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An ESPGHAN Position Paper on the Use of Breath Testing in Paediatric Gastroenterology

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Abbreviations:

AP-FGIDs: abdominal pain-related functional gastrointestinal disorders

BT: breath test

CFU: colony forming unit

CSID: congenital sucrase-isomaltase deficiency

DBPC: double-blinded placebo controlled

EMA: antiendomysial antibodies

FGID: functional gastrointestinal disorders

FODMAPs: fermentable oligosaccharides, disaccharides, monosaccharides, and polyols

FOS: fructose-oligosaccharides

GHBT: glucose hydrogen breath test

GI: gastrointestinal

GIC: Gastroenterology Committee

GLUT: glucose transporter

GRADE: Grading of Recommendations, Assessment, Development and Evaluations

HBT: hydrogen breath test

HLA: human leukocyte antigen

IBS: irritable bowel syndrome

IF: intestinal failure

LHBT: lactose hydrogen breath test

LI: lactose intolerance

LoE: level of evidence

OCTT: oro-caecal transit time

PIPO: paediatric intestinal pseudo-obstruction

PPI: proton pump inhibitor

ppm: parts per million

RAP: recurrent abdominal pain

RCT: randomised controlled trial

SBS: short bowel syndrome

SIBO: small intestinal bacterial overgrowth

SoR: strength of recommendation

TGA: tissue transglutaminase antibodies

UBT: urea breath test

Abstract

Objectives: Given a lack of a systematic approach to the use of breath testing in paediatric patients, the aim of this position paper is to provide expert guidance regarding the indications for its use and practical considerations to optimise its utility and safety.

Methods: Nine clinical questions regarding methodology, interpretation, and specific indications of breath testing and treatment of carbohydrate malabsorption were addressed by members of the Gastroenterology Committee (GIC) of the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN).

A systematic literature search was performed from 1983 to 2020 using PubMed, the MEDLINE and Cochrane Database of Systematic Reviews. Grading of Recommendations, Assessment, Development, and Evaluation was applied to evaluate the outcomes.

During a consensus meeting, all recommendations were discussed and finalised. In the absence of evidence from randomised controlled trials, recommendations reflect the expert opinion of the authors.

Results: A total of 22 recommendations were voted on using the nominal voting technique. At first, recommendations on prerequisites and preparation for as well as on interpretation of breath tests are given. Then, recommendations on the usefulness of H2-lactose breath testing, H2-fructose breath testing as well as of breath tests for other types of carbohydrate malabsorption are provided. Furthermore, breath testing is recommended to diagnose small intestinal bacterial overgrowth (SIBO), to control for success of *Helicobacter pylori* eradication therapy and to diagnose and monitor therapy of exocrine pancreatic insufficiency, but not to estimate orocaecal transit time (OCTT) or to diagnose and follow-up on celiac disease.

Conclusions: Breath tests are frequently used in paediatric gastroenterology mainly assessing carbohydrate malabsorption, but also in the diagnosis of small intestinal overgrowth, fat malabsorption, *Helicobacter pylori* infection as well as for measuring gastrointestinal transit times. Interpretation of the results can be challenging and in addition, pertinent symptoms should be considered to evaluate clinical tolerance.

What is known?

- Breath tests are commonly used in paediatric gastroenterology as they are a non-invasive, relatively low in cost and an easy diagnostic method in a variety of disorders.

- Standardisation is lacking regarding indications for testing, test methodology and interpretation of results.

Learning points:

- Despite the many indications for breath testing, the results can be false positive or false negative and results should be considered carefully.

- Interpretation of breath tests for carbohydrate malabsorption should always include the evaluation of symptoms to assess (in)tolerance.

Introduction

Breath tests (BTs) are commonly used in paediatric gastroenterology as they are non-invasive, relatively low in cost and easy to use as diagnostic method in a variety of disorders. However, standardisation is lacking regarding indications for testing, test methodology and interpretation of results.

In **carbohydrate malabsorption**, hydrogen breath tests (HBTs) are used in combination with symptom assessment using a validated standardised symptom questionnaire to filter out false-positive and false-negative results. The kinetics of the breath hydrogen excretion and the occurrence of symptoms may help to distinguish carbohydrate malabsorption from functional gastrointestinal disorders (FGIDs). It is also important not to overlook dose dependency on symptom development.

Hydrogen or methane breath testing is also applied to diagnose **small intestinal bacterial overgrowth** (SIBO). The sensitivity can be increased by measuring glycaemia during the breath test (BT). There is also a place of breath testing to assess **oro-caecal transit time** (OCTT), however, this is not widely accepted and rarely used in general practice (1). One of the most frequent applications of ^{13}C-breath testing is for the detection of ***Helicobacter pylori*** in epidemiological studies or the evaluation of the results of eradication treatments. Further possible indications for breath testing are coeliac disease, fat malabsorption, sucrase-isomaltase deficiency and gastroparesis for solids or liquids.

Methodology

Under the auspices of ESPGHAN, members from the Gastroenterology Committee (GIC) including paediatric gastroenterologists and a dietitian formulated current evidence-based clinical practice guidelines. A systematic literature search was carried out using PubMed, the MEDLINE and Cochrane Database of Systematic Reviews from 1983 to 2020 applying the terms “breath test, hydrogen breath test, lactose, fructose, sorbitol, sucrose, xylose, mannitol, sucrase-isomaltase deficiency, lactulose, ^{13}C-breath test, helicobacter, small intestinal bacterial overgrowth, gastroparesis, and dysmotility”. References in these documents were also searched to ensure acquisition of relevant source data. Grading of Recommendations, Assessment, Development, and Evaluation was applied to evaluate the outcomes. Levels of evidence for each statement were based on the grading of the literature. Using the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) system, the quality of evidence was graded as follows (2–6).

1. High: Further research is unlikely to change our confidence in the estimate of effect.
2. Moderate: Further research is likely to have impact on our confidence in the estimate of effect and may change the estimate.
3. Low: Further research is likely to have an impact on our confidence in the estimate of effect and likely to change the estimate.
4. Very low: Any estimate of effect is uncertain.

The strength of recommendations was defined as follows:

Strong: when the desirable effects of an intervention clearly outweigh the undesirable effects, or they clearly do not. It should be noted that the expert group could make strong recommendations based on lesser evidence when high-quality evidence is impossible to obtain and the anticipated benefits strongly outweigh the harms. Strong recommendations are formulated as ‘‘the ESPGHAN GIC recommends (...).’’

Weak: when the trade-offs are less certain (either because of the low quality of evidence or because the evidence suggests that desirable and undesirable effects are closely balanced). Weak recommendations are formulated as ‘‘the ESPGHAN GIC suggests (...).’’

The ESPGHAN GIC anonymously voted on each recommendation. A 9-point scale was used (1 (strongly disagree) to 9 (fully agree)), and votes are reported for each recommendation. It was decided in advance that consensus was reached if >75% of the GIC members voted 6, 7, 8, or 9. Consensus was reached for all questions. Due to the heterogeneous field regarding the handling of the breath tests and the interpretation of the results clear recommendations where given despite a lack of strong evidence for most recommendations. In the absence of evidence from randomised controlled trials, the majority of recommendations reflect the expert opinion of the authors. The final draft of the clinical guideline was sent to all committee members for approval in February 2021, and then critically reviewed by a multidisciplinary panel of the GIC and members of the Council of ESPGHAN.

Q1: What is the methodology of hydrogen and methane breath testing?

HBTs are based on measurement of exhaled hydrogen by gas chromatography or electrochemical cells. Especially anaerobic bacteria in the large bowel in health and small bowel in diseased conditions produce hydrogen by fermentation of unabsorbed carbohydrates (7). In SIBO, increased bacteria within the small bowel are responsible of an early production of H_2.

Hydrogen produced by bacteria is absorbed through the intestinal wall and eventually reaches the lungs where it is exhaled and can be measured (Figure 1).

BTs assess different physiological or pathological conditions depending on the carbohydrate that is ingested. Several parameters are important to consider to reduce its variability or the rate of false positive or negative results.

The normal baseline breath hydrogen is 7±5 parts per million (ppm) (8). In order to obtain an accurate result, it is important to distinguish an increase of breath hydrogen from baseline, which should be ≥20 ppm above baseline. Baseline values should be <10 ppm, otherwise, the HBT cannot be used (9).

