar in the general population and in patients with functional bowel disorders and gas-related symptoms.[5–7] This proves true both for disaccharides hydrolysed by brush border enzymes and for sugars absorbed by diffusion (fructose, sorbitol). Evidence that restriction diets may ameliorate symptoms is scanty. It is thought that the genesis of symptoms results more likely from abnormal handling of intestinal gas content than from increased gas production.

STATEMENT

The prevalence of sugar malabsorption (lactose, fructose…) in IBS patients and gas-related symptoms is not higher than in the general population.

Level of evidence	I
Strength of recomendation	A

The real relationship between intraluminal gas production, hydrogen excretion measured by hydrogen breath tests and so-called 'gas-related symptoms' is still ill-defined, as available data are few and often conflicting. With the possible exception of lactose hydrogen breath test, data reported in the literature do not support the hypothesis that breath tests play a relevant role in the diagnostic workout of patients with gas-related symptoms.[17, 18]

Thus, available data do not indicate what substrate should be preferred for diagnosing and quantifying excess gas production in patients with gas-related symptoms.

STATEMENT

Hydrogen breath tests do not provide clear evidence that increased gas production/excretion is present in patients with gas-related symptoms.

Level of evidence	II B
Strength of reccomendation	B

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