Effects of dietary nutrients on volatile breath metabolites

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Abstract

Breath analysis is becoming increasingly established as a means of assessing metabolic, biochemical and physiological function in health and disease. The methods available for these analyses exploit a variety of complex physicochemical principles, but are becoming more easily utilised in the clinical setting. Whilst some of the factors accounting for the biological variation in breath metabolite concentrations have been clarified, there has been relatively little work on the dietary factors that may influence them. In applying breath analysis to the clinical setting, it will be important to consider how these factors may affect the interpretation of endogenous breath composition. Diet may have complex effects on the generation of breath compounds. These effects may either be due to a direct impact on metabolism, or because they alter the gastrointestinal flora. Bacteria are a major source of compounds in breath, and their generation of H₂, hydrogen cyanide, aldehydes and alkanes may be an indicator of the health of their host.

Key words: Breath analysis: Selected ion flow tube-MS: Macronutrients: Micronutrients: Gut flora

Historical background

The relationship between breath composition and health has been known for many centuries. More than 2500 years ago, the Greek physician, Hippocrates of Cos noted the importance of breath smell in the diagnosis of liver disease, using the term ‘foetor hepaticus’ to describe the characteristic breath odour associated with liver failure (Treatise on Breath Odour and Disease, 5th century BC).

The ancient Persian physician and philosopher, Ibn Sina (Avicenna) wrote that “…it is the role of the vital force (breath) to maintain a perfect equilibrium within the elements of the body, and between the elements of the body and the environment’ (The Canon of Medicine, 10th century). An important environmental determinant of breath composition is diet. Approximately 40 years ago, Pauling et al.(1) investigated the relationship between breath composition and diet and recognised the potential impact of intestinal flora as a contributing factor to breath composition. Individuals were placed on a defined elemental diet, consisting almost entirely of small molecules that the authors assumed would be absorbed from the upper gastrointestinal tract, and that intestinal flora would be reduced in the lower gastrointestinal tract because of the lack of nutrients reaching them. Using temperature-programmed gas–liquid partition chromatography, the quantitative determination of about 250 substances in a sample of human breath was possible at that time.

Introduction

Today, using exquisitely sensitive analytical techniques, more than 500 compounds have been reproducibly identified in exhaled breath(2), though as many as 3000 different compounds recognised the potential impact of intestinal flora as a contributing factor to breath composition. Individuals were placed on a defined elemental diet, consisting almost entirely of small molecules that the authors assumed would be absorbed from the upper gastrointestinal tract, and that intestinal flora would be reduced in the lower gastrointestinal tract because of the lack of nutrients reaching them. Using temperature-programmed gas–liquid partition chromatography, the quantitative determination of about 250 substances in a sample of human breath was possible at that time.

Abbreviations: ppbv, parts per billion by volume; PTR, proton transfer reaction; SIFT, selected ion flow tube; VOC, volatile organic compounds.

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have been sporadically detected in breath of different individ-
uals\(^{3,4}\). It is now possible to measure volatile organic com-
pounds (VOC) in breath with great sensitivity (down to parts
per billion by volume; ppbv) and specificity, using MS and
related analytical methods. As a consequence, breath analysis
now has a number of well-established clinical applications\(^5\)
(Table 1). It also has enormous potential value in metabolic
research, particularly when combined with stable isotope label-
ing. It has, for example, been used in kinetic studies of amino
acid metabolism\(^6\). Breath analysis may also be used for appli-
cations that would otherwise be difficult using other techniques,
for example in the assessment of whole-body oxidative stress\(^7\),
or cholesterol biosynthesis\(^8\). Breath analysis may also be a use-
ful adjunct to blood and faecal analysis in the investigation of
gut microbiota\(^9\). The present review briefly outlines the phys-
iological and dietary factors that may have an important impact
on breath compounds and the methods used for assessing
them, together with the reported concentrations of these com-
pounds in health and disease.

### Sources of volatile metabolites in exhaled breath

Volatile metabolites in exhaled breath are derived from several
sources: they may be derived from the environmental inspired
air, from cells, including micro-organisms that are located
throughout the oral/nasal cavities and the pulmonary system,
the upper and lower gastrointestinal tracts and from general
human metabolism (Fig. 1\(^{10-12}\)). For example, NO is present

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**Table 1.** Established and emerging clinical applications of breath analysis

| Breath analysis                                                                 | References                                                                                     |
|--------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| Breath H\(_2\) test for carbohydrate metabolism                                | Rumessen et al.\(^{(137)}\); Romagnuolo et al.\(^{(138)}\); Eisenmann et al.\(^{(139)}\); Bond & Levitt et al.\(^{(140,141)}\)|
| Breath NO test to monitor therapy for asthma                                   | Eisenmann et al.\(^{(139)}\); Taylor et al.\(^{(142)}\)                                       |
| Breath CO test for neonatal jaundice                                          | Stevenson et al.\(^{(143)}\)                                                                   |
| Breath test for diagnosis of *Helicobacter pylori*                            | Romagnuolo et al.\(^{(138)}\)                                                                 |
| Breath test for heart transplant rejection                                     | Phillips et al.\(^{(144)}\)                                                                    |
| Breath NH\(_3\) has been identified as an indicator of the efficacy of renal dialysis | Endre et al.\(^{(145)}\); Rolla et al.\(^{(146)}\); Narasimhan et al.\(^{(147)}\)               |
| Breath H\(_2\) and the \(^{13}\)CO\(_2\):\(^{12}\)CO\(_2\) ratio (following the ingestion of \(^{13}C\)-labelled compounds) as related to gastric emptying and bowel transit times | Bond & Levitt et al.\(^{(140)}\); Braden et al.\(^{(148)}\); Rao et al.\(^{(149)}\); Geboes et al.\(^{(150)}\) |
| Hydrogen cyanide is released by the pathogen, *Pseudomonas aeruginosa*, and the detection of high concentrations of hydrogen cyanide in breath may be used for the early detection of bacterial infection of children with cystic fibrosis | Shrestyshka et al.\(^{(151)}\); Carroll et al.\(^{(152)}\)                                       |

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**Fig. 1.** The complex interactions between diet and expired breath metabolites.
Physiological variations in breath composition

Site of exhaled breath sampling

The measured concentrations of several exhaled breath constituents differ significantly depending on their site of breath sampling; whether from the mouth, nose, or the static oral cavity. Some of these compounds, for example NH3, ethanol and hydrogen cyanide, are predominantly generated in the oral cavity in healthy subjects\(^{(15,16)}\). Hence oral health, including periodontal and dental disease, are potential confounding factors\(^{(17)}\). It has been shown that concentrations of some compounds in the exhaled breath, for example NH3 and ethanol, can be increased by sugar and urea mouth washes\(^{(18)}\). Hence, without careful preparation, mouth production of these and other compounds can compromise the quantification of endogenous trace compounds present in the alveolar breath. However, the concentrations of both the urea and sucrose solutions used in these latter studies that proved the enhancement of NH3 and ethanol levels were greater than normally present in food and beverages; thus in most situations such severe enhancements will not occur\(^{(18)}\). It is also possible to simultaneously monitor mouth and nasal concentrations of breath compounds to elucidate their source\(^{(19)}\). Furthermore, it is possible to sample end-tidal gas only\(^{(20)}\) by physically filtering gas from the oral cavity (for example, by using buffered end-tidal sampling), or by data processing post hoc\(^{(20,21)}\).

Determinants of inter-individual and intra-individual variation

Intra-individual studies that have been carried out over about 30 d have revealed the temporal variations in the concentrations of several common breath metabolites for several individuals, including: NH3, acetone, isoprene, ethanol and acetaldehyde\(^{(11,22)}\). Breath NH3, acetone and isoprene concentrations were reported to have CV of typically 0.3 over this period. No obvious correlations were found in the distributions of these particular metabolites, except that the NH3 levels were greatest in the breath of the oldest subjects\(^{(22)}\). In population (inter-individual) studies over a longer time-frame (6 months), breath methanol levels appeared to have a log-normal distribution for the study population, and did not correlate with age, breath ethanol or ethanol consumed in the previous 24 h; however, there was an inverse correlation with BMI\(^{(23)}\). Breath NH3 increased with age, and a weak but significant correlation between breath propanol and acetone levels was reported\(^{(24)}\). Breath isoprene concentrations have been studied in healthy schoolchildren between 7 and 18 years of age, and in this group there was a strong positive association with age\(^{(25)}\), possibly related to growth, or steroid hormone biosynthesis.