1. Factors influencing hydrogen levels

a. Antibiotics

Antibiotics change the composition of the colonic microbiota, which produces hydrogen. However, it is not yet possible to determine the duration of this modification and the time to recover a normal bacterial metabolic activity. In clinical practice, a 4-week interval between the antibiotic treatment and the HBT is generally proposed (9,10). This interval can be reduced to 2 weeks, e.g. to assess the success of therapy in SIBO.

b. Laxatives

Laxatives and colonic cleansing preparations modify the composition of the colonic microbiota. It is proposed to consider a 4-week interval between laxative treatment and the HBT especially when the benefit of the test outweighs the risk of stopping therapy (9–11). However, when the constipation is severe and the cessation of laxatives would not be tolerated, this interval can be reduced to 1 week with a low quality of evidence (1).

c. Probiotics

Probiotics can alter the composition of colonic microbiota and a 4-week interval should be considered between probiotic administration and the HBT especially when the benefit of the test outweighs the risk of stopping therapy (10,11).

d. Prokinetics

While no data are available, a 4-week interval is also proposed between the end of treatment and the HBT especially when the benefit of the test outweighs the risk to stop therapy (10,11). However, when the gastroparesis will not enable the cessation of prokinetics, this interval can be reduced to 1 week with a low quality of evidence (1).

e. Diet

The fermentation of malabsorbed carbohydrates can induce an H_2 rise. Malabsorbed carbohydrates are high in beans, wheat and oat flour, potatoes, and corn, but low in rice (12). In order to have a low baseline hydrogen level and an HBT of good quality, patients should

avoid these nutrients, and favour rice and meat (12). Children should fast overnight (1,11) for 8 to 12 hours, and infants <6 months old 4 to 6 hours.

f. Mouth washing

Oropharyngeal bacteria can metabolise the test solution, which results in an early peak of hydrogen production. A mouthwash with 1% chlorhexidine before the test inactivates oropharyngeal bacteria (9,13,14). It seems useful that children brush their teeth with toothpaste before the test, while the use of chlorhexidine could be debated.

g. Exercise

During exercise, there is an increased respiratory rate, which induces a decrease in hydrogen. Therefore, physical exercise should be avoided before and during the HBT (10,14). Children should at least keep quiet during the test.

h. Cigarette smoking

During smoking, breath hydrogen excretion is increased. Thus, it is useful to instruct teenagers that cigarette smoking should be avoided during the HBT (10,14).

2. Breath sampling

a. Timing

Breath hydrogen should be measured in alveolar air. The first part of the exhaled air corresponds to the respiratory dead space air, which is equivalent to approximately 2 ml/kg (1). It represents about 1/3 of the tidal volume and this ratio can increase to 1/2 in neonates.

The best respiratory technique is to inhale maximally, to hold the inhalation for 20 seconds and then to expire into the device (10,15). Breath holding reduces the heterogeneity of alveolar air and increases the reproducibility of measurements. However, in young children breath holding may not be possible.

b. Collection

Different devices are available to collect breath samples. The modified Haldane-Priestley tube, the Y-piece device and the two-bag system are equivalent. In children who are not cooperating, breath samples can be collected invasively using nasal probes or non-invasively using facial masks with detectors of respiratory phases.

Briefly, in children who are able to follow the instructions and to blow in a mouthpiece, through a connector with a flutter valve, the air of the dead space goes into a discard bag and then the alveolar air is collected in a specific bag; syringes are used to take a sample for analysis from this collection bag. Instead of the collection bag, it is also possible to collect

directly the alveolar air into glass tubes by using another kind of connector. In younger children who are not cooperating, it is possible to use face mask and smaller bags.

c. Normalisation

Breath hydrogen values can be normalised to alveolar CO_2 concentrations. CO_2 levels are stable in alveolar air at about 5% (10). Thus, a correction is applied according to CO_2 levels measured in the sample.

d. Duration of the test

Samples are collected at 0, 15, 30, 60, 90, and 120 min (9). The North American Consensus on hydrogen- and methane-based BTs proposes a duration of 2 hours for glucose or lactulose breath tests to assess for SIBO and 3 hours for fructose and lactose breath test (1). However, there is a wide variation in the studies and the duration of the HBT can vary between 2 and 3 hours, and the intervals between 15 and 30 minutes.

e. Symptom record

In order to appropriately analyse the HBT, it is recommended to record general (fatigue, chills), gastrointestinal (hyperperistalsis, bloating, nausea, belching, heartburn, abdominal pain, diarrhoea, indigestion), and neurological symptoms (dizziness, headache) that could be associated with carbohydrate malabsorption (9).

f. Storage

Breath samples can be stored in plastic syringes or in collection tubes. To avoid gas leakage plastic syringes have to be stored at -20°C. Hydrogen concentration drops by 30% after 5 days at room temperature, while there is no loss after 2 days and only a 5 to 7% decrease after 15 days at -20°C (10).

3. Methane

Intraluminal methane (CH_4) reduces the number of atoms of hydrogen available for hydrogen excretion. The predominance of methane producing bacteria in the colon can result in false negative breath hydrogen results, with a reduced breath hydrogen peak. The prevalence of non-hydrogen-producers varies widely between 3 and 25% (11). The analysis of methane exhalation in the CH_4 breath test is recommended in the interpretation of negative HBTs due to non-producer status. Moreover, a delayed OCTT can also result in breath hydrogen false negative results (16). In this case, breath sampling can be prolonged until 4 hours (1,10).

4. Safety

BTs are safe. Due to the radioactive load, BTs are not using ^{14}C but ^{13}C; ^{14}C-lactose and ^{14}C-xylose have been replaced by ^{13}C-lactose and ^{13}C-xylose (9,17).

Adverse events can occur during HBTs, however, medical emergencies do not (9). Contraindications for HBTs are hereditary fructose intolerance (using fructose or sorbitol load) and known or suspected postprandial hypoglycaemia (9).

Recommendations:

1. The ESPGHAN GIC recommends that antibiotics, probiotics and laxatives should be stopped for at least 4 weeks before testing (LoE low, SoR strong, voting: 7,8,8,9,9,8,9,8,9,8,8,8).
2. The ESPGHAN GIC recommends avoiding exercise and smoking before and during the test (LoE low, SoR weak, voting: 9,7,8,9,8,9,8,9,8,7,8,8) .
3. The ESPGHAN GIC recommends that children should fast for >12 h and infants younger than 6 months for >6 h (LoE very low, SoR strong, voting: 9,9,8,9,9,9,7,9,8,8,9).
4. The ESPGHAN GIC recommends that children should avoid fermentable foods the day before testing (LoE low, SoR strong, voting: 9,8,8,9,8,9,8,6,7,8,9,8).
5. The ESPGHAN GIC recommends performing H_2 and CH_4 measurements to improve BT accuracy (LoE moderate, SoR strong, voting: 9,8,6,9,8,8,8,9,9,8,7,9,8).

Q2: How do you interpret hydrogen and methane breath tests and what are the pit falls?

The correct interpretation of BTs requires the correct methodology and preparation as described in question 1. For hydrogen- and methane-based BTs in adults the recommended doses for lactulose, glucose, fructose and lactose are 10, 75, 25, and 50 g, respectively (1) (Table 1). There are only few published data on recommended doses in children which suggest 1 g/kg with maximum 50 g loading dose for lactose and glucose. More studies are needed to validate these weight adapted dosages and more caution should be sought in interpreration of breath tests in children (9,18).

The interpretation of the HBTs is based on three factors: 1) H_2 exhalation level, 2) symptoms, and 3) time-dependent change of these two factors during the test period. The most recently agreed cut off values for hydrogen and methane are (1):

a) A rise of ≥20 ppm from baseline in hydrogen for fructose and lactose breath testing.

b) Until further data is available, a level of ≥10 ppm for methane on a breath test (1).

Two classic examples of typical HBT curves are described in Figure 2 and 3.

1. The curve demonstrates a negative BT as there is no H_2 increase and the patient remains asymptomatic (Figure 2).

2. The curve demonstrates a positive BT (rise of exhaled H_2 ≥20 ppm and presence of symptoms) (Figure 3).

If the H_2 concentration rises more than 10 ppm but less than 20 ppm above the basal value, the test is considered negative. A rise of ≥20 ppm from baseline within 90 minutes should be considered in SIBO, although poor oral hygiene or rapid intestinal transit might affect the result as well (11,19,20). It is recommended that SIBO be ruled out before performing lactose or fructose breath testing. A curve with two peaks is not required for the diagnosis of SIBO (1) and only one peak ≥20 ppm in hydrogen from baseline by 90 minutes should be considered diagnostic for SIBO (that also includes the scenario of a double peak with both peaks ≥20 ppm or only one of them ≥20 ppm).

