Breath Analysis Overview

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History of breath analysis

- Classical medicine, since the time of Hippocrates, has used subjective impressions of the odors of the human body, i.e., sweat, urine, feces, or breath to diagnose disease.

- Additionally, water vapor in breath has been used for centuries to detect presence of life.

- Lavoisier first detected carbon dioxide in human breath in 1784.

- Earliest modern day publications on breath analysis - Davidson; Chen et al; Pauling et al; and Riely et al; date from late 40s to the early 70s and mirror the development of modern analytical chemistry, particularly chromatography.
Breath analysis in the recent past

- Michael Phillips, MD – has been the pioneering breath researcher for more than 30 years his first paper was published in 1981

- Lars Gustafsson, MD & Phillip Silkoff, MD – identified endogenous nitric oxide in human breath and developed the first successful human nitric oxide breath test respectively


- Anton Amann, PhD – organized International Association of Breath Research, organized the Journal of Breath Research and organizes the annual international meetings on Breath Analysis.
What have we learned from prior research

- Breath analysis has two critical components—sampling and analysis: neglect of either one and you are analyzing garbage.

- Breathing is normally under autonomic control: asking a study subject to provide a breath sample causes them to be aware of their breathing and as a result hyperventilate.

- Breath can be collected into: inert gas sampling bags, evacuated stainless steel canisters, or adsorbed onto adsorbent surfaces. Analysis of collected breath is limited to molecules that are stable.

- Breath can be analyzed on-line without regard to molecular stability.
**Typical breathing parameters for a healthy subject**

Young male, 75 kg, 1.7 $m^2$ body surface area, and BMI of 25, whose resting breathing is under autonomic control

- Tidal volume 0.6 L, respiratory rate 10-12 /min
- Anatomic dead space 0.13 L
- Alveolar gas ventilation 4.7 L/min
- Inhales 300 L of ambient air/h when breathing tidally

- Breath components/composition will change during the breathing cycle (inspiratory air, airway gas, mixed expired gas)
- Pure end tidal gas can only be obtained by sampling with a bronchoscope
Sampling multiple breaths from spontaneously breathing subjects

- Monitor tidal volume of each breath and breathing frequency (paced breathing with visual prompts)
- Monitor the concentration of carbon dioxide continuously, i.e., determine end-tidal and steady state concentrations of each breath
- Monitor mouth pressure continuously
- Monitor pulse

Sample multiple breaths and the resulting analysis is independent of individual breaths
Sampling a single breath from a spontaneously breathing subject

- Control pressure (flow using critical orifice)
- Monitor pressure continuously (subject controls pressure using visual prompts)
- Monitor the concentration of carbon dioxide continuously

Analyze a single breath and the quality of breath can be quantified on the basis of:

mean ± sd of mouth pressure and mean ± sd [CO₂]
What is breath?

Breath is a complex mixture of gases, vapors and aerosols.

Breath contains:

- molecules or their metabolites originating from inhaled air (current or historical exposure) or from dermal absorption *Exposome*
- molecules or their metabolites derived from foods and beverages *Metabolome*
- molecules produced by anabolic or catabolic reactions of foreign organisms (viruses, bacteria, fungi) throughout the body (gut, mouth, lungs, etc.) *Microbiome*
- molecules produced by anabolic or catabolic reactions that occur in tissues or cells throughout the body *Human Metabolome*
Exogenous molecules in breath

- Samples of exhaled breath are always contaminated with inspiratory gas (immediate or previous)
  - There is no accepted method to background correct for room air - alveolar gradient method assumes background contaminants are present in current room air

- Samples of exhaled breath can be contaminated with molecules absorbed through the skin
  - There is no accepted method to correct for these molecules

- Samples of exhaled breath can be contaminated with molecules derived from foods and beverages
  - There is no accepted method to correct for these molecules
Analytical methods for discovery of biomarkers of disease in human breath

Analysis of collected breath -- molecular profiles
- Gas solid chromatography with various detectors (TSD, FID, etc.)
- Capillary gas chromatography with various detectors (FID, ECD, MS, etc.)
- 2-dimensional gas chromatography with various detectors

Real time analysis of molecules in breath
- Mass spectrometry using ion molecule reactions to ease interpretation of the resulting mass spectra (PTR-MS, SIFT-MS, SYFT-MS)
- Mass spectrometry-mass spectrometry
- Fast GC-Ion mobility spectrometry
- Fast GC-Differential mobility spectrometry
## Typical molecules found in human breath