Fasting and the acute effects of feeding on breath content

Effects of the fasted or fed state on breath constituents are complex. Breath acetone, NH3, ethanol, isoprene and methanol have been measured during single exhalations whilst fasting and following feeding with a liquid protein-energy meal\(^{(26)}\). Breath acetone concentrations fell from a maximum during fasting, reaching their nadir between 4 and 5 h after feeding. Changes in breath NH3 concentrations were biphasic, possibly related to changes in portal blood flow, with a rapid fall to approximately 50 % of their fasting levels before rising to two or three times their baseline values at 5 h\(^{(20)}\). A brief increase in breath ethanol concentrations was found after feeding, and this is probably related to the ethanol content of some foods. Subsequently, breath ethanol levels remained low throughout the experimental period. Isoprene concentrations did not change significantly\(^{(26)}\). Levels of breath ethanol increased if a sweet drink or food had been consumed within 2 h before providing a breath sample, but surprisingly no increase in breath ethanol was apparent when modest alcohol consumption had occurred the previous evening. Endogenous breath ethanol and acetaldehyde levels were not significantly correlated with each other\(^{(27)}\). It has recently been reported that breath hydrogen cyanide may rise following the consumption of food or drink\(^{(28)}\).

Effect of exercise and the breath cycle on breath content

Alveolar breath isoprene and methyl acetate have been reported to increase immediately after moderate exercise, returning to baseline soon thereafter\(^{(29,30)}\). We have recently reported that breath isoprene concentrations rise rapidly after commencing exercise, and then decrease during the period of exercise. Plasma cholesterol levels were not obviously correlated with isoprene concentration in breath. Also, isoprene levels were not found to be directly related to sex, age or BMI in this study of adults\(^{(30)}\). The changes in breath isoprene during exercise have been attributed to changes in tissue fractional perfusion\(^{(31)}\), and the changes in expiratory breath NO observed during exercise have been reported to be due to changes in air flow rate, rather than increased NO production\(^{(32)}\). The exercise-related changes in NH3 concentrations in breath exhaled via the nose appear to vary with age, with a several-fold increase in concentrations persisting into the post-exercise period\(^{(31)}\). These changes may be dependent on renal function. Breath composition changes during the breathing cycle. For example, the variation observed in breath acetone appears to be dependent on exhaled volume, but not flow\(^{(33)}\).

Molecules directly or indirectly derived from food, beverages and medicines

Following the ingestion of some compounds, there are wide inter-individual variations in their appearance in the breath.
For example, following the ingestion of eucalyptol, a constituent of proprietary medications, its appearance in breath varies between 1 and 5 h after ingestion, showing wide inter-subject variations too. Green tea was very effective in reducing volatile sulfur compounds (hydrogen sulfide and methyl sulfide) in mouth breath, this being attributed to its disinfectant properties. The kinetics of the acute release of aromas from food or beverages is complex, being dependent on the physiological processes involved in swallowing, the lipid content of the food and the vapour pressure of the compound. There are a number of volatile compounds in food that may rapidly appear in the breath following their consumption.

Breath alkanes, smoking, other causes of oxidant stress and dietary antioxidants

Smoking is known to induce a state of oxidative stress that is associated with lipid peroxidation and has been shown to be associated with substantial changes in breath composition. Oxidative stress has the potential to damage cells, tissues and organs via the production of reactive oxygen species such as superoxide, H$_2$O$_2$ and the hydroxyl radical. Oxidative stress may be estimated through breath measurements of biomarkers that include ethane, ethylene and pentane. Although these hydrocarbons only represent a small and possibly variable proportion of the total amount of peroxidised PUFA, their determination in exhaled breath enables an assessment of oxidative stress in vivo. Do et al. have previously reported that non-smokers have very low baseline levels of ethane, whilst ethane production correlated with active (packs per d) and lifelong (pack-years) tobacco consumption. Miller et al. have reported similar findings and also report that breath ethane concentrations are related to the time interval between the last cigarette smoked and breath sampling. Do et al. have also shown that antioxidant vitamin supplementation resulted in attenuation of smoking-related lipid peroxidation with a significant decrease in breath ethane production. Aghdassi & Allard assessed oxidative stress using breath alkane output and other markers of lipid peroxidation in several conditions associated with inflammation, including smoking. Lipid peroxidation was significantly higher and antioxidant vitamin status significantly lower in smokers compared with non-smokers. β-Carotene or vitamin E supplementation significantly reduced lipid peroxidation, whilst vitamin C supplementation had no significant effect. These findings are consistent with those of Hoshino et al. and Allard et al.

In an animal model of vitamin E deficiency, the increased peroxidation of tissue lipids leads to an increased level of breath pentane. However, in their paper, Gelmont et al. reported that pentane production was also dependent on dietary linoleate. Breath pentane in the study animals was reduced by removal of linoleate from their diet, by starvation, antibiotic treatment or the addition of vitamin C to their food or water. Breath pentane was increased by the removal of vitamin E from the diet. The authors concluded that intestinal bacteria were a major source of breath pentane in addition to endogenous membrane lipid peroxidation. In recent studies we have found, using selected ion flow tube (SIFT)-MS, that breath pentane is elevated in patients with inflammatory bowel disease such as ulcerative colitis.

The effects of a restricted-energy diet have also been investigated in the rat model. A significant decrease in ethane generation was found in the rats receiving an energy-restricted diet compared with those fed ad libitum, supporting the hypothesis that energy restriction reduces the level of oxidative stress.

Breath pentane is derived from the oxidation of n-3 and n-6 fatty acids which appear to be transferred from mother to fetus during pregnancy. However, in women in their last trimester, who have smoked during pregnancy, it has been reported that breath ethane was higher than for a control group of non-smokers, and inversely related to serum vitamin C. Dietary n-3 fatty acid supplementation also appeared to increase lipid peroxidation as assessed by breath alkane output, and this was not prevented by co-administration of vitamin E.

Dietary studies

The effects of dietary constituents on breath composition are complex, as alluded to in Fig. 2. The acute effects of diet on breath have been described briefly above. Medium- and longer-term effects may be mediated by changes of flora in the gastrointestinal tract and direct or indirect effects on gastro-caecal transit time, together with effects on metabolism, systemic inflammation and redox state.

Effects of macronutrients and dietary energy restriction

High- and low-fat diets. Rosenkranz et al. have investigated the acute effects of a high-fat meal on pulmonary function and expiratory NO. They found that a high-fat meal was associated with increased expiratory NO, but had no effect on a systemic marker of inflammation, or pulmonary function in normal individuals, and the authors concluded that a high-fat diet may contribute to inflammation within the airway. Studies in patients with asthma have found that a diet containing a high n-6:n-3 fatty acid ratio was associated with worsening of asthma control and higher concentrations of NO in exhaled breath.

Ketogenic diets are high in fat, low in carbohydrate and contain adequate levels of protein. Under these conditions fat is metabolised in preference to carbohydrates, and ketone bodies (acetone, acetoacetate and β-hydroxybutyrate) are generated in the liver, leading to ketosis. In these circumstances, expiratory breath acetone concentrations are increased substantially. Even in healthy subjects, breath acetone has been reported to rise more than five-fold following a ketogenic diet. Breath acetone appears to be indicative of systemic ketosis associated with a ketogenic diet. Under certain circumstances acetone is reduced to isopropanol by hepatic alcohol dehydrogenase and this then also appears in the breath.
Simple carbohydrates and alcohol. H₂ breath tests have been used for the assessment of carbohydrate malabsorption and abnormal bacterial colonisation of the gut for many years. Basal breath H₂ is dependent on dietary carbohydrate. H₂ production in man is primarily dependent upon the delivery of ingested, fermentable substrates to an abundant intestinal flora that is normally present only in the colon. In the normal intestine, more than 99% of H₂ production appears to be of colonic origin, but small-bowel production may be increased in a patient with excessive numbers of small-bowel bacteria. H₂ breath tests are based on the fact that there is no source for H₂ gas in humans other than bacterial metabolism of carbohydrates. Respiratory H₂ excretion can therefore be used as an indicator of intestinal H₂ production. In carbohydrate tolerance tests, different carbohydrates are administered orally and the concentration of H₂ is measured in expired air. When defective sugar absorption is present, unabsorbed sugars are available in the colon for bacterial fermentation. Approximately 14% of the total H₂ production is excreted by the lungs, and rates of breath H₂ excretion and production correlates well. Smoking raises and exercise lowers H₂ concentrations and is therefore not allowed during these tests.

Oroecal transit time is increased in subjects with alcoholism, but it also appears to be increased among individuals who drink moderate amounts of alcohol as assessed by the H₂ breath test. Clearly, this has the potential to alter the occurrence of specific breath constituents and the overall postprandial breath profile. Alcohol is largely metabolised to acetaldehyde by dehydrogenase enzymes, leading to the appearance of high concentrations of acetaldehyde in the breath after alcohol consumption. Somatic cells and microbes representing normal human gut flora are also able to produce acetaldehyde from ethanol. After the ingestion of alcoholic beverages, there are high local acetaldehyde concentrations in the saliva, gastric juice and the contents of the large intestine. In addition, microbes may produce acetaldehyde endogenously in the absence of exogenous alcohol administration.