False-negative HBTs result where the colonic microbiome does not produce sufficient hydrogen (11,16). Another reason could be that hydrogen excretion tends to be lower in methanogenic patients (cf. question 1) (21). The North American Consensus recommends, that hydrogen, methane and carbon dioxide should be measured simultaneously during breath testing (1). The measurement of methane improves sensitivity in hydrogen non-excretors (22). The North American Consensus and recent evidence suggest a cut off of >10 ppm for excessive methane production (1,23). Furthermore, the rise of methane is not as sharp as hydrogen (23). Methane and carbon dioxide measurements increase the complexity and the cost of BTs and are not readily available. In addition to a careful nutritional history, measurement of H_2 and CH_4 as well as documentation of symptoms may be helpful in interpreting BT results more accurately.

Recommendations:

6. The ESPGHAN GIC recommends that a rise of ≥20 ppm from baseline in hydrogen during the test should be considered positive for fructose and lactose breath testing (LoE low, SoR strong, voting: 9,9,6,9,9,8,8,9,9,8,9,8).
7. The ESPGHAN GIC recommends that until better data are available for clinical and research purposes, a rise ≥20 ppm from baseline in hydrogen by 90 minutes should be considered a positive test to suggest the presence of SIBO (LoE low, SoR strong, voting: 9,7,7,8,9,9,8,9,8,8,8,8).
8. The ESPGHAN GIC recommends that, in the absence of underlying GI motility disorders, if the level at baseline is ≥20 ppm, the test should be stopped and a new breath test needs to be rescheduled (LoE low, SoR strong, voting: 9,9,7,9,9,9,9,9,8,8,9,9).
9. The ESPGHAN GIC recommends that two peaks on breath test are not required for the diagnosis of SIBO (LoE low, SoR strong, voting: 9,9,7,8,9,9,8,9,9,8,9,9).
10. The ESPGHAN GIC recommends that until further data are available a level of ≥10 ppm be considered positive for methane on a breath test (LoE low, SoR strong, voting: 9,8,7,9,8,9,8,9,8,8,9,8).

11. The ESPGHAN GIC recommends that until further data are available for clinical purposes of breath testing in children a loading dose of 1 g/kg with a maximum of 50 g for lactose and glucose may be considered (LoE low, SoR strong, voting: 9,8,9,6,9,8,9,9,8,8,9,8).

Q3: What are the aetiology, epidemiology, symptoms and prognosis of lactose intolerance?

Definition

Lactose intolerance (LI) is a common disorder characterised by the inability to digest lactose and may be primary or secondary. Congenital LI is an extremely rare autosomal recessive disorder, in which severe symptoms (profuse vomiting and diarrhoea) appear from birth.

Aetiology

Primary LI is the most frequent disaccharide deficiency (24). Lactase has a maximum level immediately after birth and a genetically programmed progressive decrease begins at around 2 years of age and becomes clinically apparent after the age of 5 to 6 years (25).

The lactase gene (LCT) has 50 kb and is located on the long arm of chromosome 2 (26). Two polymorphisms are responsible for the persistence of lactase, namely C/T13910 and G/A22018. C/T13910 appears to be the dominant polymorphism with C being responsible for decreasing lactase expression (26,27). In adulthood, heterozygous individuals have moderately low lactase activity, and homozygous (CC, GG, respectively) have undetectable levels of lactase at the surface of the intestinal mucosa. TT or AA genotypes correlate with lactose tolerance (26,27).

Secondary LI is found in patients with intestinal diseases such as acute gastroenteritis, giardiasis, coeliac disease, Crohn's disease and drug- or radiation-induced enteritis, which affect a large part of the mucosal surface resulting in lower lactose digestion capacity.

Epidemiology

It is estimated that up to 75% of the world's population is lactose intolerant (28). Primary LI is more common in non-Caucasian populations (75 to 90%) compared to Caucasians (25%). In Europe, primary LI has a rising prevalence form North to South (25). In a recent systematic review, Harvey et al. found a prevalence of 0 to 17.9% for primary LI and 0 to 19% for secondary LI in children aged 1 to 5 years (29).

Symptoms

Symptoms suggesting LI appear a few hours after ingesting lactose and include abdominal pain, nausea and vomiting, meteorism, borborygmi and diarrhoea (26).

Secondary LI which may appear at any age is often transient, depending on the therapeutic control of the underlying disease (30,31). Pawlowska et al. performed HBTs in 232 children with organic and functional gastrointestinal diseases showing that 86 (37.08%) children had a positive HBT (32). More positive tests were found in children with IBD and malabsorption syndromes when compared to children presenting with a FGID (32). The development of symptoms depends on multiple factors such as the amount of ingested lactose, intestinal transit time or associated FGID (31,33).

Primary LI is frequently found in children with chronic abdominal pain, but causality is often not proven. It is known that any type of intervention, whether lactase supplementation or exclusion diet, has an important placebo effect in individuals with FGID, leading to up to 50% improvement of symptoms. However, the placebo effect of a lactase intervention or diet in this population is very difficult to evaluate. Gijsbers et al. investigated primary LI in 210 children presenting with recurrent abdominal pain, aged 4 to 16 years, of whom 57 tested positive on the HBT. In 19 of these children, symptoms resolved without diet. The rest of the children (n=38) started a lactose-free diet and 24/38 reported resolution of symptoms. Remarkably, the open provocation test was positive in just 7/23 children. The double-blinded placebo-controlled test was performed in only 10% of the children with a positive lactose breath test and it was negative in all 6 children. The authors concluded that primary LI could not be established as the cause of the recurrent abdominal pain (34). The poor results of the provocation tests, whether open or blinded, suggests that symptoms do not correlate with the outcome of the HBT.

In another study, HBTs 66% of 95 children aged 6 to 18 years had a positive lactose HBT. There was no difference in LI symptoms between children with positive and negative tests prior to performing the HBT. During the HBT, diarrhoea and flatulence were significantly more frequent in the group with a positive HBT compared to those with a negative test (31.7% vs. 9.4%, p=0.016 and 69.8% vs. 40.6%, p=0.006, respectively). Surprisingly, the frequency of abdominal pain and bloating was similar in both groups. The response to a lactose-free diet was similar between those groups (35). Abdominal pain was the least specific symptom of LI and, therefore, the HBT should be performed only in children with symptoms of LI (35).

Furthermore, in a more recent study, Posovszky et al. showed that 114 of 253 with chronic abdominal pain aged 7 to 12 years reported a relationship between abdominal pain and lactose ingestion. Only 18% (20/114) of these children had a positive HBT and only 3 reported pain relief after a lactose-free diet. Based on these data the evidence of the HBT for diagnosing primary LI in children with chronic abdominal pain is low (36).

Role of investigations in the diagnosis of PLI

There are five methods to diagnose LI: genetic tests that identify polymorphisms associated with primary LI; measurement of lactase activity on intestinal biopsies; the HBT; the lactose tolerance test and the gaxilose test (31). Current evidence does not support breath testing for diagnosing primary LI in children with chronic or recurrent abdominal pain (32,34–36). Furthermore, breath tests do not identify children with chronic or recurrent abdominal pain who will benefit from a lactose-free diet (34–36).

Genetic tests use sequencing or real-time PCR on DNA extracted from buccal swab or venous blood. These tests are useful in epidemiological studies. In Caucasians, primary LI may be identified. However, the genetic profile is more complex in patients with African or Asian heritage. Thus, genetic tests are not recommended in these populations in clinical settings (25,31). Furthermore, secondary LI cannot be detected by genetic tests.

Measuring lactase activity on intestinal biopsies detects both primary and secondary LI (25,31). Lactase activity is patchy and several biopsies are required for best accuracy (31). Still, upper GI endoscopy for measuring lactase activity on intestinal biopsies is not routinely indicated (25,31).

The lactose tolerance test measures serum glucose at different times after lactose ingestion. Although the lactose tolerance test has the lowest costs and may be performed even in low resource settings, its invasiveness limits its utility (25,31).

Similarly, the gaxilose test involves the administration of gaxilose (4-galactosylxylose) with measurement of D-xylose in urine or blood. Theoretically, this test is ideal for the assessment of intestinal lactase since it measures lactase activity over the entire small bowel (31). At this point, its use is still debated and further evidence is needed in order to make a firm recommendation (31).

Currently, none of the above described test are recommended routinely in clinical practice for diagnosing LI.

