European Review for Medical and Pharmacological Sciences 2013; 17(Suppl 2): 2-10

Intestinal gas production and gastrointestinal symptoms: from pathogenesis to clinical implication

F. SCALDAFERRI[1], O. NARDONE[1], L.R. LOPETUSO[1], V. PETITO[1], S. BIBBÒ[1], L. LATERZA[1], V. GERARDI[1], G. BRUNO[1], I. SCOLERI[1], A. DIROMA[1], A. SGAMBATO[2], E. GAETANI[1], G. CAMMAROTA[1], A. GASBARRINI[1]

[1]Medical Pathology, Department of Internal Medicine, Gastroenterology Division, Policlinico Universitario A. Gemelli, Catholic University of Sacred Heart, Rome, Italy
[2]General Pathology, Policlinico Universitario A. Gemelli, Catholic University of Sacred Heart, Rome, Italy

Abstract. **Intestinal gases are the expression of metabolic activity of gut microbiota in the gut, particularly carbohydrates in the case of H_2, CH_4. Alterations in composition of gases and air handling, directly or upon challenge with food are relevant for GI and extra-GI diseases. Assessing gas composition in breath can be a very useful tool for clinic, but technical issues are crucial (breath sampling, storing and analyzing).**

Aim of the present review is to summarize the understanding of the importance of intestinal gases in gastro-intestinal physiology and patho-physiology. Practical considerations on how to collect samples and instruments available for the clinic have also been provided.

Keywords:
Methane, Hydrogen, Carbohydrates, Breath test, Gut microbiota, Intestinal gases.

Introduction

The presence of hydrogen and methane in intestinal lumen has been suspected since 1816, when Magendie hypothesized that these gases were present in the intestine of guilotined convicts[1].

Later on, the report of explosions during colonic surgery supported the notion that the gut may contain combustibles gas[2]. Colonic gas explosion, although rare, is one of the most frightening iatrogenic complications during colonoscopy with electrocautery[3]. The explosion results from the accumulation of colonic gases at explosive concentrations and may be prevented by meticulous bowel preparation. Three factors are involved to trigger an explosion of colonic gases: 1) presence of combustible gases (hydrogen and methane) produced by colonic bacteria fermentation of non-absorbable carbohydrates; 2) presence of combustive gas (oxygen), and 3) application of a heat source (electrocautery or argon plasma coagulation). Concentrations of hydrogen more than 4% and/or methane more than 5% are considered potentially explosive. Almost half of the patients (42.8%) with unprepared colon have potentially explosive concentrations of hydrogen and methane. Nevertheless, an explosion may occur only when the oxygen concentration is over 5%. So adequate colonic cleansing is crucial for the safety of this procedure[4].

Earlier than 1980's mannitol was considered as the reference agent for colonic preparation. In 1979 the first colonic explosion was reported during colonoscopic polypectomy after mannitol preparation and its use is now avoided as cleansing colonic solution[2].

Since 1990 a major progress occurred with new agents for bowel cleaning such as polyethylene glycol electrolyte lavage solution (PEG-ELS) or oral sodium phosphate (NaP) solutions, that provide a climate safe for electrocautery during colonoscopy and enema preparation to choose for the lesions located up to the level of sigmoid colon and needed electrocautery[4].

In 1992, a prospective study on 30 patients undergoing flexible sigmoidoscopy after phosphoda enemas and 30 patients undergoing colonoscopy after PEG-ELS preparation, showed that the concentrations of hydrogen and methane remained below combustible levels in all patients in the PEG-ELS group, while three out of 30 patients (10%) had combustible levels of either hydrogen

Corresponding Author: Franco Scaldaferri, MD; Ph.D; francoscadaferri@gmail.com

or methane in the phosphoda enemas group. So, due to the clinically significant risk of explosion, electrocautery should not be performed during routine flexible sigmoidoscopy after the standard phosphosoda enema preparation. Another important observation of this elegant study was that during colonoscopy even segments of colon with excess retained stools did not have combustible levels of these two gases. It was conceivable that insufflations of air during colonoscopy equalized the distribution of combustible gases, overcoming the compartmentation of the colon[3].

A systematic review of the medical research published from 1952 to October 2006 reported a total of 20 cases of colonic gas explosion, eleven cases during surgery and 9 cases during colonoscopy.

Argon plasma coagulation provided the initiating heat source in five of the nine colonoscopic cases whereas the remaining four cases were associated with endoscopic polypectomy. Nine out of 20 cases (45%) of gas explosion were complicated with colon perforation. Perforation was observed in all of the four polypectomy cases, using argon plasma coagulation in two cases and electrosurgery in three cases. Bowel preparation by ingestion of a mannitol solution was used in 14 cases and sorbitol solution in one case. Preparation by enemas contain no fermentable agent was used in all five cases treated with argon plasma coagulation.