Complex carbohydrates and fibre. Complex carbohydrate and fibre increase gut transit time and therefore increase the quantity of fermentable, non-absorbed carbohydrate reaching the distal intestine, and hence increase the production of gut-derived H₂ and CH₄. In some groups of subjects, there appears to be an adaptation to high intakes of resistant starch over time and an apparent relationship with insulin sensitivity. Using breath H₂ analysis, Strocchi & Levitt found that 5–10% of starch in wheat, potatoes and maize is not absorbed by healthy subjects, while rice starch is nearly completely absorbed. The physiological effects of dietary fibre are not always predictable.
from their physicochemical properties. For example, maize fibre has been reported to resist fermentation better than potato fibre and to have a lower digestibility. However, both dietary fibres increased faecal output of DM, neutral sugars and water. Orocaecal transit time is increased by potato fibre, and it is reported to reduce postprandial plasma levels of total and esterified cholesterol. In contrast, maize fibre has been reported to lower fasting blood cholesterol concentrations and increase the non-esterified cholesterol ratio. A class of non-digestible but fermentable oligosaccharides, trans-galacto-oligosaccharides, was found to increase the concentration of breath H₂ and the N density of the faeces, whilst dietary fibre from maize, cassava and amaranth all increased faecal energy loss. Expired breath H₂ was highest for those individuals consuming maize or cassava. In critically ill patients receiving jejunal feeding with a semi-elemental diet, fibre supplementation appeared to improve microbiota mass and function, being associated with increased carbohydrate fermentation, measured as breath H₂ and CH₄.

Protein. The ingestion of a high protein-energy meal is associated with some complex changes in breath compounds; the changes in exhaled acetone, NH₃ and ethanol concentrations have been discussed above.

Effects of entire diets. A diet that is chronically energy restricted is associated with longevity, which is probably related to a reduction in oxidant stress, such a diet is also associated with low breath ethane concentrations, that again may relate to reduced oxidant stress.

Kundu et al. have found that the amount of breath acetone in exhaled breath was correlated with the rate of fat loss in subjects on a restricted-energy weight-loss programme.

Using a randomised controlled design of the effects of a diet rich in fruit and vegetables, with or without low-fat dairy products for 8 weeks’ duration, Miller et al. found that breath ethane was significantly reduced in patients on both fruit- and vegetable-rich diets, but particularly in subjects on a low-fat dairy diet. The endogenous production of methanol is increased after the consumption of fruit, its concentrations increasing by as much as an order of magnitude. This is thought to be due to the degradation of natural pectin (which is esterified with methyl alcohol) in the colon. In vitro studies showed that pectin in either a pure form or a natural form (in 1 kg of apples) induces a significant increase of methanol in the breath (and by inference in the blood) up to a level similar to that seen following the consumption of alcohol spirits.

Effects of pre- and probiotics. In vitro studies of isolated bacterial cultures have demonstrated that the VOC profile that they produce is distinctive, and may be used to differentiate bacterial species. Whilst some of these molecules, for example ethanol and acetone, are produced by their human host, other trace gases, for example H₂ and indole, are only produced in detectable quantities by bacteria. Furthermore, the gases emitted by bacteria also appear to be dependent on the strain of the bacterial isolate and culture conditions. Bartram et al. have reported that daily yogurt enriched with Bifidobacterium longum and 5 g lactulose/1 increased breath H₂ exhalation and mouth-to-caecum transit time.

SCFA are produced by bacterial fermentation of carbohydrates in the colon, influence gastrointestinal motility, and can affect motility at a distance from their site of production. The mechanisms of action of SCFA on gastrointestinal motility have not been completely elucidated. They may involve systemic humoral and neural pathways as well as local reflexes and myogenic responses.

Cellulose has a β-1,4 linkage, so it is resistant to hydrolysis by human small-intestinal disaccharidase and, hence, reaches the colon undigested. The excretion of breath H₂ gas after cellulose ingestion was found to be significantly greater than after glucose ingestion. In another study, prebiotic treatment increased breath H₂ excretion by 3-fold and reduced hunger. The AUC for plasma glucagon-like peptide 1 and the volatile release curve for breath-H₂ excretion measured after the meal were significantly correlated with each other.

Dietary micro nutrients. Micronutrients have the potential to affect redox status and prevailing inflammation, or they may have direct effects on constituents within breath. Furthermore, lung function (forced expiratory volume) appears to be related to dietary vitamin C and fruit intake, although the latter study was in children and so the findings may not be the same for adults. Increased concentrations of breath alkanes are associated with reduced antioxidant micronutrient status. Supplementation with a cocktail of antioxidant vitamins (vitamin C, vitamin E and β-carotene) has been reported to be associated with reduced breath pentane in smokers. In contrast, Fe supplements have been found to increase breath ethane concentrations in young women. The amount of breath dimethyl selenide has been reported to increase after the ingestion of Se supplements and substantial amounts are found in the breath of individuals with Se toxicity, and this may account for the characteristic breath odour in individuals with this condition.

Principles of measurement. The ability to accurately measure concentrations of trace gases in humid breath has only been possible in the last 20–30 years. GC-MS has been used widely used for breath analysis and continues to be vigorously exploited to great effect for this purpose. In GC-MS, breath samples are collected and volatile compounds extracted and pre-concentrated before offline analysis. Whilst GC-MS has allowed the identification of compounds in breath it is not possible to use this technique in real time. It is disturbed by the large amount of water vapour present in humid exhaled breath.
More recent analytical advances include SIFT-MS, proton transfer reaction (PTR)-MS and various optical spectroscopic or electronic ‘nose’ devices; these are techniques that have allowed real-time analysis of breath\(^{(120-123)}\). Spectroscopic detection methods have been designed to detect specific simple molecules of permanent gases, such as NO and ethane\(^{(121)}\) rather than a profile of VOC in breath, but are amenable to real-time applications. The physicochemical principle of electronic nose devices is that exposure of the detector to specific compounds is associated with a change in surface conductivity of the sensor; however, interpretation may be complicated for humid samples\(^{(123)}\) and they generally lack positive identification.

**Methods commonly used for breath analysis**

Those methods used for breath analysis mentioned above are briefly described below and summarised in Table 2. The most widely reported breath analytes are shown in Table 3, together with the methods used to detect them, their concentrations, sources and potential confounding factors.

**Ion mobility spectrometry**

The aim of ion mobility spectrometry is to identify trace gases by the mobility of their characteristic gas-phase ions or their derivatives in a buffer/carrying gas\(^{(124)}\). These ions are produced by exposing the carrier gas/tracer gas to a radioactive source or electrical discharge when chemical ionisation reactions result in the analytical drifting ions. The movement of these ions is dependent on their mass and molecular geometry, and their dwell times are used to characterise the original mixture of trace gases. Whilst this approach is not recommended for the identification of unknown compounds, it has been used to determine differences in breath metabolite profiles associated with specific diseases\(^{(123)}\).

**Proton transfer reaction-MS and proton transfer reaction-time of flight**

In these techniques, precursor hydronium ions (H\(_3\)O\(^+\)) are injected into the buffer gas, which is usually the gas sample to be analysed, and react with the trace gas present in the sample. The precursor ions react with the trace gas species, producing characteristic ion products that are detected and quantified using a down-stream analytical MS. PTR-MS is sensitive to down to and below ppbv\(^{(125)}\). The precursor molecules react with most trace gas molecules to produce a protonated molecule (MH\(^+\)). However, the latter nascent ion may be unstable for some compounds, for example, when M is an alcohol. Furthermore, when the carrier gas in PTR-MS is humid breath, this leads to the formation of cluster ions, for example H\(_3\)O\(^+\)(H\(_2\)O)\(_{1,2,3}\), that may make quantitative analysis more complex, although this cluster ion formation is inhibited by the presence of the axial electric field along the flow tube\(^{(126)}\). In PTR-time-of-flight analysis, ions are accelerated to uniform energy by an electric field, and subsequently traverse a defined distance. The time of flight of the ion is directly related to the ion’s mass/charge ratio, and this allows a mass resolution that is substantially better than for conventional PTR-MS\(^{(129)}\). Whilst the original instruments relied on long integration times to attain sufficient sensitivity, recently developed PTR-time of flight instrumentation has improved sensitivity\(^{(130)}\), with integration times of 1 s and a corresponding limit of detection approaching 100 parts per trillion for most compounds, allowing online breath analysis\(^{(129)}\).
Table 3. Summary of breath analytes with reported ranges and sources