Prognosis

Management of lactose intolerance consists of dietary dairy avoidance. In primary LI, dairy products should be avoided for 2 to 4 weeks, the time needed for remission of symptoms. After remission, a gradual, individual reintroduction of dairy low in lactose is recommended (30). Only about 50% of individuals with genetic predisposition to primary LI are symptomatic (37) and individual thresholds for tolerance vary (30,38). Many individuals with primary LI can consume dairy without symptoms, while others show substantial improvement by a dairy free diet (24,26). Most individuals tolerate up to 12 g of lactose (approximately 250 ml milk) per day, especially if it is spread out throughout the day or it is consumed with food (31,33). The type of ingested dairy is also important for tolerance. For example, yoghurt that contains viable live bacteria with beta-galactosidase activity is much

better tolerated than pasteurized yoghurt (limited beta-galactosidase activity) (30,31). Lactase supplementation leads to improvement of symptoms and may be a therapeutic option (31).

Since dairy is an important source of calcium, its supplementation is recommended during a lactose free diet (30).

Primary LI may have a negative impact on the quality of life and may lead to anxiety in relation to lactose ingestion and, in severe cases, to restrictive food intake disorder (31).

Recommendations:

12. The ESPGHAN GIC recommends to not using the lactose BT in the diagnostic work-up of children with abdominal pain-related functional gastrointestinal disorders (AP-FGIDs) (LoE moderate, SoR strong, voting: 9,7,9,8,9,9,9,9,8,9,7,8).
13. The ESPGHAN GIC recommends that unless the child is unable to comply with a breath test the following are not routinely used in the work up of children with suspected LI: genetic testing; intestinal biopsy lactase activity; lactose tolerance test or the gaxilose test (LoE moderate, SoR strong, voting: voting: 9,9,7,9,8,9,7,9,9,8,9,8).

Q4: What are the aetiology, epidemiology, symptoms and prognosis of fructose malabsorption?

Definition

Fructose malabsorption, also referred to as fructose intolerance, should not be confused with hereditary fructose intolerance (a rare, autosomal recessive disorder with a prevalence of 1 per 25000 persons), in which a lack of functional aldolase B results in an accumulation of fructose-1-phosphate in the liver, kidneys, and intestine, causing hypoglycaemia, nausea, bloating, abdominal pain, diarrhoea, and vomiting (30,39). Fructose malabsorption is caused by fermentative metabolism of fructose by luminal bacteria resulting in production of hydrogen, carbon dioxide, methane and short-chain fatty acids causing symptoms (40). If symptoms occur after consumption of less than 25 to 30 g fructose, the patient has symptomatic primary fructose malabsorption. In secondary fructose malabsorption, the morphological damage of the epithelium or the reduction of the intestinal surface may cause a functional transport disorder of fructose (41).

Aetiology

Fructose is a six-carbon monosaccharide that exists in three forms: as pure monosaccharide, as disaccharide (sucrose, where fructose is complexed with glucose) and as polymerised forms (oligosaccharides and polysaccharides) (42). Fructose exists in food naturally or as a sweetening additive (43). The exact mechanism of fructose transport across the intestinal mucosa has not been completely explained and this has hampered identification of a possible defect in transport (18). Different from sucrose or lactose, which are digested by sucrase or

lactase on the intestinal brush border, fructose is absorbed by the passive glucose transporter GLUT-5 and the active glucose transporter GLUT-2. The uptake of fructose is dose dependent (42). Fructose is mainly transported across the apical membrane of intestinal epithelial cells by the facilitative transporter GLUT-5 (44). In human jejunum, GLUT-5 has also been detected on the basolateral membrane (45). GLUT-2 is a facilitative transporter of glucose, galactose, and fructose, carrying monosaccharides across the basolateral membrane, although in specific condition the enzyme may be expressed on the apical membrane (39,43,46). GLUT-5 has a low, saturable uptake capacity. If fructose consumption exceeds 30 to 50 g per hour, osmotically active fructose remains in the intestinal lumen. However, uptake may be enhanced by glucose or amino acids. Intensive physical training, a low-glucose diet, and interaction of the fructose transporter with other osmotic substances (mannitol, xylitol) may inhibit fructose transport. In addition, sorbitol can be transformed into fructose within the intestine, blocking GLUT-5. This leads to aggravation of the fructose uptake disorder (41). Whether defective fructose transporters are involved in the pathogenesis of fructose malabsorption is still matter of debate (40).

Epidemiology

Free fructose has limited absorption in the small intestine, with up to one half of the population unable to completely absorb a load of 25 g (39). The rate of children with a positive fructose HBT has been shown to be significantly higher in younger age groups (18), namely 70% between the ages of 1 and 3 years compared to 27% between 4 and 5 years when given a dose of 1 g/kg fructose (47). Symptomatic children between 2 months and 15 years tested with consistent dosage [0.5 g/kg body weight of fructose (maximum of 10 g)] showed a fructose malabsorption decreasing from 88% in children younger than 1 year to 30% at the age of 10 years (18). This decrease in fructose malabsorption with age may suggest a normal developmental maturation of fructose absorption (18).

Symptoms

Fructose malabsorption may be the cause of abdominal complaints and diarrhoea, symptoms indistinguishable from those of FGIDs, including irritable bowel syndrome (IBS), functional diarrhoea, or functional abdominal bloating (48).

Role of investigations in the diagnosis of fructose malabsorption

Up to now, two randomised controlled trials have been published on children with AP-FGIDs. In a double-blinded placebo controlled (DBPC) trial, fructose malabsorption was diagnosed in 79 of 121 children with recurrent abdominal pain (RAP). Fructose malabsorption in RAP was evaluated by HBT, elimination and DBPC provocation, relying on consistency of symptoms. Forty-nine out of 79 children received a fructose elimination diet. Thirty-two out of 49 children (65%) reported absence of symptoms during the diet, but only 13 out of 31 children (41%) responded to an open provocation with fructose. Finally, DBPC provocations in 8 out 13 patients were negative. In conclusion, fructose malabsorption does

not explain functional abdominal pain in their cohort (34). However, the reason why children with a positive fructose HBT have no symptoms during DBPC with fructose could be explained by the dose as during the BT, children received a maximum of 50 g fructose (2 g/kg) while in the DBPC trial, 25 g was consumed over the whole day. In addition, considering that the elimination and provocation was performed at home, it is probable that children did not strictly follow the guidelines leading to controversial results (40). In a more recent prospective randomised controlled trial, 103 children with AP-FGIDs were randomised to either a fructose-restricted diet (n=51) or to placebo (n = 52) for 2 weeks. Children on the fructose-restricted diet (regardless of the HBT result) showed less pain intensity; nevertheless, they did not show a reduction in pain frequency (49). In contrast, in a prospective observational study, 75 children with AP-FGIDs and a positive fructose HBT received a restricted fructose diet. Overall pain frequency and pain severity decreased while on the exclusion diet (50). Another recent prospective observational study was aimed to analyse the role of lactose or fructose malabsorption as a cause of chronic abdominal pain by an HBT or diagnostic elimination diet. Fructose malabsorption diagnosed by HBT was demonstrated in 30% (35/118) of patients, whereas lactose malabsorption in 18% (20/114). Pain relief during a diagnostic elimination diet was reported in 46% (25/54) of children. Overall, 17 patients had lactose malabsorption, 29 fructose malabsorption, and 9 combined carbohydrate malabsorption. However, carbohydrate intolerance as a cause of chronic abdominal pain was diagnosed at follow-up in only 18% (10/55) of children. Therefore, carbohydrate malabsorption seems to be an incidental finding in children with functional abdominal pain disorders, rather than its cause (36). Based on these studies, the value of fructose BTs in children with AP-FGIDs is controversial.

Prognosis

The treatment consists of a reduction of fructose intake to <10 g/day with a complete exclusion of sugar alcohols and alcoholic beverages. Furthermore, it is also important to balance the consumption of glucose and fructose in order to increase fructose uptake. Using these dietetic strategies, it is possible to obtain remission of symptoms in 60 to 90% (41). A fructose reduced diet is not clearly defined and varies from total exclusion of all fruits, vegetables with fructose, and honey to excluding fruits and foods with a higher content of fructose than glucose (30).

Nutritional concerns would be a deficiency of vitamin C along with fibres and antioxidants. However, there are no studies on the nutritional impact of fructose exclusion.

Recommendation

14. The ESPGHAN GIC recommends to not using fructose BTs in the diagnostic work-up of children with AP-FGIDs (LoE moderate, SoR strong, voting: 9,8,9,8,9,9,9,9,8,9,7,9).

Q5: What are the aetiology, epidemiology, symptoms and prognosis of other carbohydrate malabsorption syndromes? Which breath tests for carbohydrate malabsorption exist?