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc (v/v)</th>
<th>Physiological Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetaldehyde</td>
<td>ppb</td>
<td>ethanol metabolism, lipid peroxidation</td>
</tr>
<tr>
<td>acetone</td>
<td>ppb</td>
<td>fatty acid metabolism</td>
</tr>
<tr>
<td>ammonia</td>
<td>ppb</td>
<td>protein metabolism</td>
</tr>
<tr>
<td>carbon dioxide</td>
<td>%</td>
<td>respiration</td>
</tr>
<tr>
<td>carbon monoxide</td>
<td>ppm</td>
<td>heme catabolism catalyzed by <em>heme oxygenase</em>, cytoprotective role</td>
</tr>
<tr>
<td>carbonyl sulfide</td>
<td>ppb</td>
<td>gut bacterial oxidation of reduced sulfur species</td>
</tr>
<tr>
<td>ethane</td>
<td>ppb</td>
<td>lipid peroxidation</td>
</tr>
<tr>
<td>ethanol</td>
<td>ppb</td>
<td>gut bacterial metabolism of sugars</td>
</tr>
<tr>
<td>ethylene</td>
<td>ppb</td>
<td>lipid peroxidation, molecular signaling</td>
</tr>
<tr>
<td>hydrogen</td>
<td>ppm</td>
<td>gut bacterial metabolism of carbohydrates</td>
</tr>
</tbody>
</table>
## Typical molecules found in human breath

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<th>Compound</th>
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<th>Physiological Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydrogen cyanide</td>
<td>ppb</td>
<td>synthesized by <em>P. aeruginosa</em></td>
</tr>
<tr>
<td>hydrogen sulfide</td>
<td>ppb</td>
<td>bacterial metabolism of thiol proteins; mediator of brain, gastrointestinal and liver function</td>
</tr>
<tr>
<td>isoprene</td>
<td>ppb</td>
<td>cholesterol biosynthesis; may be involved in regulation of <em>HMG CoA reductase</em></td>
</tr>
<tr>
<td>methane</td>
<td>ppm</td>
<td>gut metabolism of carbohydrates</td>
</tr>
<tr>
<td>methanethiol</td>
<td>ppb</td>
<td>methionine metabolism</td>
</tr>
<tr>
<td>methylamine</td>
<td>ppb</td>
<td>protein metabolism</td>
</tr>
<tr>
<td>nitric oxide</td>
<td>ppb</td>
<td>catalyzed by <em>nitric oxide synthases</em>; involved in vasodilation or neurotransmission</td>
</tr>
<tr>
<td>1-pentane</td>
<td>ppb</td>
<td>lipid peroxidation</td>
</tr>
<tr>
<td>water</td>
<td>%</td>
<td>respiration</td>
</tr>
</tbody>
</table>
Do unique breath biomarkers for diseases exist?

Unique biomarkers in breath can only originate from:

- ingestion, inhalation or dermal absorption of foreign substances (exposome, metabolome)
- bacterial, viral, or fungal metabolism (microbiome)

The onset of disease results in changes in the concentrations of breath molecules and not the production of unique breath biomarkers - disease does not produce novel biochemistry it inhibits or induces enzyme systems (human metabolome)
What is the process of biomarker discovery

- Select a disease and control study population that are age, gender, ethnicity matched if possible
  - The number of study subjects is critical
  - Compare breath profiles for disease *versus* healthy controls
  - Identify differences between populations -- single or multiple biomarkers
  - The normal concentration in healthy subjects must be known as a function of age, gender, and ethnicity
  - The biochemical pathway for the production of potential biomarkers must be known
  - Identify if there is a reasonable biochemical basis for the biomarker and whether other diseases can involve the same biomarker
Lipid peroxidation of PUFA by ROS result in the production of hydrocarbons (ethane, ethylene, pentane)

**Elevated levels of breath ethane**

- Exposure to solvents, ionizing radiation, exogenous sources of ethane
- Bad personal habits – poor diet, smoking
- Diseases such as: cancer, Alzheimer's disease, amyotrophic lateral sclerosis, scleroderma, pulmonary disease, diabetes, liver disease, Parkinson disease, cardiovascular diseases, airway reactivity (asthma) etc.,
- Having an active infection - viral, fungal, or bacterial (host response to infection)
- Being premature, growing old
- Ischemia/reperfusion injury observed during surgery - also observed during sickle cell anemia crises.

_Elevated breath ethane provides no definitive diagnostic information it is suggestive that something is going on--systemic temperature measurement_
Breath ammonia in humans

Normal catabolism of amino acids in proteins produces ammonia and urea.

Urease producing gut flora will convert urea to ammonia.

Elevated levels of breath ammonia:
- Patients with severe impairment of metabolic liver function
- Patients with genetic disorders of the urea cycle
- Patients with end-stage renal disease i.e., decreased excretion of urine
- Subjects who have just exercised
- Subjects with periodontal disease

Elevated breath ammonia could be due to liver disease, kidney disease, genetic diseases, periodontal disease, exercise.
Breath acetone in humans

Acetone together with other ketone bodies is produced by hepatocytes from excess acetyl CoA.
Acetone (ketone bodies) can be produced by the degradation of lipids (lipolysis).
Ketone bodies diffuse from the hepatocytes and are oxidized via the Krebs cycle in peripheral tissue.

Elevated levels of breath acetone
- Patients presenting with diabetes
- Study subjects dieting (Adkins Diet)
- Study subjects fasting
- Study subjects under stress
- Study subjects after exercise

Elevated breath acetone could be due to a disease or activity.
Breath isoprene in humans

Isoprene is biosynthesized from DL-mevalonate in the liver. Isoprene is produced during the biosynthesis of cholesterol.