| Compound            | Study                        | Number of subjects | Method                  | Concentration (ppbv) | Source                          | Comments                                                                 |
|---------------------|------------------------------|--------------------|-------------------------|-----------------------|--------------------------------|--------------------------------------------------------------------------|
| Acetaldehyde        | Diskin et al. (22)           | 5                  | SIFT-MS                 | Range 2–5             | Ethanol metabolism (160)       | Oral microbes                                                            |
|                     | Fuchs et al. (161)           | 12 lung cancer patients, 12 smokers, 12 healthy volunteers | GC-MS                   | Mean >200              | Carbohydrate metabolism, ambient air | No significant difference in exhaled acetaldehyde concentrations in all subject groups |
|                     | Turner et al. (27)           | 30                 | SIFT-MS                 | Mean 24 (range 0–104) | Ethanol metabolism            | Increased levels detected with consumption of sweet drinks/food 2 h before |
| Acetone             | Turner et al. (24)           | 30                 | SIFT-MS, H3O+O2+        | Mean 477 (range 148–2744) | Decarboxylation of acetoacetate, dehydrogenation of isopropanol Related to blood glucose in some studies (159) | Levels strongly influenced by physiological factors other than diet |
| Smith et al. (26)   |                              | 6                  | SIFT-MS                 | Pre-meal: range 200–600 5 h Post-meal: mean about 200 | | |
| NH₃                 | Diskin et al. (22)           | 5                  | SIFT-MS, H₂O + O₂+     | Range 422–2389         | Protein metabolism (160)       | Not much day-to-day variation in individuals |
|                     | Turner et al. (24)           | 30                 | SIFT-MS                 | Mean 833 (range 248–2935) | Protein metabolism            | Breath concentrations influenced by age and background air |
|                     | Španièl et al. (163)         | 6                  | SIFT-MS                 | Range 200–1750         | Protein metabolism            | Protein diet |
| Smith et al. (26)   |                              | 6                  | SIFT-MS                 | Pre-meal: range 300–600 5 h Post-meal: range 600–1800 | | |
| Allyl sulfides      | Rosen et al. (164)           | Simulated          | Thermal desorption GC-MS | | Garlic (165,166) | Allicin decomposes in gastric acid leading to the formation of allyl sulfides (165,166) |
| Carbon disulfide    | Phillips (169)               | 42                 | GC-MS                   | Range 0.005–0.008      | Atmospheric (169)              | |
|                     | Ciaffoni et al. (169)        | Laser absorption spectroscopy | | | Gut bacteria (170) | |
| Carbonyl sulfide    | Wysocki et al. (171)         | Simulated          | Pulsed quantum cascade-based sensor | | Gut bacteria (172) | |
|                     | Halmer et al. (172)          | Simulated          | Mid-cavity leak out spectroscopy | | | |
| CO                  | Middleton & Morice (179)     | 65                 | Electronic nose device | Non-smokers: mean 1830 | Haem catabolism (174) | Formation catalysed by haem oxygenase (179) and CO production is increased by haemolysis (174) |
|                     | Paredi et al. (175)          | 37                 | Electrochemical         | Non-smokers: mean 17400 | | |
|                     | Costello et al. (177)        | 10                 | Electrochemical detector | Non-smokers: mean 2100 (range 600–4900) Smokers: mean 12800 (range 8300–18700) | | |
| Compound               | Method                                                                 | Normal: mean 7-6 (so 0-6) | Methionine metabolism | Production is dependent on intestinal bacteria(170) |
|------------------------|------------------------------------------------------------------------|---------------------------|-----------------------|---------------------------------------------------|
| Dimethyl sulfide       | Tangerman et al. (170)                                                 |                           |                       |                                                   |
| Ethane                 | Azad et al. (170)                                                      | Simulated                 | GC                    |                                                   |
|                        | Paredi et al. (110)                                                    | 22                        | Chemiluminescence     |                                                   |
| Ethanol                | Diskin et al. (122)                                                    | 5                         | SIFT-MS               |                                                   |
|                        |                                                                       | 6                         | GC                    |                                                   |
| Ethylene               | Smith et al. (210)                                                     | Photo-acoustic spectroscopy | Mean 20               |                                                   |
|                        | Dumitras et al. (180)                                                  |                           |                       |                                                   |
|                        | Costello et al. (177)                                                  | 10                        |                       |                                                   |
| Hydrogen cyanide       | Schmidt et al. (130)                                                   | 19                        | Cavity ring down spectroscopy | Mean 7100                                      |
|                        | Španěl et al. (110)                                                    | 26                        | SIFT-MS               |                                                   |
|                        | Enderby et al. (180)                                                   | 16 children with cystic fibrosis | Mean 8               |                                                   |
|                        |                                                                       | 9 adults                  | SIFT-MS               |                                                   |
|                        |                                                                       | 21 children with asthma   |                       |                                                   |
| Hydrogen sulfide       | Costello et al. (177)                                                  | 10                        | Electrochemical detector | Mean 330 (range 0–1300) |
| Isoprene               | Tumer et al. (23,30)                                                   | 20                        | SIFT-MS               | Oral flora                                         |
|                        |                                                                       |                           | NO*                   |                                                   |
|                        | Jones et al. (192)                                                     | 16                        | Thermal desorption GC and diode array UV detection | Range 36–231                                   |
|                        | Smith et al. (21)                                                     | 200                       | SIFT-MS               | Cholesterol metabolism(180,190)                   |
|                        | Davies et al. (171)                                                   | 19                        | SIFT-MS               |                                                      |
|                        | Taucher et al. (194)                                                  |                           | PTR-MS                |                                                      |
|                        |                                                                       |                           | Range 100–1000        |                                                      |
| Methane                | Dryahina et al. (193)                                                  | 75                        | SIFT-MS               | Cholesterol metabolism(180,190)                   |
| Methanol               | Tumer et al. (23)                                                     | 30                        | SIFT-MS               | No statistical difference in isoprene concentrations between men and women Age dependent |
|                        |                                                                       |                           | O₂                    |                                                      |
|                        | Taucher et al. (194)                                                  |                           | PTR-MS                |                                                      |
|                        |                                                                       |                           | Range 6–30            |                                                      |
| Methylamine            | Marinov et al. (120)                                                   | Simulated                 | Near-IR laser spectrometer based on the cavity ring down detection | Range 0.005–0.010                               |
| Methyl nitrate         | Novak et al. (201)                                                    | 10 T1DM children          | GC offline using electron capture(210) | Oxidative processes                              |
| Methyl mercaptan       | Chen et al. (170)                                                     |                           |                        | Methionine metabolism(170)                        |
| NO                     | Paredi et al. (202,203)                                               | Chemiluminescence, following its reaction with O₂(210) | Mean 6.7              | NO synthase and arginine(205–208)                 |

**Continued**
Selected ion flow tube-MS

SIFT-MS combines the fast flow tube technique, chemical ionisation using selected precursor ions, either H$_3$O$^+$, NO$^+$ or O$_2^+$, and quantitative MS that allows online, real-time quantitative analysis of the trace gases (such as ethanol, acetaldehyde, NH$_3$, acetone and isoprene, etc.) in single breath exhalations down to concentrations in the ppbv range in a timescale of seconds (131). SIFT-MS relies on chemical ionisation by the chosen precursor ions of the trace gas molecules in air/breath samples introduced into He carrier gas. These reactions proceed for an accurately defined time, the precursor and product ions being detected and counted by a downstream quadrupole mass spectrometer, thus effecting quantification. Because the absolute concentrations of trace gases in single breath exhalations can be determined by SIFT-MS down to ppbv concentrations, this obviates the need for offline sample collection for the most common breath trace gases. A numerical algorithm allows the calculation, in real time, of absolute concentrations of trace gases, including VOC and water vapor (132).

Optical and laser spectroscopic detection

Laser spectroscopic detection techniques have high sensitivity and high selectivity, but also the advantageous features of near real-time response and low instrument cost. Of approximately thirty-five biomarkers quantified using this method, fourteen species have been analysed in exhaled human breath by high-sensitivity laser spectroscopic techniques, for example acetone, NH$_3$, CO$_2$, ethane, CH$_4$ and NO. The spectral fingerprints of these potentially useful biomarkers span from the UV to the mid-IR spectral regions and the detection limits achieved by the laser techniques range from parts per million by volume to ppbv. Sensors using the laser spectroscopic techniques are already commercially available for a few breath biomarkers, for example CO$_2$ and NO (121).