Congenital **sucrose-isomaltase** deficiency is a rare autosomal recessive inherited disease resulting from mutations in sucrose-isomaltase, an enzyme complex responsible for catalysing the hydrolysis of dietary sucrose and starch. Prevalence has been estimated at 1 in 5000 among the European population, with higher rates among indigenous populations of Alaska, Greenland, and Canada (51). Patients with CSID have a decreased or absent sucrase and/or isomaltase enzymatic activity, with several phenotypes described after investigations at the subcellular and molecular levels in intestinal biopsies, differing in transport efficiency, processing, and sorting of the protein, which result in impaired physiological functions (30). Clinical manifestations depend both on the degree of enzyme deficiency and on the amount of sugar and starch consumed. Gastrointestinal symptoms usually begin after weaning off breast milk, when firstly exposed to sucrose and starch. Failure to absorb starch and disaccharides can also impair the absorption of other nutrients and the hormonal regulation of gastrointestinal function. Therefore, patients are at risk for chronic malnutrition and failure to thrive. Symptoms are usually more severe in infants due to the shorter length of the small intestine. Mutations within the sucrase isomaltase gene are responsible for the phenotype of CSID. These mutations prevent normal synthesis and transport of the protein responsible for sucrase and isomaltase and 80% of maltose digestion. The gene is located at chromosome 3q26.1, is approximately 100 kb in size, consists of 48 exons, and encodes a protein of 1827 amino acids. Isomaltase remains contiguous with the apical border of the villous cells, but sucrase may be cleaved from pro-sucrase isomaltase by trypsin. The enzyme is anchored in the cytoplasm and cell membrane (amino acids 2–32) and has a short stalk region (amino acids 33–109) with isomaltase (amino acids 110–1007) and sucrase (amino acids 1008–1827) extending into the intestinal lumen. The four most common genetic mutations (p.Val577Gly, p.Gly1073Asp, and p.Phe1745Cys in the sucrase domain, and p.Arg1124X in the isomaltase domain) can be found in 80% of the patients (52). Alternative diagnostic tools as the sucrose HBT and intestinal disaccharidase activity in intestinal biopsies are less used, presently. Treatment of CSID is based on dietary restriction and the administration of an oral solution containing sacrosidase (Sucraid®, Invertase®) as enzyme replacement therapy. This enzyme is generally well tolerated and induces a reduction of symptoms (30).

Sorbitol and **mannitol** are six-carbon polyol isomers with a similar molecular weight and size, differing only in the orientation of a hydroxyl group. Sorbitol is naturally present in fruits and juices and, because of its sweetening power; it is widely used as a sugar substitute in drugs, sweets, dietetic foods and beverages, and chewing gum. It does not cause a rise in blood sugar when taken orally as it is poorly absorbed from the small intestine. At a dose as low as 5 g, 50% of subjects test positive on hydrogen breath testing (53). Sorbitol absorption occurs by a not mediated diffusion pathway, it is dose and concentration related, and depends on the entity of intestinal absorption surface. Whereas it is estimated that ingestion of 20 to 30 g can produce osmotic diarrhoea in most subjects, in patients with malabsorption as a result of untreated coeliac disease, ingestion of the smallest and least concentrated dose used

(5 g in a 2% solution), provokes a highly significant increase in H_2 excretion (54). Therefore, the sorbitol HBT is effective in detecting small bowel damage with a relevant reduction of absorption surface, but it is not specific for any condition responsible for intestinal malabsorption. Therefore, the HBT is not recommended in clinical practice, while its use may be indicated for research purposes. Some studies have shown superior diagnostic properties when evaluating malabsorption of a 1 hour isotope ^{13}C-sorbitol BT compared to the HBT (55,56).

Mannitol, mainly present in vegetables, is absorbed via passive diffusion across the small intestinal epithelium. Some studies have extrapolated sorbitol findings to mannitol, assuming that a similar proportion of mannitol is absorbed. However, literature on mannitol absorption is scarce. A randomised, double-blinded, placebo-controlled cross-over study evaluating the differences in the small intestinal handling of sorbitol and mannitol between healthy and individuals with inflammatory bowel disease showed similar extent of absorption of both polyols in healthy subjects. Patients with IBS not only showed a greater ability to absorb both polyols, but also absorbed mannitol more readily than sorbitol (57).

Xylose is a monosaccharide with five carbon atoms and a functional aldehyde group. D-xylose and its hydrogenated form xylitol are used as sweeteners in food and beverages due to its low impact on blood sugar and insulin secretion and its minimal caloric value (2.4 calories/g). D-xylose is primarily absorbed in the proximal small intestine and partially absorbed and excreted in the urine. The D-xylose BT is based on elevated breath $^{14}CO_2$ concentrations after ^{14}C-D xylose administration because of its increased small intestinal bacterial catabolism. Under normal conditions, the proportion of the absorbed compound excreted in the air and urine remains constant but depends on gut transit and the bacterial population of the small intestine. These properties allow the test to be useful in the evaluation and diagnosis of intestinal malabsorption and inappropriate translocation of colonic flora. Aside from SIBO, the ^{14}C-D xylose test has been used in the evaluation of conditions such as coeliac disease, tropical sprue, Crohn's disease, immunoglobulin deficiencies, blind loop syndrome, and radiation enteritis (58). The stable isotope ^{13}C-xylose has shown comparable diagnostic accuracy as the radioactive isotope ^{14}C-xylose and should be preferred (17).

Role of investigations in the diagnosis of other carbohydrate malabsorption syndromes

Congenital sucrose-isomaltase deficiency is an inherited enzymatic defect that can lead to diarrhoea and failure to drive after the first exposure to sucrose and starch. The association with the time of introduction of supplementary feeding after weaning from human milk is suggestive of this defect that should be confirmed by identification of the genetic mutations.

Secondary malabsorption to carbohydrates such as sorbitol, mannitol and xylose can be present in different syndromes causing intestinal damage. Therefore, breath tests using those carbohydrates as substrates are quite unspecific and should not be used in the clinical practice.

Recommendations:

15. The ESPGHAN GIC recommends, that the diagnosis of congenital sucrose-isomaltase deficiency is usually made with genetic testing after appearance of a malabsorption syndrome when firstly exposed to sucrose and starch in the diet (LoE moderate, SoR strong, voting: 9,9,9,5,9,9,8,9,9,9,9,9).
16. The ESPGHAN GIC recommends, that sorbitol, mannitol and xylose BTs are not helpful to differentiate between the causes of intestinal damage driving carbohydrate malabsorption (LoE low, SoR strong, voting: 9,9,9,7,9,8,9,9,8,9,8,9).

Q6: What are the aetiology, epidemiology, symptoms, treatment, and prognosis of small intestinal bacterial overgrowth? What are the indications, sensitivity and the value of breath testing in small intestinal bacterial overgrowth?

SIBO is characterised by GI signs and symptoms due to an excessive bacterial concentration in the small bowel (59). SIBO was initially described as $>10^5$ colony forming units (CFUs) per ml of fluid obtained by direct jejunal aspiration, but a recent North American Consensus has defined a new cut-off level of $>10^3$ CFU/ml of duodenal aspirate, based on data obtained in healthy adult subjects (1). However, metagenomic studies have recently questioned the culture-based approach, as it significantly underestimates both the amount and the diversity of bacteria in the small intestine.

Aetiology and Prevalence

Several mechanisms prevent small bowel bacterial colonisation, including gastric acidity, normal small bowel motility, pancreatic and biliary secretion, systemic and local immunity and anatomic integrity (60,61). The dysfunction of one of the aforementioned mechanisms might lead to SIBO.

Whilst a recent meta-analysis of adult studies showed that proton pump inhibitor (PPI) use moderately increases the risk of SIBO (OR 1.71, 95% confidence interval 1.20-2.43), the results in children are conflicting (62–65). Both primary and secondary GI neuromuscular disorders might predispose to SIBO; however data in children are lacking. Both congenital and acquired anatomical abnormalities, such as stricturing and fistulating Crohn's disease, presence of ostomies, previous surgeries associated with the creation of a blind loop (Billroth II and Roux-en-Y) and short bowel syndrome (SBS) with intestinal failure (IF), might predispose to SIBO (66,67). The prevalence of SIBO in children with IF ranges between 34% and 71% (68,69). Many other conditions including immune dysregulation, cystic fibrosis, intrahepatic cholestasis, overweight and obesity, abnormal pancreatico-biliary secretions, autism, poor socio-economic status, constipation and other FGIDs are associated with SIBO (70–78).