Argon plasma coagulation carries an increased risk of explosion during sigmoidoscopy following enemas, and it should only be performed after full bowel preparation. Cleansing purgatives (PEG,NaP) that make the bowel safe for electrocautery by decreasing the concentrations of the combustible gases are adequate for colon preparation while cleansing solutions containing mannitol or other malabsorbed carbohydrates (e.g. sorbitol) should be avoided since intracolonic concentrations of hydrogen or/and methane CH_4 could gain combustible levels. Even during standard enema preparation concentration of hydrogen or/and methane CH_4 could be at explosive levels and the presence of residual stools above the lesions could enhance gas production and explain gas explosion[4].

Intestinal gases characterization

Among the first complete reports characterizing intestinal gases contents, it should be mentioned the important work of Levitt and Kirk[5,6].

They identified five major components of intestinal gases (estimated concentration is reported in brackets)[5,6]:

- Nitrogen – N_2 (23 to 80%)
- Oxygen – O_2 (0.1 to 2.3%)
- Hydrogen – H_2 (0.06 to 47%)
- Methane – CH4 (0 to 26%)
- Carbon dioxide – CO_2 (5.1 to 29%)

Hydrogen and methane are the two major combustible gases found in the normal colon.

They are produced in the colonic lumen from fermentation of non absorbable (e.g. lactulose, mannitol) or incompletely absorbed (lactose, fructose, sorbitol) carbohydrates by the colonic flora, from air swallowing (i.e. absence of the gastric bubble in subjects with advanced achalasia), from CO_2 produced by interaction of bicarbonate and acid in duodenum, and from diffusivity of a gas across the mucosa of the gastro intestinal tract (CO2 diffuses much more rapidly than H_2, CH_4, N_2, and O_2)[7].

Since 1974 it is known that no mammalian cell is capable of producing H_2 or CH_4, but bacteria do it by fermentation of appropriate substrates under anaerobic conditions[1].

In this light 64 strains of intestinal bacteria were cultured under anaerobic conditions in lactulose-containing media to assess their ability to ferment lactulose. Some organisms were unable to metabolize disaccharide, while others, e.g. clostridia and lactobacilli, extensively metabolized lactulose.

Intestinal gases, however, are not the only metabolites originating from bacterial fermentations of indigestible carbohydrates. Qualitative analyses of the fermentation products in vitro indicated that the major non-gaseous metabolites were acetic, lactic and butyric acids, that are characteristically produced by clostridia. Bacteroides predominantly metabolized lactulose to acetic and succinic acids, but produced smaller quantities of higher fatty acids during lactulose fermentation than with basal medium alone. Hydrogen and carbon dioxide were the only gases detected[8,9].

Starting from this settings, it is easy to understand that hydrogen and methane are just two components of the complex activity of the metabolic gut microbiota activity involving "indigestible" carbohydrates which are part of the human diet.

Hydrogen as cause of intestinal discomfort

In 1974, Newman et al found that after feeding baked beans to volunteers, H_2 appeared in exhaled breath and that the rise in breath H_2 concentration paralleled the subjects' abdominal discomfort.

In vitro studies further demonstrated that fecal or ileal flora, incubated with various substrates produced striking amounts of CO_2 and H_2. When stacchyose, a sugar abundantly present in baked beans, was incubated with ileal or colonic flora as much CO_2 or H_2 were evolved as when glucose, galactose, or other common sugars were incubated. This was of particular interest since stacchyose is an oligosaccharide hydrolyzed by an enzyme not present in human intestine but possessed by enteric bacteria, that are able to split stacchyose into fermentable monosaccharides[1].

It is likely that the wind-producing potential of a food is related to its content of non-absorbable fermentable substrates, most probably oligosaccharidic and fibrous in nature.

As far as concern the diet, it is common folklore, verified by old studies, that apple, grape and prune juices, all-Bran cereals more than refined wheat or bland formula diets, soyabeans, lima beans are all gas inducer food; in contrast orange, apricot, pineapples and peanuts are poor gases inducers in humans[10,11].

Studies from same period showed that a minority of people would display an excessive production of gas because of carbohydrate malabsorption (e.g., lactose malabsorption or celiac disease)[7]: these studies brought over time the definition of carbohydrates malabsorption.

Both Levitt and Calloway, in fact, reported an excellent correlation between lactose tolerance tests and breath H_2 measurements after lactose ingestion. Levitt has shown that as little as 5 g of lactose was followed by a rise in breath H_2 in severely hypolactasic subjects, while Calloway has established that a rise in breath H_2 greater than 20 ppm after ingestion of 0.5 g lactose/kg was as accurate as a lactose tolerance test in diagnosing lactose malabsorption. In addition the amount of lactose absorbed was dose dependent and there was no detectable H_2 in breath in some lactase-deficient subjects when the test dose was halved, though, as showed by Levitt, some subjects were exquisitely intolerant to the sugar[1].