Electronic nose detection

Electronic noses, or artificial sensors of volatiles including odorants, have been developed over the last 10 years to perform a variety of identification tasks in various industries. Electronic noses produce a chemical fingerprint of the sample, and this is matched to a reference database (133). This powerful technology is only beginning to be introduced in the field of medicine, but is promising in its potential to assist in diagnosis (134).

Chemiluminescence

Chemiluminescence (CL) is a powerful analytical tool in trace gas analysis. CL monitoring has been used as universal nitrogen and sulfur detectors for GC and capillary electrophoresis (135). CL detection can be used as the basis of compact and sensitive analyzers for real-time analysis. isotopes and sulfur compounds in expired breath and atmospheric samples have been successfully measured by couplings to a small collection system. Short-term adsorbent collection enhances the sensitivity and considerably reduces interference. The organosulfur compounds, for example, can be quantified by GC and CL detection techniques.

Table 3. Continued

| Compound | Study | Number of subjects | Method               | Concentration (ppbv) | Source | Comments |
|----------|-------|--------------------|----------------------|----------------------|--------|----------|
| Pentane  | Cobos Barroso et al. (210) | 12 patients (acute asthma) 11 patients (stable asthma) 17 volunteers | Chemiluminescence | Mean 9.7 | Lipid peroxidation (46,48,50) | Concentration in children >17 years predictive of asthma |
| Pentane  | Olopade et al. (211) | 12 patients (acute asthma) 11 patients (stable asthma) 17 volunteers | GC | Mean 188 (SEM 65) Mean 81 (SEM 9) Mean 58 (SEM 4) | Ethane and pentane production is dependent on antioxidant status (181,182) |
| Propanol | 30 | SIFT-MS H$_3$O$^+$ | Mean 18 (range 0–135) | Acetone metabolism (212) | Acetone | Natural levels in the body similar to methanol levels |
| Propanol | 46 | PTR-MS | Mean 120 (range 50–250) | | | |

ppbv, Parts per billion by volume; SIFT, selected ion flow tube; PTR, proton transfer reaction; T1DM, type 1 diabetes mellitus.
compounds methyl mercaptan and dimethyl sulfide can be separated on the same column that is used for collection(136).

Conclusions

Breath analysis is becoming more accessible for clinical and physiological applications. Expired breath is a complex mixture of low-molecular-weight volatile compounds that are derived from diet and endogenous metabolism, or from micro-organisms in the gastrointestinal and respiratory tracts. Metabolic, inflammatory and neoplastic conditions are reported to be associated with characteristic breath profiles, and breath analysis has been promoted as a potentially simple, non-invasive method for screening and monitoring conditions such as asthma, diabetes mellitus and lung cancer. However, there are a number of factors that affect the concentrations of compounds in breath, including diet, physical activity and smoking habit, and it will be important to better understand how these factors influence breath composition as the applications of breath analysis broaden in scope. In order to apply breath analysis to investigations of human nutrition, it would be important to consider any concomitant co-morbidity, including renal and liver dysfunction, neoplastic disease, infection and inflammation. Breath sampling should probably take place under standardised conditions, for example after an overnight fast, and involve diurnal and longitudinal monitoring. A method should be used that is less sensitive to the local release of compounds from the oral cavity. Whichever methods are used should probably also have defined age-related reference ranges.

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References

1. Pauling L, Robinson AB, Teranish R, et al. (1971) Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. Proc Natl Acad Sci U S A 68, 2374–2376.
2. Risby T & Solga S (2006) Current status of clinical breath analysis. Appl Phys B 85, 421–426.
3. Phillips M (1997) Method for the collection and assay of volatile organic compounds in breath. Anal Biochem 247, 272–278.
4. Phillips M, Herrera J, Krishnan S, et al. (1999) Variation in volatile organic compounds in the breath of normal humans. J Chromatogr B Biomed Sci Appl 729, 75–88.
5. Španěl P & Smith D (2011) Volatile compounds in health and disease. Curr Opin Clin Nutr Metab Care 14, 455–460.
6. Terashi T, Ozeki Y, Hori H, et al. (2012) C-13-phenylalanine breath test detects altered phenylalanine kinetics in schizophrenia patients. Transl Psychiatry 2, e119.
7. Louhelainen N, Myllärmä M, Rahman I, et al. (2008) Airway biomarkers of the oxidant burden in asthma and chronic obstructive pulmonary disease: current and future perspectives. Int J Chron Obstruct Pulmon Dis 3, 585–603.
8. Salerno-Kennedy R & Cashman KD (2005) Potential applications of breath isoprene as a biomarker in modern medicine: a concise overview. Wien Klin Wochenschr 117, 180–186.
9. Hamer HM, De Preter V, Windey K, et al. (2012) Functional analysis of colonic bacterial metabolism: relevant to health? Am J Physiol Gastrointest Liver Physiol 302, G1–G9.
10. Popov TA (2011) Human exhaled breath analysis. Ann Allergy Asthma Immunol 106, 451–466.
11. Smith D, Turner C & Španěl P (2007) Volatile metabolites in the exhaled breath of healthy volunteers: their levels and distributions. J Breath Res 1, 014004.
12. Zhang Z & Li G (2010) A review of advances and new developments in the analysis of biological volatile organic compounds. Microchem J 95, 127–139.
13. Singh S & Evans TW (1997) Nitric oxide, the biological mediator of the decade: fact or fiction? Eur Respir J 10, 699–707.
14. Weinberg JB (1998) Nitric oxide production and nitric oxide synthase type 2 expression by human mononuclear phagocytes: a review. Mol Med 4, 557–591.
15. Wang T, Pysanenko A, Dryahina K, et al. (2008) Analysis of breath, exhaled via the mouth and nose, and the air in the oral cavity. J Breath Res 2, 037013.
16. Smith D, Wang T, Pysanenko A, et al. (2008) A selected ion flow tube mass spectrometry study of ammonia in mouth- and nose-exhaled breath and in the oral cavity. Rapid Commun Mass Spectrom 22, 783–789.
17. van den Broek AM, Feenstra L & de Baat C (2007) A review of functional real-time breath-gas analyzers using selected-ion ow-tube mass spectrometry. Rapid Commun Mass Spectrom 21, 321–331.
18. Španěl P, Turner C, Wang TS, et al. (2006) Generation of volatile compounds on mouth exposure to urea and sucrose: implications for exhaled breath analysis. Physiol Meas 27, N7–N17.
19. Hodgson M, Linforth RST & Taylor AJ (2003) Simultaneous real-time measurements of mastication, swallowing, nasal airflow, and aroma release. J Agric Food Chem 51, 5052–5057.
20. Herbig J, Titzmann T, Beauchamp J, et al. (2008) Buffered end-tidal (BET) sampling – a novel method for real-time breath-gas analysis. J Breath Res 2, 037008.
21. Birken T, Schubert J, Miekisch W, et al. (2006) A novel visually CO2 controlled alveolar breath sampling technique. Tamed Health Care 14, 499–506.
22. Diskin AM, Španěl P & Smith D (2003) Time variation of ammonia, acetone, isoprene and ethanol in breath: a quantitative SIFT-MS study over 30 days. Physiol Meas 24, 107–119.
23. Turner C, Španěl P & Smith D (2006) A longitudinal study of methanol in the exhaled breath of 30 healthy volunteers using selected ion flow tube mass spectrometry, SIFT-MS. Physiol Meas 27, 637–648.
24. Turner C, Španěl P & Smith D (2006) A longitudinal study of ammonia, acetone and propanol in the exhaled breath of 30 subjects using selected ion flow tube mass spectrometry, SIFT-MS. Physiol Meas 27, 321–337.
25. Smith D, Španěl P, Enderby B, et al. (2010) Isoprene levels in the exhaled breath of 200 healthy pupils within the age range 7–18 years studied using SIFT-MS. J Breath Res 4, 017101.
26. Smith D, Španěl P & Davies S (1999) Trace gases in breath of healthy volunteers when fasting and after a protein-calorie meal: a preliminary study. J Appl Physiol 87, 1584–1588.
27. Turner C, Španěl P & Smith D (2006) A longitudinal study of ethanol and acetaldehyde in the exhaled breath of healthy volunteers using selected-ion flow-tube mass spectrometry. Rapid Commun Mass Spectrom 20, 61–68.
28. Schmidt F, Metsala M, Vaittinen O, et al. (2011) Background levels and diurnal variations of hydrogen cyanide in breath and emitted from skin. J Breath Res 5, 046004.
29. King J, Mochaliski P, Kupferthaler A, et al. (2010) Dynamic profiles of volatile organic compounds in exhaled breath as
75. Barros R, Moreira A, Fonseca J, et al. (2011) Dietary intake of α-linolenic acid and low ratio of n-6-to-3 PUFA are associated with decreased exhaled NO and improved asthma control. Br J Nutr 106, 441–150.