Clinical Manifestations

The clinical manifestations of SIBO are quite variable ranging from mild non-specific GI symptoms, such as appetite loss, belching, nausea, diarrhoea, abdominal distension and pain and flatulence, to severe complications, such as malnutrition and growth failure. However, discrimination whether symptoms are due to SIBO or the underlying clinical conditions is often challenging. The effect of bacterial proliferation on intestinal mucosa and the impact of bacterial metabolism on host absorptive and digestive mechanisms have been advocated in explaining how SIBO might generate the aforementioned symptoms (79).

Treatment

The use of antibiotics has become the cornerstone of treatment. Ideally, antibiotics should selectively modify the GI microecology, but due to its impracticality, it is common practice in children to use broad-spectrum antibiotics such as rifaximin, trimethoprim/sulfamethoxazole, metronidazole, amoxicillin-clavulanic acid, gentamicin, neomycin, and ciprofloxacin. The evidence is scarce and there is no agreement on both treatment dose and duration. Moreover, SIBO commonly reoccurs in several conditions such as SBS and PIPO after the first antibiotics course and despite the absence of evidence a repeated course of the same antibiotic or the use of empiric cycling regimes has become common practice. In children, the only available data on efficacy of antibiotic treatment is related to the use of rifaximin and combined treatment with metronidazole and trimethoprim/sulfamethoxazole (71,80,81). However, evidence on rifaximin is still conflicting.

Non-pharmacologic treatments, such as probiotics and dietary interventions have been suggested as therapeutic options for SIBO. Different from adult data, only few studies on the prevention of PPI-induced SIBO are available in children showing conflicting results. Decrease in carbohydrate intake, low FODMAP diet and elemental diet have also been advocated in adults based on the idea of decreasing fermentable products, but no data are available in children (59).

Role of investigations in the diagnosis of SIBO

Cultures of jejunal aspirate are the gold standard for the diagnosis of SIBO. However, it is costly and time consuming, it requires an upper endoscopy and it has several pitfalls, including a high number of false positive and false negative results, a lack of protocol standardisation and a low yield in identifying all small bowel bacterial species (59).

Due to their inexpensiveness and non-invasiveness, the glucose hydrogen breath test (GHBT) and the lactulose HBT have become the most common used tests for detecting SIBO. As reported before, a rise of $\geq$20 ppm hydrogen from baseline by 90 minutes should be considered both a positive GHBT and lactulose HBT, whilst a rise of $\geq$10 ppm is considered a positive methane breath test. It is a matter of debate whether an increased baseline hydrogen level ($\geq$20 ppm) represents the consequence of an ongoing bacterial fermentation in the small

intestine or it is the result of poor adherence to the pre-test recommendations, such as poor oral hygiene, short fasting time and excessive intake of carbohydrate. In the absence of underlying conditions associated with SIBO, baseline levels ≥20 ppm should lead to stopping and rescheduling the HBT (82).

Although studies in adults have shown that the accuracy of the GHBT and the LHBT is quite variable, the former has a better performance. Compared to jejunal aspirates, the sensitivity and specificity of the GHBT range from 20 to 93% and from 30 to 86%, respectively, whilst the sensitivity and specificity of the LHBT range from 31 to 68% and from 44 to 100%, respectively (59). A recent systematic review with data pooled analysis has confirmed the highest diagnostic yield of the GHBT compared to the LHBT, showing both higher sensitivity (55% vs. 42%) and higher specificity (83% vs. 71%) (83). Although in the last decades the validity of BTs in the diagnosis of SIBO has been significantly questioned and the level of evidence is low, breath testing should be considered in children with non-specific GI symptoms and predisposing conditions (1). Breath testing should also be considered in symptomatic children with functional abdominal pain disorders such as IBS and on PPI therapy, whilst there is no current indication for its use in asymptomatic children on PPIs (1).

Urinary testing of bacterial metabolites has been used as surrogate markers for SIBO. Urinary indole lactic acid, phenyl lactic acid, fumaric acid, 4-hydroxyphenylacetic acid and formic acid is increased in jejunal cultures of patients with a malabsorption syndrome (84). Although urine tests are very attractive in infants and young children unable to undergo breath testing, these tests are not validated.

Prognosis

SIBO is a relapsing condition, mainly in the presence of predisposing factors requiring either a repeated or prolonged course of antibiotics. Moreover, long-term consequences on the changes in gut microbiota either due to SIBO or its treatment in children are currently unknown.

Recommendations:

17. The ESPGHAN GIC recommends to use the glucose hydrogen breath test (GHBT) and the lactulose breath test for diagnosing SIBO (LoE moderate, SoR strong, voting: 9,8,8,9,9,9,8,9,7,9,8,8).

Q7: What are the indications, sensitivity, and the value of breath testing in the estimation of oro-caecal transit time?

The indications for the lactulose breath test are limited and slow transit small bowel issues are infrequent. It does not seem a good test for colonic slow transit and indeed this is aimed at estimating oro-caecal transit (85). Significantly compromised gastric emptying may affect interpretation in respect of small bowel dysmotility or slow transit. As mentioned before, if OCTT is prolonged testing up to 4 hours may be needed.

The most important limitations of the lactulose HBT are its low specificity and sensitivity due to dose-dependent accelerations of OCTT, interfering with the H_2-rise from malabsorbed dietary fibre and H_2-non-producers. In contrast, lactose-[^{13}C]ureide $^{13}CO_2$-BT may avoid these disadvantages (64). The ^{13}C-lactose BT allows non-invasive measurement of liquid gastric emptying time, while the H_2-lactulose BT measures OCTT. Because of different test principles, both tests can generally be combined. This would not only spare time and resources but may also deliver additional information on the integrated regulation of gastrointestinal motor functions (86). Physiological alterations, such as those encountered by children who are critically ill, may further compromise the accuracy of the lactulose HBT in the assessment of OCTT, although evidence for this came from a very small study (87). Inulin may be a better alternative (88).

Hydrogen and methane production do not differ significantly by IBS subtype. Methane production may correlate positively with whole intestinal transit time but in one study methane production (threshold 3 ppm) as a marker for identifying IBS subtype constipation had a sensitivity of 60% and specificity of 42.9% (89). Furthermore, in children with IBS, the lactulose breath test hydrogen and methane production did not, however, correlate with abdominal pain, IBS subtype, or psychosocial distress (89). Generally, because of the physiological variation and component of SIBO as a confounding variable, it would be apparent from evidence in the literature that the utility of HBT for assessment of OCTT compared to scintigraphy is relatively limited in adults and the evidence in children is further limited by paucity (20). Hence, we conclude that there is little or no value in the use of breath testing in assessment of OCTT in children.

Recommendation:

18. The ESPGHAN GIC recommends not using breath testing for estimation of OCTT in children (LoE low, SoR strong, voting: 9,8,9,9,8,9,8,9,8,9,8,9).

Q8: What are the indications, sensitivity and the value of ^{13}C-breath testing in *H. pylori* infection?

Helicobacter pylori (*H. pylori*), is a spiral Gram-negative bacterium that colonizes the mucosa of the human stomach and is the major cause of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma in children and in adults (90). A meta-analysis reported that the prevalence of *H. pylori* infection varies from as low as 18.9% in Switzerland to 87.7% in Nigeria (90). In children specifically, the prevalence of *H. pylori* varies from 2.5% in Japan to 34.6% in Ethiopia (91). Seroprevalence increases with age, decreases with higher income and is predominantly acquired in early childhood via person-to-person contact (mother-child, grandmothers-child) (92,93).

The majority of people, including children, infected with *H. pylori* have no significant symptoms and remain symptom-free throughout life (94–96). However, the recent updated

ESPGHAN/NASPGHAN guideline strongly states that diagnostic testing for *H. pylori* infection in children with functional abdominal pain disorders is not needed, since studies are uncontrolled, of poor quality, or do not include sufficient patients (97). In addition, evidence is lacking that children with periodontal disease, otitis media, upper respiratory tract infections, food allergy, sudden infant death syndrome and short stature should be tested for *H. pylori* (97). In contrast, in children in whom the father or the mother is affected by gastric cancer, testing for *H. pylori* using a noninvasive test may be considered (97). In addition, it has been recommended to test for *H. pylori* infection in children with chronic immune thrombocytopenia (97). In summary, diagnosing *H. pylori* infection in children is only required when symptoms, such as vomiting, persistent abdominal pain and gastrointestinal bleeding, can justify esophagogastroduodenoscopy with histological examinations and microbial detection or culture, because it is important to determine the underlying cause of the symptoms and not solely focus on the presence of *H. pylori* infection (97). This subsequently implies that "test and treat" strategies are not recommended for *H. pylori* infection in children (97).