Methane as a metabolic pathway originating by hydrogen

Concerning CH_4, the world's population may be divided into CH_4 'producers' and 'non- CH_4 producers', with some familial tendency towards CH_4 production, but with no evidence that spouses share the propensity. Producers usually exhale a concentration of more than 23 ppm while 'non-producers' exhale less than 3 or 4 ppm. CH_4 production never begins before the age of 2. It was observed that the pattern of CH_4 exhalation is fairly constant in CH_4 producers over the course of a 24-hour day, thus apparently not depending on an exogenous substrate: in fact it was hypothesized and then demonstrated that CH_4 is generated under strictly anaerobic conditions as the result of the reduction of CO_2 with H_2, arising from the fermentative action of bacteria[1]. The main CH_4 producing organism in humans is *Methanobrevibacter smithii*, but other microorganisms in the human gut, such as certain Clostridium and Bacteroides species, are capable of producing CH_4[12,13].

It is estimated that the conversion of hydrogen in methane is a reaction associated to a clear reduction of intestinal gas volume: in fact 4 moles of hydrogen and 1 of CO_2 are metabolized in order to produce 1 mole of methane and 2 of water. In addition, if H_2 is not metabolized, the volume of gas accumulating in the gut will be substantially greater than if CH_4 is produced[14].

Different catabolic pathways of hydrogen within the gut

Hydrogen could be metabolized not just in methane by gut bacteria but also through a variety of other pathways, including sulphate reduction and acetogenesis[14].

An interesting paper assessed in vitro factors associated to a different catabolic activity. In this paper, stools were taken from 30 healthy subjects and incubated as 5% (w/v) slurries with Lintner's starch. On the basis of methanogenesis rates and numbers of sulphate reducing bacteria (SRB) in faces, the subjects were divided into two groups A that had less than 107 SRB/g dry weight faeces and B that had more than 107 SRB/g of faces. Most subjects (group A; n=23) shared high rates of fecal methanogenesis. In this group, 21 out of 23 subjects had methane in the breath. None of the subjects in group B (n=7) had methane in the breath and produced methane in vitro, while had high rates of sulphate reduction in feces and higher concentrations of sulphide. Considerable methane production occurred only when sulphate reducing bacteria were not active. The SRB were found using lactate as a source of carbon and energy and their counts showed a strongly positive association with H_2S concentrations in faces.

So sulphate reduction and methanogenesis seems to be mutually exclusive in the colon and

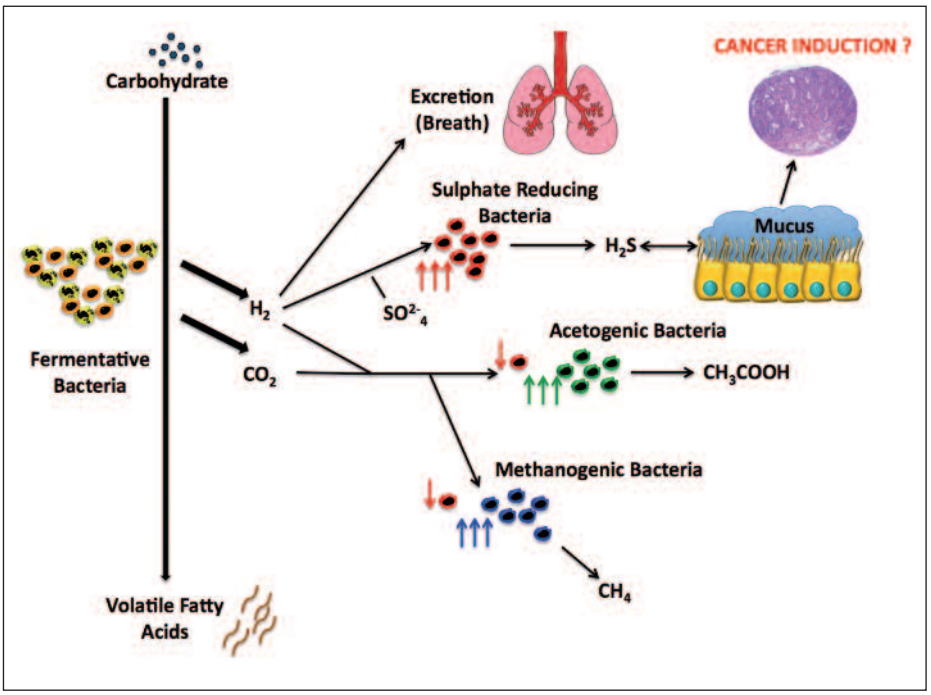

Figure 1. Intestinal gases as markers of catabolism indigestible carbohydrates by gut microbiota (fermentative bacteria). Main element produced by carbohydrate catabolism is hidrogen, which can stay as it is and exaled in the breath, utilized for H2S production or to produce acetate or methane. Volatile fatty acid are other important catabolites also produced from carbohydrates.

this is probably linked to sulphate availability. When sulphate is available, SRB are known to have higher substrate affinity for hydrogen and H_2S is produced.