76. Kalaposp M (2003) On the mammalian acetone metabolism: from chemistry to clinical implications. Biochim Biophys Acta 1621, 122–139.

77. Musa-Veloso K, Lkhodii SS & Cunnane SC (2002) Breath acetone is a reliable indicator of ketosis in adults consuming ketogenic meals. Am J Clin Nutr 76, 65–70.

78. Spaniel P, Dryahina K, Rejskova A, et al. (2011) Breath acetone concentration, biological variability and the influence of diet. Physiol Meas 32, N23–N31.

79. Musa-Veloso K, Rarama E, Comeau F, et al. (2002) Epilepsy and the ketogenic diet: assessment of ketosis in children using breath acetone. Pediatr Res 52, 443–448.

80. Jones A & Rossner S (2007) False-positive breath-alcohol test after a ketogenic diet. Int J Obes 31, 559–561.

81. Levitt MD (1969) Production and excretion of hydrogen gas in healthy volunteers. Br J Nutr 24, 37–42.

82. Avallone EV, De Carolis A, Loizos P, et al. (2010) Hydrogen breath test–diet and basal H2 excretion: a technical note. Digestion 82, 39–41.

83. Rumessen JJ, Hamberg O & Gudmandhoyer E (1990) Interval sampling of end-expiratory hydrogen (H2) concentrations to quantify carbohydrate malabsorption. Gut 30, 811–814.

84. Rumessen JJ, Hamberg O & Gudmandhoyer E (1990) Interval sampling of end-expiratory hydrogen (H2) concentrations to quantify carbohydrate malabsorption by means of lactulose standards. Gut 31, 37–42.

85. Simren M & Stotzer PO (2006) Use and abuse of hydrogen breath tests. Gut 55, 297–303.

86. Addolorato G, Montalto M, Capristo E, et al. (1997) Influence of alcohol on gastrointestinal motility: lactulose breath hydrogen testing in oroccal transit time in chronic alcoholics, social drinkers and teetotaler subjects. Hepatogastroenterology 44, 1076–1081.

87. Wegener M, Schaffstein J, Dilger U, et al. (1991) Gastrointestinal transit of solid liquid meal in chronic alcoholics. Dig Dis Sci 36, 917–923.

88. Smith D, Wang TS & Španiel P (2002) On-line, simultaneous quantification of ethanol, some metabolites and water vapour in breath following the ingestion of alcohol. Physiol Meas 23, 477–489.

89. Mitsubayashi K, Matsunaga H, Nishio G, et al. (2005) Bioelectronic sniffers for ethanol and acetaldehyde in breath air. Biosens Bioelectron 20, 1573–1579.

90. Salaspuro MP (2003) Acetaldehyde, microbes, and cancer of the digestive tract. Crit Rev Clin Lab Sci 40, 183–208.

91. Fritz M, Siebert G & Kasper H (1985) Dose dependence of breath hydrogen and methane in healthy-volunteers after ingestion of a commercial disaccharide mixture, Palatinit. Br J Nutr 54, 389–400.

92. Fritz M, Kasper H, Schrezenmier J, et al. (1985) Effect of acarbose on the production of hydrogen and methane and on hormonal parameters in young adults under standardized low-fiber mixed diets. Z Ernahrungsphysiolog 24, 1–18.

93. Madsen HJ, Linnet J & Rumessen JJ (2006) Effect of nonabsorbed amounts of a fructose–sorbitol mixture on small intestinal transit in healthy volunteers. Dig Dis Sci 51, 147–153.

94. Behall KM & Howe JC (1997) Breath-hydrogen production and amylase content of the diet. Am J Clin Nutr 65, 1783–1789.

95. Stroechl A & Levit MD (1991) Measurement of starch absorption in humans. Can J Physiol Pharmacol 69, 108–110.

96. Cherbut C, Aube AC, Mekki N, et al. (1997) Digestive and metabolic effects of potato and maize fibres in human subjects. Br J Nutr 77, 33–46.

97. Alles MS, Hartemink R, Meyboom S, et al. (1999) Effect of transgalactooligosaccharides on the composition of the human intestinal microflora and on putative risk markers for colon cancer. Am J Clin Nutr 69, 980–991.

98. Hamaker BR, Rivera K, Morales E, et al. (1991) Effect of dietary fiber and starch on fecal composition in preschool children consuming maize, amaranth, or cassava flour. J Pediatr Gastroenterol Nutr 13, 59–66.

99. O’Keefe SJ, Ou J, Delany JP, et al. (2011) Effect of fiber supplementation on the microbiota in critically ill patients. World J Gastroenterol Pathobiol 2, 138–145.

100. Beckman KB & Arnes BN (1998) The free radical theory of aging mutations. Physiol Rev 78, 547–581.

101. Trepanowski JF, Canale RE, Marshall KE, et al. (2011) Impact of caloric and dietary restriction regimens on markers of health and longevity in humans and animals: a summary of available findings. Nutr J 10, 107.

102. Habib MP, Dickerson F & Mooradian AD (1990) Ethane production rate in vivo is reduced with dietary restriction. J Appl Physiol 68, 2588–2590.

103. Kundu SK, Brueck JA, Nair R, et al. (1993) Breath acetone analyzer: diagnostic tool to monitor dietary fat loss. Clin Chem 39, 87–92.

104. Miller ER, Appel LJ & Risby TH (1998) Effect of dietary patterns on measures of lipid peroxidation: results from a randomized clinical trial. Circulation 98, 2390–2395.

105. Lindinger W, Taucher J, Jordan A, et al. (1997) Endogenous production of methanol after the consumption of fruit. Alcohol Clin Exp Res 21, 939–943.

106. Taucher J, Lagg A, Hansel A, et al. (1995) Methanol in human breath. Alcohol Clin Exp Res 19, 1147–1150.

107. Zhu J, Bean HD, Xiu YM, et al. (2010) Fast detection of volatile organic compounds from bacterial cultures by secondary electro-spray ionization-mass spectrometry. J Clin Microbiol 48, 4426–4431.

108. Shestivska V, Španiel P, Dryahina K, et al. (2012) Variability in the concentrations of volatile metabolites emitted by genotypically different strains of Pseudomonas aeruginosa. J Appl Microbiol 113, 701–713.

109. Chippendale TW, Španiel P & Smith D (2011) Time-resolved selected ion flow tube mass spectrometric quantification of the volatile compounds generated by E. coli JM109 cultured in two different media. Rapid Commun Mass Spectrom 25, 2163–2172.

110. Bartram HP, Scheppach W, Gerlach S, et al. (1994) Does yogurt enriched with Bifidobacterium longum affect colonic microbiology and fecal metabolites in healthy-subjects? Am J Clin Nutr 59, 426–432.

111. Cherbut C, Aube AC, Blottiere HM, et al. (1997) Effects of short-chain fatty acids on gastrointestinal motility. Scand J Gastroenterol 32, 58–61.

112. Nakamura S, Oka T & Ichinose A (2004) Bioavailability of cellobiose by tolerance test and breath hydrogen excretion in humans. Nutrition 20, 979–983.

113. Cani PD, Loccuff E, Dewulf EM, et al. (2009) Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. Am J Clin Nutr 90, 1236–1243.

114. Britton JR, Pavord ID, Richards KA, et al. (1995) Dietary antioxidant vitamin intake and lung function in the general population. Am J Respir Crit Care Med 151, 1383–1387.

115. Cook DG, Carey IM, Whineup PH, et al. (1997) Effect of fresh fruit consumption on lung function and wheeze in children. Thorax 52, 628–633.

116. Steinberg FM & Chait A (1998) Antioxidant vitamin supplementation and lipid peroxidation in smokers. Am J Clin Nutr 68, 319–327.

117. Mertz SD, Woodhouse LR, Donangelo CM, et al. (1999) Breath ethane excretion rate in young women is increased by daily iron but not by daily zinc supplementation. FASEB J 13, A241.

118. Kremer D, Ilgen G & Feldmann J (2005) GC-ICP-MS determination of dimethylselenide in human breath after ingestion of Se-77-enriched selenium: monitoring of in vivo methylation of selenium. Anal Bioanal Chem 383, 509–515.

119. Barceloux DG (1999) Selenium. J Toxicol Clin Toxicol 37, 145–172.
120. Smith D & Španěl P (2011) Direct, rapid quantitative analyses of BVOCs using SIFT-MS and PTR-MS obviating sample collection. *Trends Anal Chem* 30, 945–959.

121. Wang CJ & Sahay P (2009) Breath analysis using laser spectroscopic techniques: breath biomarkers, spectral fingerprints, and detection limits. *Sensory* 9, 8230–8262.