Noninvasive tests for active infection before and after therapy include the stool antigen and PCR test and the ^{13}C-urea breath test (UBT), of which the latter is considered as safe and the most accurate non-invasive method to diagnose *H. pylori* infection in children older than 2 years of age (98). The UBT involves the ingestion of ^{13}C-labeled urea; if *H. pylori* is present, bacterial urease (urea-amidohydrolase), an enzyme that is needed for the bacteria to colonize the acidic stomach environment, releases the label, which is measured and compared with a baseline value (99). Breakdown of labeled urea by *H. pylori*-derived urease results in the production of labeled carbon dioxide, which subsequently can be measured in expired breath samples. A simplified test protocol is available for children older than 2 years of age (98). After a 2 hour fast, the UBT is performed by collecting a baseline sample of expired air, followed by the ingestion of ^{13}C-urea (50 mg for children <50 kg and 75 mg for children >50 kg) with 50 mg of a glucose polymer in 5 to 10 ml of water. It is important that the solution of urea be swallowed quickly and not held in the mouth, where urease-producing organisms in the oral microflora can cause a false positive test result. Another reason for a false-positive result is the lower distribution volume and a different CO_2 production rate in younger children, which can be adjusted for (100). A second expired breath sample is then collected 30 minutes later. The ratio of ^{12}C to ^{13}C is measured in baseline and 30-minute samples, with the difference between samples calculated by subtraction. The UBT has excellent sensitivity and specificity for the diagnosis of *H. pylori* infection in older children. A recent meta-analysis showed a sensitivity of 96.6% and a specificity of 97.7% in children older than 6 years of age. In children younger than 2 years of age, however, the UBT may have reduced specificity (101,102). A study in 1499 German children found a positive and negative predictive value of 98% and 100% respectively whereas this was 69% and 100%, respectively, in children younger than 6 years of age (103). It is recommended that clinicians wait at least 2 weeks after stopping PPIs and 4 weeks after stopping antibiotics before performing a UBT (97).

Recommendations:

19. The ESPGHAN GIC recommends that the ^{13}C-breath test for *H. pylori* should not be applied to diagnose *H. pylori* infection, but only control success of the eradication treatment (LoE low, SoR strong, voting: 9,9,8,9,9,8,9,9,9,9,9,9).
20. The ESPGHAN GIC recommends that the success of eradication therapy should be monitored 4 to 6 weeks after stopping antibiotics and at least 2 weeks after stopping PPIs (LoE low, SoR strong, voting: 9,9,9,7,9,9,9,9,9,9,8,9).

Q9: What are sensitivity and the value of breath testing for other indications (coeliac disease, fat malabsorption)?

Coeliac disease

Coeliac disease is defined as an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals as characterised by the presence of gluten-dependent clinical manifestations, coeliac disease-specific antibodies, a specific genetic background, and enteropathy (104,105).

Coeliac disease enteropathy is characterised by a reduction in absorptive surface of the small intestine, which results in malabsorption of major nutrients including carbohydrates. It is not surprising that testing for disaccharide malabsorption including hydrogen breath testing was introduced early as a potentially useful diagnostic tool for coeliac disease (106). Later reports proposed other BTs, such as the ^{13}C-sorbitol BT, which, however, did not show any advantage over coeliac disease specific serological tests (55). There is some evidence that the sorbitol BT possibly correlates better with intestinal damage than tissue transglutaminase antibodies (TGA) and antiendomysial antibodies (EMA) (54,107). However, these tests have not been validated.

There have also been attempts to use volatile chemicals in human breath as a marker of carbohydrate malabsorption in coeliac disease, however, this method was not able to discriminate coeliac disease patients from controls (108).

The decision to perform BTs in patients who potentially have coeliac disease must be made with much care especially in atypical cases, and results interpreted with caution. Due to the non-invasive nature of BTs there is a risk of overuse and overinterpretation (109) and other more specific tests may not be ordered and treatment (e.g. a carbohydrate elimination diet) may be based solely on a positive result of the BT. Relief of symptoms, which can occur, might erroneously lead to the belief that a carbohydrate such as lactose is a sole reason for patient's illness and this can cause dangerous delays in coeliac disease diagnosis, which are already known to be long in many regions (110).

Despite those limitations, BTs can be of some value in case of symptom persistence despite strict gluten-free diet to detect concomitant primary or secondary carbohydrate intolerance

(111).

In summary, BTs lack specificity and do not have any advantage in diagnosing coeliac disease over the disease specific serological tests such as TGA and EMA.

Fat malabsorption

Pancreatic insufficiency commonly results in fat malabsorption. BTs using radioisotopic carbon labelled triglycerides have been repeatedly applied in patients with fat malabsorption to evaluate pancreatic function (112).

The BTs are based on the principle that intestinal triglyceride absorption requires prior hydrolysis by lipase to produce free fatty acids and monoacylglycerol. These metabolites are absorbed and metabolised to produce labelled CO_2, which is exhaled. Increase in labelled CO_2 correlates with intestinal lipid degradation and absorption. These tests can be used instead of the cumbersome direct measurement of faecal fat excretion (113,114).

Cystic fibrosis is a typical disease with pancreatic involvement often since early childhood. Several ^{13}C-labelled lipid-based substrates that are digested by pancreatic enzymes have been proposed for BTs. These substrates assess the intraluminal activity of pancreatic enzymes and thus pancreatic exocrine function (115).

Particularly in children, ^{13}C-BTs are suitable not only for the diagnosis of pancreatic exocrine insufficiency, but also for therapy control under pancreatic enzyme substitution (116–118).

However, $^{13}CO_2$ excretion also depends on other sources of lipolytic activity (such as gastric lipase), biliary secretion that is needed for formation of micelles, gastrointestinal transit and absorption as well as hepatic and lung function. Therefore, the test cannot be 100% specific for pancreatic exocrine insufficiency and is not 100% sensitive either, particularly not in mild pancreatic exocrine insufficiency (117,119,120).

In conclusion, lipid-based ^{13}C-BTs, particularly the ^{13}C-mixed triglyceride BT, reliably detects severe pancreatic exocrine insufficiency. It can be used to monitor pancreatic enzyme replacement therapy especially in children with chronic pancreatic insufficiency.

Recommendations:

21. The ESPGHAN GIC does not recommend the use of hydrogen breath testing neither in the diagnostic approach nor in the follow up of coeliac disease (LoE low, SoR strong, voting: 9,9,8,9,9,9,9,9,9,9,9,9).
22. The ESPGHAN GIC recommends using the ^{13}C-mixed triglyceride BT for the diagnosis and therapeutic monitoring of exocrine pancreatic insufficiency (LoE moderate, SoR strong, voting: 9,9,7,7,9,9,8,9,7,9,8,8).

ESPGHAN disclaimer:

ESPGHAN is not responsible for the practices of physicians and provides guidelines and position papers as indicators of best practice only. Diagnosis and treatment are at the discretion of physicians.

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Figure 1. Schematic diagram that shows the principle of the hydrogen breath test (HBT). SIBO: small intestinal bacterial overgrowth; ppm parts per million (adapted from (121)).