In conditions of low sulphate availability methanogenic bacteria and acetogenic bacteria are able to combine H_2 with CO_2 to form methane and acetate respectively[15] (Figure 1).

Bjorneklett and Jenssen have shown that subjects who produce methane during fermentation, produce appreciably less H_2 in breath in response to a standard dose of lactulose.

Secondly, if H_2 is not further metabolized, fermentation may be incomplete and intermediates such as lactate, succinate, and ethanol are likely to accumulate[19] (Figure 1).

D-lactate, produced by colonic bacteria, is only partially metabolized in humans and can cause severe metabolic disturbance in certain situations.

The end products of these terminal oxidative reactions differ in their toxicity.

Methane is a harmless gas, readily expelled and acetate is absorbed and metabolized by peripheral tissues such as muscle, but H_2S is highly toxic and may poison colonic epithelial cells if not oxidized rapidly after absorption[15,16].

The capacity for high rates of H_2S production exists in some people and it may be that SRB play a part in the etiology of some intestinal and extra-intestinal disorders[15].

Indeed, disorders in H_2 and CH_4 pathways, with or without intestinal symptoms, have been also detected in several diseases, including endocrinological (thyroid, diabetes, etc), neurological (Parkinson disease, etc), auto-immune disorders (psoriasis, etc), infectious diseases and iatrogenic diseases (chemotherapy or surgery)[17-19].

Recent studies suggest that enteric bacteria play a crucial role in H_2 pathways dis-metabolism.

In fact H_2 breath tests are more frequently altered in subjects with irritable bowel syndrome (IBS), which also display several alteration in gut microbiota composition.

This concept was initially based on the common finding of an abnormal lactulose breath test, suggesting the presence of small intestinal bacterial overgrowth in IBS patients[20]. A meta-analysis by Shah showed that an altered breath test is more common in IBS patients compared to control subjects and the prevalence of abnormal breath test was even more significant when examining high quality aged and sex-matched studies[21]. The abnormal fermentation timing and dynamics of the breath test findings support a role for an abnormal intestinal bacterial distribution in IBS. However many bacteria in the gut utilize hydrogen gas for their energy source including methanogens and SRB. The presence of these bacteria can significantly impair the accurate detection of hydrogen[21,22].

Physiological and pathological implication related to gastro-intestinal gases

The volume of each gas within the intestinal lumen reflects the balance between the input and output of that gas. Input may result from swallowing, chemical reactions, bacterial fermentation, and diffusion from the blood, whereas output involves belching, bacterial consumption, absorption into the blood, and anal evacuation[23].

Measurements of intestinal gas volume, originally obtained using a body plethysmograph and later using a washout technique, indicated that the volume of intestinal gas in healthy subjects is approximately 200 mL[7]. Similar data have been reported using a specifically designed and validated computed tomography (CT) technique[24]. In the fasting state, the healthy gastrointestinal tract contains about 100 mL of gas, distributed almost equally among six compartments: stomach, small intestine, ascending colon, transverse colon, descending colon, and distal (pelvic) colon. Postprandially, the volume of gas increases by 65%, primarily in the pelvic colon[23]. Gas enters the stomach primarily via air swallowing and a sizable fraction is eructated. Some oxygen in swallowed air diffuses into the gastric mucosa. The reaction of acid and bicarbonate in the duodenum yields copious CO_2, which diffuses into the blood, while N_2 diffuses into the lumen down the gradient established by CO_2 production. In the colon, bacterial metabolism of fermentable substrates releases CO_2, H_2, and CH_4, as well as a variety of trace gases. Fractions of these bacteria-derived gases are absorbed and metabolized or excreted in expired air. In addition, a large proportion of H_2 is consumed by other bacteria to reduce sulfate to sulfide, CO_2 to acetate, and CO_2 to CH_4, thereby reducing the net volume of gas derived from bacterial metabolism. N_2 and O_2 diffuse from the blood into the colonic lumen down a gradient created by the production of gas by bacteria.

Gas ordinarily is propelled through the gastrointestinal tract and excreted per rectum.

The net result of these processes determines the volume and composition of intestinal gas[7,25,26].

Symptoms commonly attributed to too much gas, such as abdominal bloating and distention, are among the most frequently encountered gastrointestinal complaints[27,28].

Bloating refers to subjective sensations of a swollen abdomen, full belly, abdominal pressure, or excess gas. Abdominal distention refers to an objective increase in girth. Distention usually develops following meals or at the end of the day and resolves after an overnight rest. Some IBS patients, particularly those with rectal hypersensitivity, however, complain of bloating in the absence of objective distention[28].