122. McGrady MR, Bakhirin Y, Wysocki G, et al. (2007) Recent advances of laser spectroscopy-based techniques for applications in breath analysis. *J Breath Res* 1, 014001.

123. Smith D & Španěl P (2007) The challenge of breath analysis for clinical diagnosis and therapeutic monitoring. *Analyst* 132, 390–396.

124. Ruszvány V, Baumbach JI, Silemann S, et al. (2005) Detection of human metabolites using multi-capillary columns coupled to ion mobility spectrometers. *J Chromatogr A* 1084, 145–151.

125. Moser B, Bodrogi F, Eibl G, et al. (2005) Mass spectrometric profile of exhaled breath – field study by PTR-MS. *Respir Physiol Neurobiol* 145, 295–300.

126. Lirk P, Bodrogi F & Rieder J (2004) Medical applications of proton transfer reaction-mass spectrometry: ambient air monitoring and breath analysis. *Int J Mass Spectrom* 239, 221–226.

127. Jordan A, Haideracher S, Hanel G, et al. (2009) An online ultra-high sensitivity proton-transfer-reaction mass spectrometer combined with switchable reagent ion capability (PTR + SRI-MS). *Int J Mass Spectrom* 286, 32–38.

128. Blake RS, Monks PS & Ellis AM (2009) Proton-transfer reaction mass spectrometry. *Chem Rev* 109, 861–896.

129. Herbig J, Muller M, Schallhart S, et al. (2009) On-line breath analysis with PTR-TOF. *J Breath Res* 3, 027004.

130. Jordan A, Haideracher S, Hanel G, et al. (2009) A high resolution and high sensitivity proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF-MS). *Int J Mass Spectrom* 286, 122–128.

131. Wang TS (2005) The selected ion flow tube mass spectrometry and its applications to trace gas analysis. *Chinese J Anal Chem* 33, 887–893.

132. Španěl P, Drahalyka K & Smith D (2006) A general method for the calculation of absolute trace gas concentrations in air and breath from selected ion flow tube mass spectrometry data. *Int J Mass Spectrom* 249, 230–239.

133. Di Francesco F, Fuoco R, Trivella MG, et al. (2005) Breath analysis: trends in techniques and clinical applications. *Mass Spectrom* 79, 405–410.

134. Thaker ER, Kennedy DW & Hanson CW (2001) Medical applications of electronic nose technology: review of current status. *Am J Rhinol* 15, 291–295.

135. Syc WF & Cheng MF (1997) The analysis of sulfur compounds by solid adsorbent Tenax GR preconcentration and gas chromatography with flameless sulfur chemiluminescence detection. *J Chinese Chem Soc* 44, 107–114.

136. Toda K & Dasgupta PK (2008) New applications of chemiluminescence for selective gas analysis. *Chem Eng Commun* 195, 82–97.

137. Rumensen JJ, Kolholm G & Gudmandhoyer E (1987) Methodological aspects of breath hydrogen (H₂) analysis – evaluation of a H₂ monitor and interpretation of the breath H₂ test. *Scand J Clin Lab Invest* 47, 555–560.

138. Romagnuolo J, Schiller D & Bailey RJ (2002) Using breath tests wisely in a gastroenterology practice: an evidence-based review of indications and pitfalls in interpretation. *Am J Gastroenterol* 97, 1113–1126.

139. Eisenmann A, Amann A, Said M, et al. (2008) Implementation and interpretation of hydrogen breath tests. *J Breath Res* 2, 046002.

140. Bond JH & Levitt MD (1971) Quantitative measurement of carboxyhaemoglobin malabsorption using respiratory hydrogen (H₂) excretion. *Gastroenterology* 60, 765.

141. Bond JH & Levitt MD (1977) Use of breath hydrogen (H₂) in the study of carboxyhaemoglobin absorption. *Am J Dig Dis* 22, 379–382.

142. Taylor D, Pijnenburg M, Smith A, et al. (2006) Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 61, 817–827.

143. Stevenson DK, Fanaroff AA, Maisels MJ, et al. (2001) Prediction of hyperbilirubinemia in near-term and term infants. *Pediatrics* 108, 31–39.

144. Phillips M, Boehmer JP, Cataneo RN, et al. (2004) Prediction of heart transplant rejection with a breath test for markers of oxidative stress. *Am J Cardiol* 94, 1593–1594.

145. Endre Z, Pickering J, Storer M, et al. (2011) Breath ammonia and trimethylamine allow real-time monitoring of haemodialysis efficacy. *Physiol Meas* 32, 115–130.

146. Rolla G, Bruno M, Bommartino L, et al. (2008) Breath analysis in patients with end-stage renal disease: effect of haemodialysis. *Eur J Clin Invest* 38, 728–733.

147. Narasimhan LR, Goodman W & Patel CKN (2001) Correlation of breath ammonia with blood urea nitrogen and creatinine during hemodialysis. *Pneum Allergol Immunol* 15, 467–4621.

148. Braden B, Lembecke B, Kük B, et al. (2007) 13C breath tests: current state of the art and future directions. *Dig Liver Dis* 39, 795–805.

149. Rao S, Camilleri M, Hasler W, et al. (2011) Evaluation of gastrointestinal transit in clinical practice: position paper of the American and European Neurogastroenterology and Motility Societies. *Neurogastroenterol Motil* 23, 8–23.

150. Gehoes KP, Luypaerts A, Rutgeerts P, et al. (2003) Inulin is an ideal substrate for a hydrogen breath test to measure the oro caecal transit time. *Aliment Pharmacol Ther* 18, 721–729.

151. Shestivska V, Nemec A, Drevinek P, et al. (2011) Quantification of methyl thio cyanate in the head space of *Pseudomonas aeruginosa* cultures and in the breath of cystic fibrosis patients by selected ion flow tube mass spectrometry. *Rapid Commun Mass Spectrom* 25, 2459–2467.

152. Carroll W, Lenney W, Wang TS, et al. (2005) Detection of volatile compounds emitted by *Pseudomonas aeruginosa* using selected ion flow tube mass spectrometry. *Pediatr Pulmonol* 39, 452–456.

153. Sanchez JM & Sacks RD (2003) GC analysis of human breath with a series-coupled column ensemble and a multibed sorption trap. *Anal Chem* 75, 2231–2236.

154. Smith D, Španěl P, Thompson JM, et al. (1998) The selected ion flow tube method for workplace analyses of trace gases in air and breath: its scope, validation and applications. *Appl Occup Environ Hygiene* 13, 817–823.

155. Smith D & Španěl P (1996) Application of ion chemistry and the SIFT technique to the quantitative analysis of trace gases in air and on breath. *Int Rev Physiol* 15, 231–271.

156. Smith D & Španěl P (2005) Selected ion flow tube mass spectrometry (SIFT-MS) for on-line trace gas analysis. *Mass Spectrom Rev* 24, 661–700.

157. Boshier PR, Marczin N & Hanna GB (2010) Repeatability of the measurement of exhaled volatile metabolites using selected ion flow tube mass spectrometry. *J Am Soc Mass Spectrom* 21, 1070–1074.

158. Oh EH, Song HS & Park TH (2011) Recent advances in electronic and bioelectronic noses and their biomedical applications. *Enzyme Microb Technol* 48, 427–437.

159. Lewicki R, Wysocki G, Kosterev A, et al. (2007) Carbon dioxide and ammonia detection using 2 µm diode laser based quartz-enhanced photoacoustic spectroscopy. *Appl Phys B* 7, 152, 157–162.

160. Dahneke H, Kleine D, Hering P, et al. (2001) Real-time monitoring of ethanol in human breath using mid-infrared cavity leak-out spectroscopy. *Appl Phys B* 72, 971–975.

161. Fuchs P, Loesecken C, Schubert JK, et al. (2010) Breath gas aldehydes as biomarkers of lung cancer. *Int J Cancer* 126, 2663–2670.

162. Henderson MJ, Karger BA & Wrenshall GA (1952) Acetone in the breath; a study of acetone exhalation in diabetic and nondiabetic human subjects. *Diabetes* 1, 188–193.

163. Španěl P, Davies S & Smith D (1998) Quantification of ammonia in human breath by the selected ion flow tube analytical method using H₂O²⁻ and O₂⁻ precursor ions. *Rapid Commun Mass Spectrom* 12, 763–766.

164. Rosen RT, Hiserodt RD, Fukuda EK, et al. (2000) The determination of metabolites of garlic preparations in breath and human plasma. *Biofactors* 13, 241–249.

165. Lawson LD & Hughes BG (1992) Characterization of the formation of alllicin and other thiosulfinates from garlic. *Planta Med* 58, 345–350.
Lawson LD & Gardner CD (2005) Composition, stability, and bioavailability of garlic products used in a clinical trial. J Agric Food Chem 53, 6254–6261.