Elevated levels of breath isoprene

- Patients presenting with familial hypercholesterolemia
- Patients with familial combined hyperlipidemia
- Patients presenting with certain muscle dystrophies
- Subjects who have been exercising
- Elderly study subjects
- Smokers

Elevated breath isoprene could be due to a disease or activity.
Breath sulfur compounds in humans

Reduced sulfur compounds are produced by the incomplete catabolism of methionine in the liver.
Reduced sulfur compounds are produced by microbiome in the gut and mouth.

Elevated levels of breath sulfur compounds

- Patients presenting with liver diseases
- Patients presenting with bacterial overgrowth in the gut
- Acute rejection of organ transplants
- Subjects who have periodontal disease

Elevated breath sulfur compounds could be due to a variety of diseases or conditions
Breath ethanol in humans

Ethanol is produced by the microbiome in the gut

**Elevated levels of breath ethanol**

- Some patients who are obese have elevated levels of ethanol in their breath due to the gut microbiome and decreased gut motility. This increased level of ethanol may be the cause of steatohepatitis (fatty liver disease) in abstinent patients (NAFLD, NASH)
- Subjects who have drunk alcohol
- Patients who have used hand sanitizers
- Room air contamination due to the use of hand sanitizers
- Patients with yeast overgrowth

**Elevated breath ethanol may be a risk factor for the development of cirrhosis the liver, steatohepatitis but it could be due to environmental contamination or personal habits**
Breath hydrogen cyanide in humans

- Hydrogen cyanide has been suggested to be produced by *P. aeruginosa*

**Elevated levels of breath hydrogen cyanide**
- Patients presenting with pneumonias
- Patients presenting with pneumonias secondary to cystic fibrosis, or with lung allografts
- Patients presenting with urinary tract infections
- Patients presenting with renal infections
- Patients presenting with septic shock
- Patients presenting with necrotising enterocolitis (NEC)
- Burns victims and patients with wound infections

**Elevated breath hydrogen cyanide may appear to be correlated with a disease although this relationship is due to an active bacterial infection that may not be disease related**
## Current clinical breath tests

<table>
<thead>
<tr>
<th>Clinical test</th>
<th>Molecule used in test</th>
</tr>
</thead>
<tbody>
<tr>
<td>capnography</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>gastrointestinal diagnoses (disaccharide deficiency, GI transit time, bacterial over growth, intestinal statis)</td>
<td>hydrogen, methane</td>
</tr>
<tr>
<td>heart transplant rejection (Heartsbreath)</td>
<td>branched chain H/C</td>
</tr>
<tr>
<td>carbon monoxide toxicity, smoking cessation</td>
<td>carbon monoxide</td>
</tr>
<tr>
<td>airway reactivity (asthma)</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>metabolism of labeled urea for the diagnosis of <em>H pylori</em> infection</td>
<td>$^{13}$C carbon dioxide</td>
</tr>
</tbody>
</table>
# Potential clinical breath tests

<table>
<thead>
<tr>
<th>Clinical test</th>
<th>Molecule to be used in test</th>
</tr>
</thead>
<tbody>
<tr>
<td>metabolism of labeled drugs or enzyme substrates (liver function, renal function, etc.)</td>
<td>$^{13}$C carbon dioxide</td>
</tr>
<tr>
<td>neonatal jaundice</td>
<td>carbon monoxide</td>
</tr>
<tr>
<td>oxidative stress (acute or chronic disease)</td>
<td>hydrocarbons, aldehydes</td>
</tr>
<tr>
<td>cholesterol biosynthesis, monitoring <em>HMG CoA reductase</em> inhibitors</td>
<td>isoprene</td>
</tr>
<tr>
<td>renal function</td>
<td>ammonia, alkylamines</td>
</tr>
<tr>
<td>hepatic function</td>
<td>ammonia, carbonyl sulfide, methyl sulfide, methanethiol</td>
</tr>
<tr>
<td>host response to infection</td>
<td>hydrocarbons, aldehydes</td>
</tr>
<tr>
<td>oxidative stress</td>
<td>carbon monoxide, nitric oxide</td>
</tr>
<tr>
<td>induction of antioxidant defenses</td>
<td></td>
</tr>
<tr>
<td>Urea cycle disorder, hepatic encephalitis, exercise physiology</td>
<td>ammonia</td>
</tr>
<tr>
<td>diabetes, fasting/dieting, weight loss</td>
<td>acetone, (ethanol)</td>
</tr>
</tbody>
</table>
What are the future directions for breath analysis

- Based upon all available information, breath analysis can **currently** be used to follow therapy/pharmacologic intervention but not diagnosis.
- However, breath analysis will have a **future role** in clinical diagnosis providing the concentration of biomarker(s) can be definitely related to disease if it:
  - Provides novel information or diagnoses
  - Provides information quicker than traditional tests (real time)
- Diagnosis based upon breath analysis will probably be based upon real time analysis – future use for lasers
Acknowledgements

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  - Colleagues and Students at Johns Hopkins Medical Institutions

- **Study Subjects**
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