A major question is to what extent subjective bloating and objective distention are associated with or caused by an increased rate of production or volume of intestinal gas[18].

The role of intestinal gas in functional abdominal pain has been studied since 1975.

By using a washout technique with intestinal infusion of an inert gas mixture in 12 fasting patients with chronic complaints, the volume of gas excess did not differ significantly from that of 10 controls. Similarly there was no difference in the composition or accumulation rate of intestinal gas. However, more gas tended to reflux back in to stomach in patients who complained of abdominal pain[7]. More recently Hernando-Harder et al[29] soughs to evaluate colonic gas accommodation, ileo-cecal competence, and colonic clearance in subgroups patients with abdominal bloating. Thirty-six patients complaining of abdominal bloating (12 constipation-predominant IBS (IBS-C), 12 diarrhea-predominant IBS (IBS-D), and 12 functional bloating) and 18 healthy controls were studied. Abdominal perception and girth were measured during: (i) 1h continuous infusion of gas at 24 ml/min into the rectum (accommodation period) and (ii) 30 min free rectal gas evacuation (clearance period). In eight patients and eight healthy subjects, the gas infused was labeled with radioactive xenon (74MBq 133Xe), and gas distribution was determined by scintigraphy.

The results indicate that colonic gas accommodation produced significantly more abdominal symptoms and distension in patients than in healthy subjects. Scintigraphy showed no differences in colonic gas distribution and no ileal gas reflux, but patients exhibited impaired gas clearance from the proximal colon, resulting in more residual gas perception and girth increment. The authors concluded that patients with abdominal bloating had normal colonic accommodation and ileo-cecal competence but impaired gas clearance from the proximal colon after retrograde infusion, in relation to bowel habit[29].

Bowel habit is strictly reliant on intestinal transit time[30].

Other reports make the story more complex showing that intestinal gases could display a direct effects on transit time. In particular three independent studies reported slower intestinal transit time in subjects with known production of methane compared to non methane producers[30-32].

Furthermore, the presence of methane on lactulose breath test among IBS patients is highly associated with constipation.

The role of methane in showering down the transit time was shown by Pimentel et al[33], using an interesting and well-characterized canine model. Briefly, two chronic small intestinal fistulas were created surgically, at 10 cm distal to the bile and pancreatic ducts and 160 cm (midgut fistula) from the pylorus. To test for the effect of gas on transit, room air or methane was delivered into the distal half of the gut. Luminal methane infusion reduced radioactive marker recovery in all dogs compared with room air by an average of 59%[33].

If it is true that methane is modifying gastrointestinal transit time it is also true, according to other reports that gastro-intestinal transit time could influence methane and gas production.

El Oufir et al, in fact, have investigated the relations between transit time, fermentation products and hydrogen consuming flora in healthy humans[34]. Eight healthy volunteers, four methane excretors and four non methane-excretors were studied for three week periods during which they received a controlled diet alone and then the same diet with cisapride or loperamide. At the end of each period mean transit time (MTT) was estimated and H_2 lactulose breath test was performed.

Cisapride and loperamide induced MTT changes but did not affect the number of viable anaerobes per g of faces. Cisapride administration induced a significant decrease in MMT and a significant increase in H_2 excretion in breath while methane excretion was significantly reduced during cisapride administration. No significant effect in H_2 excretion but significant methane excretion was observed with loperamide administration. The authors concluded that MTT was inversely related to the volume of H_2 excreted in breath test after lactulose ingestion. Methane excretion in breath was at a higher level during loperamide administration while the volume of exhaled H_2 was hardly reduced[34].

Measuring intestinal gases in clinics

Three methods are currently available for the measurement of intestinal gases in humans:

1. *in vitro* by fecal culturing;
2. *in vivo* by analyzing rectal air and
3. *ex vivo* by breath analysis.

Breath analysis has a number of advantages as compared with others.

The diffusivity of a gas across the mucosa of the gastrointestinal tract depends on its solubility in water; for a given partial pressure difference, CO_2 diffuses much more rapidly than H_2, CH_4, N_2, and O_2. The rate and direction of diffusion of each gas is a function of the diffusivity, partial pressure difference between lumen and blood, and exposure of the gas to the mucosal surface.

H_2 and CH_4 absorbed from the bowel are not metabolized thus excreted in expired air, and breath analysis provides a simple means of assessing the volume of these gases in the gastrointestinal tract because it equals the their rate of absorption[35].

H_2 excretion contained in the breath is the results of the alveolar ventilation rate and alveolar H_2 concentration. Over the last few years breath test analysis tried to interpret the finding of several gases and products not mentioned in this review, but the lack of standardized systems of sampling made difficult to interpret the results[36].

H_2, CO_2 and CH_4 measurement, on the contrary, are commonly measured through relatively well-standardized procedure and technical instrumentation.