Rosen RT, Hiserodt RD, Fukuda EK, et al. (2001) Determination of allin, S-allylcyysteine and volatile metabolites of garlic in breath, plasma or simulated gastric fluids. J Nutr 131, 968S–971S.

Phillips M (1992) Detection of carbon-disulfide in breath and air: a possible new risk factor for coronary artery disease. Int Arch Occup Environ Health 64, 119–123.

Ciaffoni L, Peverall R & Ritchie GAD (2011) Laser spectroscopy on volatile sulfur compounds: possibilities for breath analysis. J Breath Res 5, 024002.

Chen S, Zieve L. & Mahaladev V (1970) Merecaptans and dimethyl sulfide in breath of patients with cirrhosis of liver. Effect of feeding methionine. J Lab Clin Med 75, 628–635.

Wysocki G, McCurdy M, So S, et al. (2004) Pulsed quantum-cascade laser-based sensor for trace-gas detection of carbonyl sulfide. Appl Opt 43, 6040–6046.

Halmer D, von Basum G, Hering P, et al. (2005) Mid-infrared cavity leak-out spectroscopy for ultrasensitive detection of carbonyl sulfide. Opt Lett 30, 2314–2316.

Middleton ET & Morice AH (2000) Breath carbon monoxide as an indication of smoking habit. Chest 117, 758–763.

Tenhunen R, Marver HS & Schmid R (1968) The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. Pnu Metab 7, 748–755.

Ryter SW, Alam J & Choi AMK (2006) Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol Rev 86, 883–650.

Paredi P, Biernacki W, Invernizzi G, et al. (1999) Exhaled carbon monoxide levels elevated in diabetes and correlated with glucose concentration in blood: a new test for monitoring the disease?. Chest 116, 1007.

Costello B, Ewen R & Ratcliffe N (2008) A sensor system for monitoring the simple gases hydrogen, carbon monoxide, hydrogen sulfide, ammonia and ethanol in exhaled breath. J Breath Res 2, 037001.

Tangerman A, Meuwesearends MT & Vantongeren JHM (1983) A new sensitive assay for measuring volatile sulfur compounds in human breath by Tenax trapping and gas-chromatography and its application in liver cirrhosis. Clinica Chimica Acta 130, 103–110.

Azad M, Obira A, Nishimura K, et al. (2006) Single column trapping/separation and chemiluminescence detection for on-site measurement of methyl mercaptan and dimethyl sulfide. Anal Chem 78, 6252–6259.

Paredi P, Khartonov SA, Leak D, et al. (2000) Exhaled ethane, a marker of lipid peroxidation, is elevated in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 162, 369–373.

Dillard CJ, Dumelin EE & Tappel AL (1977) Effect of dietary vitamin E on expiration of pentane and ethane by rat. Lipids 12, 109–114.

Dumelin EE & Tappel AL (1977) Hydrocarbon gases produced during in vitro peroxidation of polyunsaturated fatty acids and decomposition of preformed hydroperoxides. Lipids 12, 894–900.

Dumitras DC, Giubileo G & Piuu A (2005) Investigation of human biomarkers in exhaled breath by laser photoacoustic spectroscopy. Proc SPIE 5850, 111–121.

Perrin JA, Muller S, Barr RG, et al. (1984) Fasting breath hydrogen concentration: normal values and clinical application. Gastroenterology 87, 1358–1363.

Christl SU, Murgatroyd PR, Gibson GR, et al. (1992) Production, metabolism, and excretion of hydrogen in the large intestine. Gastroenterology 102, 1269–1277.

Lundquist P, Rosling H & Sorbo B (1988) The origin of hydrogen cyanide in breath. Arch Toxicol 61, 270–274.

Spanel P, Dryahina K & Smith D (2007) Acetone, ammonia and hydrogen cyanide in exhaled breath of several volunteers aged 4–83 years. J Breath Res 1, 011001.

Enderby B, Smith D, Carroll W, et al. (2009) Hydrogen cyanide as a biomarker for Pseudomonas aeruginosa in the breath of children with cystic fibrosis. Pediatr Pulmonol 44, 142–147.

Gelman D, Stein RA & Mead JF (1981) Isoprene: the main hydroxycarbon in human breath. Biochem Biophys Res Commun 99, 1456–1460.

Karl T, Praezeller P, Mayr D, et al. (2001) Human breath isoprene and its relation to blood cholesterol levels: new measurements and modeling. J Appl Physiol 91, 762–770.

Stone BG, Besse TJ, Duane WC, et al. (1993) Effect of regulating cholesterol biosynthesis on breath isoprene excretion in men. Lipids 28, 705–708.

Jones AW, Lagesson V & Tagesson C (1995) Origins of breath isoprene. J Clin Pathol 48, 979–980.

Davies S, Spanel P & Smith D (2001) A new ‘online’ method to measure increased exhaled isoprene in end-stage renal failure. Nephrol Dial Transplant 16, 838–839.

Taucher J, Hansel A, Jordan A, et al. (1997) Detection of isoprene in expired air from human subjects using proton-transfer-reaction mass spectrometry. Rapid Commun Mass Spectrom 11, 1230–1234.

Dryahina K, Smith D & Spanel P (2010) Quantification of methane in humid air and exhaled breath using selected ion flow tube mass spectrometry. Rapid Commun Mass Spectrom 24, 1296–1304.

Rumessen JJ (1992) Hydrogen and methane breath tests for evaluation of resistant carbohydrates. Eur J Clin Nutr 46, 577–590.

Rumessen JJ, Nordgaard Andersen I & Gudmandhoye E (1994) Carbohydrate malabsorption – quantification by methane and hydrogen breath tests. Scand J Gastroenterol 29, 826–832.

Sahakian AB, Jee SR & Pimentel M (2010) Methane and the gastrointestinal tract. Dig Dis Sci 55, 2135–2143.

Miller TL, Wolin MJ, Demacario EC, et al. (1982) Isolation of Methanobrevibacter smithii from human feces. Appl Environ Microbiol 43, 227–232.

Marinov D, Rey JM, Mueller MG, et al. (2007) Spectroscopic investigation of methylamines by a cavity-ringdown-based spectrometer. Appl Opt 46, 3981–3986.

Novak BJ, Blake DR, Meinardi S, et al. (2007) Exhaled methyl nitrate as a noninvasive marker of hyperglycemia in type 1 diabetes. Pnu Metab 104, 15613–15618.

Paredi P, Ward S, Cramer D, et al. (2007) Normal bronchial blood flow in COPD is unaffected by inhaled corticosteroids and correlates with exhaled nitric oxide. Chest 131, 1075–1081.

Paredi P, Kharitonov SA & Barnes PJ (2001) Direct methods for the measurement of nitric oxide. Monaldi Arch Chest Dise 56, 88–90.

American Thoracic Society Workshop (2006) ATS workshop proceedings: exhaled nitric oxide and nitric oxide oxidative metabolites in exhaled breath condensate: executive summary. Am J Respir Crit Care Med 173, 811–813.

Lundberg JON, Weitzberg E, Lundberg JM, et al. (1996) Nitric oxide in exhaled air. Eur Respir J 9, 2671–2680.

Lundberg J (1996) Airborne nitric oxide: inflammatory marker and aerocine messenger in man. Acta Physiol Scand Suppl 575, 1–27.

Hibbs JB (1991) Synthesis of nitric oxide from L-arginine: a recently discovered pathway induced by cytokines with antitumor and antimicrobial activity. Res Immunol 142, 565–569.

Hibbs JB, Westenberg C, Taïnnot R, et al. (1992) Evidence for cytokine-inducible nitric oxide synthesis from L-arginine in patients receiving interleukin-2 therapy. J Clin Invest 90, 867–877.

Silicoff PE, McLean PA, Shanks AS, et al. (1997) Marked flow dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. Am J Respir Crit Care Med 155, 260–267.

Cobos Barroso N, Perez-Yarza EG, Sardon Prado O, et al. (2008) Exhaled nitric oxide in children: a noninvasive marker of airway inflammation (article in Spanish). Arch Bronconeumol 44, 41–51.

Olopad O, Zakkar M, Swedler WI, et al. (1997) Exhaled pentane levels in acute asthma. Chest 111, 862–865.

Lewis GD, Laufer MK, Meanall BH, et al. (1984) Metabolism of acetone to isopropyl alcohol in rats and humans. J Forensic Sci 29, 541–549.

Warncke C, Kuczynska J, Hansel A, et al. (1996) Proton transfer reaction mass spectrometry (PTR-MS): propanol in human breath. Int J Mass Spectrom 154, 61–70.