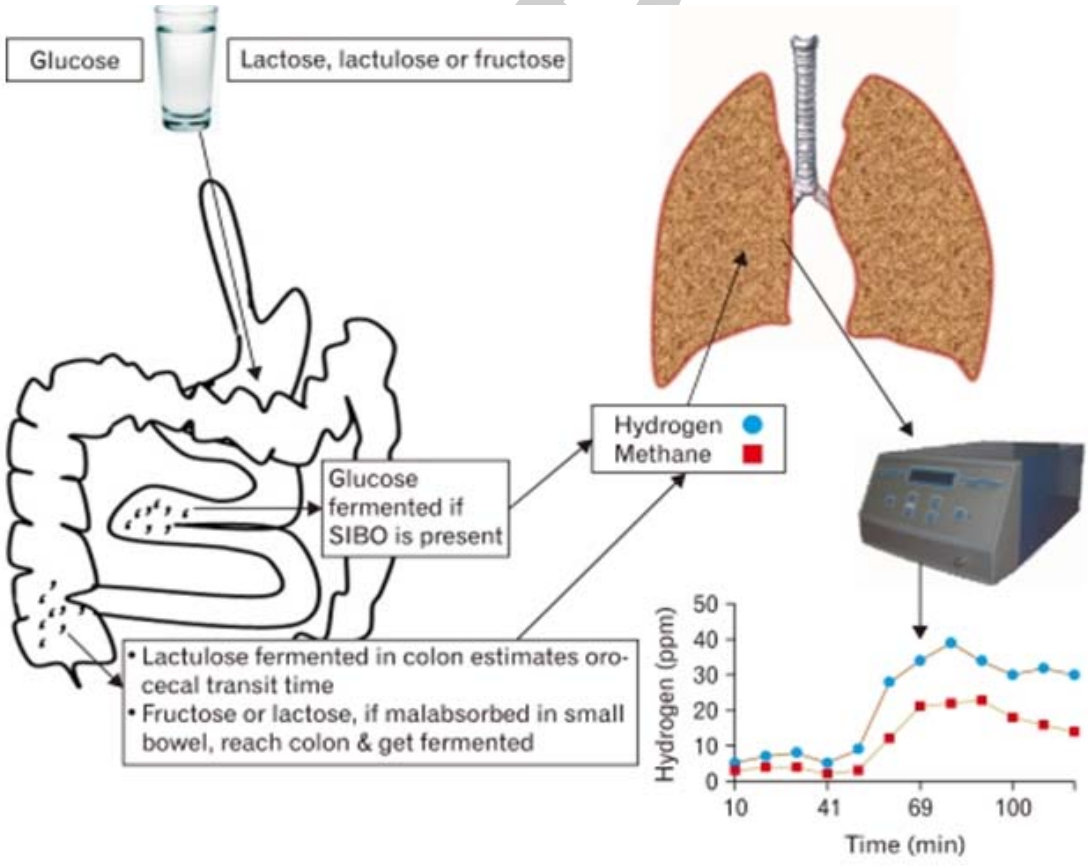

Figure 2. Negative BT (adapted from (9)). No H_2 increase, no symptoms. Interpretation: normal.

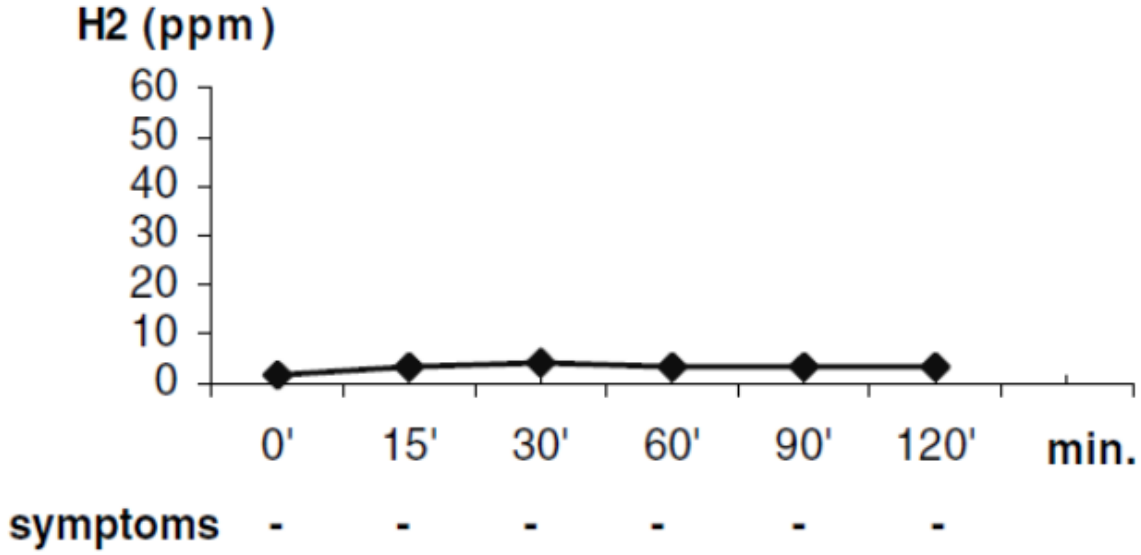

Figure 3. Positive BT and symptoms (adapted from (9)). H_2 increase and presence of symptoms both after 60 minutes. Interpretation: intestinal intolerance of the test substance.

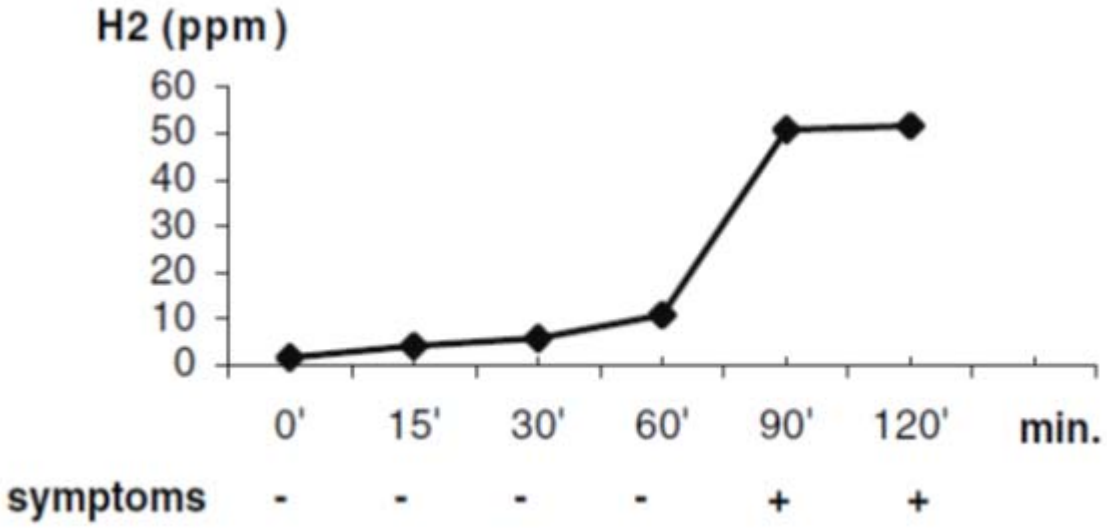

Table 1. Breath test, equipment to be used, dose of the testing material, indications, contraindications and the panel recommendations.

Breath test	**Dose of testing material**	**Indications**	**Absolute contraindications[1]**	**Panel recommendation**
H_2-lactose BT	1 g/kg with maximum of 50 g	Lactose intolerance	Known or suspected (postprandial) hypoglycaemia	Not recommended in AP-FGID
H_2-fructose BT	0.5 g/kg with maximum of 25 g	Fructose malabsorption	Hereditary fructose intolerance; known or suspected (postprandial) hypoglycaemia	Not recommended in AP-FGID
H_2-glucose BT	1 g/kg with maximum of 50 g	SIBO	Known or suspected (postprandial) hypoglycaemia	Recommended for diagnosis of SIBO
H_2-lactulose BT	10 g	SIBO, OCTT	Known or suspected (postprandial) hypoglycaemia	Recommended for diagnosis of SIBO, not recommended to measure OCTT
H_2-sorbitol BT or ^{13}C-sorbitol BT	0.2 g/kg	Small bowel damage with a relevant reduction of absorption surface	Hereditary fructose intolerance; known or suspected (postprandial) hypoglycaemia	Not recommended to assess small bowel damage
H_2-sucrose BT	Not defined	CSID		Not recommended to diagnose CSID
H_2-mannitol BT	Not defined	Small bowel damage with a relevant reduction of absorption surface		Not recommended to assess small bowel damage
^{13}C-xylose BT	1 g	Small bowel damage with a relevant reduction of absorption		Not recommended to assess small bowel damage

		surface		
^{13}C-urea BT	50 mg <50 kg; 75 mg >50 kg	H. pylori gastritis	Within 4 to 6 weeks after antibiotic treatment and within 2 after weeks PPI treatment	Recommended to assess success of *H. pylori* eradication therapy
^{13}C-sorbitol BT	0.2 g/kg with a maximum of 10 g	Celiac disease		Not recommend for diagnosis or follow up of coeliac disease
^{13}C-mixed triglyceride BT	10-20 mg/kg <30 kg; 5 mg/kg >30 kg in liquid test meal with 0.7 g/kg fat	pancreatic exocrine insufficiency		Recommended for diagnosis and therapeutic monitoring of exocrine pancreatic insufficiency

[1]relative contraindications for H2-breath tests are: antibiotics, bowel cleansing, and probiotic treatment in the last 4 weeks.

AP-FGID: abdominal pain-related functional gastrointestinal disorders; BT: breath test; CSID: Congenital sucrose-isomaltase deficiency; OCTT: oro-caecal transit time; PPI: proton pump inhibitor; SIBO: small intestinal bacterial overgrowth.