The correct measurement of these gases, however, needs to consider pulmonary physiology and in particular the assumption that blood concentration, which is in equilibrium with intestinal concentration of the gases, is in equilibrium with alveolar concentration of gases.

Exhaled air is a mixture of alveolar air and ambient air retained in the respiratory dead space. Alveolar air is a part of exhaled air, which has been in contact with blood inside alveoli. Dead space is the volume of air which is inhaled that does not take part in the gas exchange, either because it remains in the conducting airways (anatomical dead space) and it reaches alveoli that are not perfused or poorly perfused (physiological dead space)[37,38]. This volume is equal to approximately 2 mL/kg of body weight and with a normal 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.

In order to facilitating the collection of alveolar air, three collecting systems have been developed: the modified Haldane-Priestley tube, the Y-piece device, bag syringe system and the two-bag system. Their comparison did not show any significant difference in terms of accuracy[37,39].

CO_2 levels in alveolar air are stable around 5%, so this parameter can be considered a marker of correct sampling and normalization of breath hydrogen values to an alveolar concentration using the observed carbon dioxide concentration[38].

The crucial issue of the correct sampling, regardless of the utilized protocol, has been nicely shown[37]. Variability of duplicate measurements of hydrogen, methane, carbon monoxide and carbon dioxide were assessed comparing 4 different respiratory techniques:

a) 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.

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.

Available technologies to measure breath H_2, CO_2 and CH_4 and practical tricks

Hydrogen measurement in breath may be performed by two main types of gas-chromatographs: dedicated and non-dedicated.

Standard gas-chromatographs 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 simple, dedicated instruments can be stationary or portable.

Stationary dedicated gas-chromatographs with solid state sensor represent the gold standard for hydrogen determinations in breath, as they were previously validated in comparison with non-dedicated instruments, and tested in terms of linearity and reproducibility of results.

Solid state sensor is stable, accurate but costly, need time after starting the machine, while electrochemical sensor has good accuracy, it is ready to use and it is risk for drifting over time, which alters accuracy of me[36]. 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[42].

Good stationary dedicated gas-chromatographs with elettrochemical sensor have been developed mainly to reduce costs related to gases analysis. Elettrochemical sensor is based on electrochemical cells first proposed by Ross et al, then evaluated as prototype demonstrating good reproducibility[37,40,41].

Portable instruments adopt a technology closer to gas-chromatographs with elettrochemical sensor, and although precise in the origin, they require maintenance and need to be checked to ensure precision over time[37,40,41].

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[43,44].

Another definitely non-negligible source of variability of gas measurement is represented by the technique of breath sample storage[43].

Even if characterized by an appreciable stability, gas bags of Mylar-impregnated foil and gas-tight syringes are unsuitable when many samples have to be tested and also, they are too expensive. Hydrogen and methane may be preserved in vacutainer tubes as gaseous contaminants from either the silicone tube coating or the organic additives after sterilization by ionizing radiations.

Breath samples are currently stored in plastic syringes, an inexpensive method allowing the analysis of gases with no further handling[37].

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. It is also possible that the low temperature modifies the permeability of plastic syringes to gases, and is not able to reduce their diffusibility.

According to the consensus conference by Gasbarrini et al[36] the accuracy and the reproducibility of the test need a standardizing baseline. For example the use of antibiotics modifies the composition of colonic flora and may therefore be a cause of interference with the test results. 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. So the test should be made 4 weeks after suspension of antibiotics and colon cleaning procedures[37,45].

Concerning the diet, it is commonly recommended dinner without fermentable carbohydrates (rice, meat and olive oil) the night before the test because it usually normalizes baseline H_2 excretion. An oral cavity cleaning with clorexidina before substrate administration aim at the inactivation of bacterial flora thus preventing the increase in an early peak of breath hydrogen excretion which may cause false positive results[36].

Conclusions

Hydrogen, methane, H_2 and CO_2 are important gases contained within the gut lumen. They are the expression of the metabolic activity of gut microbiota upon indigestible carbohydrates. Hydrogen is the first to be produced and then physiologically utilized for methane production, reducing the gas volume and ensuring a rapid elimination of CO_2. It could be utilized in order to oxidize sulphide, which could be the result of the fermentation of proteic products. Hydrogen hyper-production in response to certain sugars like lactose could be considered the expression of an intolerance or a mal digestion of a sugar, with the following abnormal fermentation by gut microbiota. Alterations in intestinal gas pathways have been described for gastro-intestinal and also extra intestinal diseases.

Methane, on the other hand could be a marker or also a cause for a reduced gastro-intestinal transit time.

A full characterization of H_2 metabolic pathway, however, would need to consider also other gases and small chain fatty acid, but this last aspect is above the content of this review.

Measurement of these gases is a delicate issue, where the choice of appropriate instrument, the correct maintenance of it and the way of collecting breath sample could play a major role in invalidating results.

Conflict of interest

The Authors declare that they have no conflict of interests.

References

1) Newman A. Review. Breath-analysis tests in gastroenterology. Gut 1974; 15: 308-323.
2) Bigard MA, Gaucher P, Lassalle C. Fatal colonic explosion during colonoscopic polypectomy. Gastroenterology 1979; 77: 1307-1310.
3) Monahan DW, Peluso FE, Goldner F. Combustible colonic gas levels during flexible sigmoidoscopy and colonoscopy. Gastrointest Endosc 1992; 38: 40-43.
4) Ladas Sd, Karamanolis G, Ben-Soussan E. Review article: colonic gas explosion during therapeutic colonoscopy with electrocautery. World J Gastroenterol 2007; 13: 5295-5298.
5) Levitt MD, Bond JH Jr. Volume, composition, and source of intestinal gas. Gastroenterology 1970; 59: 921-929.
6) Kirk E. The quantity and composition of human colonic flatus. Gastroenterology 1949; 12: 782-794
7) Azpiroz F, Levitt MD. Intestinal GAS. Sleisenger and Fordtran's Gastrointestinal and Liver Disease, 9th ed, chapter 16, Saunders, Elsevier, 2010
8) Sahota Ss, Bramley Pm, Menzies Is. The fermentation of lactulose by colonic bacteria. J Gen Microbiol 1982; 128: 319-325.
9) Kaur A, Rose DJ, Rumpagaporn P, Patterson JA, Hamaker BR. In vitro batch fecal fermentation comparison of gas and short-chain fatty acid production using "slowly fermentable" dietary fibers. J Food Sci 2011; 76: H137-142.
10) Hickey CA, Calloway DH, Murphy EL. Intestinal gas production following ingestion of fruits and fruit juices. Am J Dig Dis 1972; 17: 383-389.
11) Le Marchand L, Wilkens LR, Harwood P, Cooney RV. Use of breath hydrogen and methane as markers of colonic fermentation in epidemiologic studies: circadian patterns of excretion. Environ Health Perspect 1992; 98: 199-202.
12) Miller TL, Wolin MJ. Enumeration of Methanobrevibacter smithii in human feces. Arch Microbiol 1982; 131: 14-18.
13) Pochart P, Lémann F, Flourié B, Pellier P, Goderel I, Rambaud JC. Pyxigraphic sampling to enumerate methanogens and anaerobes in the right colon of healthy humans. Gastroenterology 1993; 105: 1281-1285.
14) Gibson GR, Cummings JH, Macfarlane GT, Allison C, Segal I, Vorster HH, Walker ARP. Alternative pathways for hydrogen disposal during fermentation in the human colon. Gut 1990; 31: 679-683.
15) Mckay LF, Eastwood MA, Brydon WG. Methane excretion in man—a study of breath, flatus, and faeces. Gut 1985; 26: 69-74.
16) Flourie B, Pellier P, Florent C, Marteau P, Pochart P, Rambaud JC. Site and substrates for methane production in human colon. Am J Physiol 1991; 260(5 Pt1): G752-757.

17) Belson A, Shetty AK, Yorgin PD, Bujanover Y, Peled Y, Dar MH, Reif S. Colonic hydrogen elimination and methane production in infants with and without infantile colic syndrome. Dig Dis Sci 2003; 48: 1762-1766.
18) Sahakian AB, Jee SR, Pimentel M. Review article: methane and the gastrointestinal tract. Dig Dis Sci 2010; 55: 2135-2143
19) Bjorneklett A, Jenssen E. Relationships between hydrogen (H_2) and methane (CH_4) production in man. Scand J Gastroenterol 1982; 17: 985-992.
20) Youn YH, Park JS, Jahng JH, Lim HC, Kim JH, Pimentel M, Park H, Lee SI. Relationships among the lactulose breath test, intestinal gas volume, and gastrointestinal symptoms in patients with irritable bowel syndrome. Dig Dis Sci 2011; 56: 2059-2066.
21) Shah ED, Basseri RJ, Chong K, Pimentel M. Abnormal breath testing in IBS: a meta-analysis. Dig Dis Sci 2010; 55: 2441-2449.
22) Levitt MD, Furne J, Springfield J, Suarez F, Demaster E. Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa. J Clin Invest 1999; 104: 1107-1114.
23) Perez F, Accarino A, Azpiroz F, Quiroga S, Malagelada Jr. Gas distribution within the human gut: effect of meals. Am J Gastroenterol 2007; 102: 842-849.
24) Mc Williams SR, Mc Laughlin PD, O'connor OJ, Desmond AN, Ní Laoíre A, Shanahan F, Quigley EM, Maher MM. Computed tomography assessment of intestinal gas volumes in functional gastrointestinal disorders. J Neurogastroenterol Motility 2012; 18: 419-425.
25) Jones MP. Bloating and intestinal gas. Curr Treat Options Gastroenterol 2005; 8: 311-318.
26) Suarez F, Furne J, Springfield J, Levitt M. Insights into human colonic physiology obtained from the study of flatus composition. Am J Physiol 1997; 272(5 Pt 1): G1028-1033.
27) Lasser RB, Bond JH, Levitt MD. The role of intestinal gas in functional abdominal pain. N Engl J Med 1975; 293: 524-526.
28) Di Stefano M, Miceli E, Missanelli A, Mazzocchi S, Tana P, Corazza GR. Role of colonic fermentation in the perception of colonic distention in irritable bowel syndrome and functional bloating. Clin Gastroenterol Hepatol 2006; 4: 1242-1247.
29) Hernando-Harder AC, Serra J, Azpiroz F, Milà M, Aguadé S, Malagelada C, Tremolaterra F, Villoria A, Malagelada JR. Colonic responses to gas loads in subgroups of patients with abdominal bloating. Am J Gastroenterol 2010; 105: 876-882.
30) Jahng J, Jung IS, Choi EJ, Conklin JL, Park H. The effects of methane and hydrogen gases produced by enteric bacteria on ileal motility and colonic transit time. Neurogastroenterol Motil 2012; 24: 185-190.
31) Lewis S, Cochrane S. Alteration of sulfate and hydrogen metabolism in the human colon by changing intestinal transit rate. Am J Gastroenterol 2007; 102: 624-633.
32) 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.
33) Pimentel M, Lin Hc, Enayati P, van den Burg B, Lee HR, Chen JH, Park S, Kong Y, Conklin J. Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity. Am J Physiol Gastrointest Liver Physiol 2006; 290: G1089-1095.
34) El Oufir L, Flourie B, Bruley DES Varannes S, Barry JL, Cloarec D, Bornet F, Galmiche JP. Relations between transit time, fermentation products, and hydrogen consuming flora in healthy humans. Gut 1996; 38: 870-877.
35) Mckay LF, Holbrook WP, Eastwood MA. Methane and hydrogen production by human intestinal anaerobic bacteria. Acta Pathol Microbiol Immunol Scand B 1982; 90: 257-260.
36) Gasbarrini A, Corazza GR, Gasbarrini G, Montalto M, Di Stefano M, Basilisco G, Parodi A, Usai-Satta P, Vernia P, Anania C, Astegiano M, Barbara G, Benini L, Bonazzi P, Capurso G, Certo M, Colecchia A, Cuoco L, Di Sario A, Festi D, Lauritano C, Miceli E, Nardone G, Perri F, Portincasa P, Risicato R, Sorge M, Tursi A; 1st Rome H2-Breath Testing Consensus Conference Working Group. First Rome H2-Breath Testing Consensus Conference Working Group. Methodology and indications of H2-breath testing in gastrointestinal diseases: the Rome Consensus Conference. Aliment Pharmacol Ther 2009; 29(Suppl 1): 1-49.
37) Di Stefano M, Certo M, Colecchia A, Sorges M, Perri F. H2-breath tests: methodological audits in adults and children. Aliment Pharmacol Ther 2009; 29(Suppl 1): 1-49.
38) Levitt MD, Ellis C, Furne J. Influence of method of alveolar air collection on results of breath tests. Dig Dis Sci 1998; 43: 1938-1945.
39) Buszewski B, Kesy M, Ligor T, Amann A. Review article: human exhaled air analytics: biomarkers of diseases. Biomed Chromatogr 2007; 21: 553-566.
40) Ross LF. Gas chromatographic technique to simultaneously quantitate the gases produced by intestinal microorganisms from fermentation mixtures. J Chromatogr 1987; 414: 405-410.
41) Tsuji K, Shimizu M, Nishimura Y, Nakagawa Y, Ichikawa T. Simultaneous determination of hydrogen, methane and carbon dioxide of breath using gas-solid chromatography. J Nutr Sci Vitaminol (Tokyo) 1992; 38: 103-109.
42) Ellis CJ, Kneip JM, Levitt MD. Storage of breath samples for H2 analyses. Gastroenterology 1988; 94: 822-824.
43) Ross LF. Gas chromatographic technique to simultaneously quantitate the gases produced by intestinal microorganisms from fermentation mixtures. J Chromatogr 1987; 414: 405-410.
44) Tsuji K, Shimizu M, Nishimura Y, Nakagawa Y, Ichikawa T. Simultaneous determination of hydrogen, methane and carbon dioxide of breath using gas-solid chromatography. J Nutr Sci Vitaminol (Tokyo) 1992; 38: 103-109.
45) Strocchi A, Ellis C, Levitt MD. Reproducibility of measurements of trace gas concentrations in expired air. Gastroenterology 1991; 101: 175-179.