Breath Analysis: Past, Present and Future - A Personal Perspective

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Breath analysis in the past

- Classical medicine, since the time of Hippocrates, has used “subjective impressions” of the odors of the body, i.e., sweat, urine, feces, or breath to suggest diagnoses.
- Additionally, presence of water vapor in breath has been used to signify the presence of life.
- Lavoisier in collaboration with Laplace first detected carbon dioxide in breath in 1784.

- Earliest modern day publications on breath analysis by: Davidson; Chen, Mahadevan & Zieve; Pauling, Robinson, Teranishi & Cary; and Riely, Cohen & Lieberman 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

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


- Anton Amann, PhD – organized the International Association of Breath Research (IABR), organized the Journal of Breath Research and the annual international meetings on Breath Analysis starting in 2004.
What have we learned from this previous research into breath analysis?

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

- Tidal breathing is under autonomic control: asking a study subject to breathe causes them to be aware of their breathing and as a result they hyperventilate.

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

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

- Breath contains:
  - molecules or their metabolites originating from inhaled air (current or historical exposure) or from dermal absorption.
  - molecules or their metabolites derived from foods and beverages.
  - molecules produced by anabolic or catabolic reactions that occur in tissues or cells throughout the body.
Typical breathing parameters for a healthy subject

Young male, 75 kg, 1.7 m² 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 360 l of ambient air/h when breathing tidally

- Breath components/composition will change during a breathing cycle (inspiratory air, airway gas, mixed expired gas).
- Pure end tidal gas can only be sampled with a bronchoscope

- Endogenous breath molecules originate from biochemical processes occurring in cells within oral/nasal cavities, pulmonary system and organs and tissues throughout the body
Sampling multiple breaths from spontaneously breathing subjects

- Monitor tidal volume of each breath and breathing frequency
- 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
Sampling paced tidal breathing

- CO$_2$ monitor
- Pressure meter
- Flow meter
- Filter
- MOUTH

Breath
Paced breathing as a function of time
Sampling a single breath from a spontaneously breathing subject

- Control pressure (flow using critical orifice)
- Monitor pressure continuously
- Monitor the concentration of carbon dioxide continuously
Monitoring a single breath

- Pressure monitor
- CO₂ monitor
- Critical orifice
- Pressure monitor
- One-way valve
- Real-time monitor
- Mouth
Breath parameters as a function of time

**Plateau Report**

- **CO2 [mmHg]**
  - 39.2 ± 0.7

- **Pressure [cmH2O]**
  - 9.2 ± 0.4

- **Duration [sec]**
  - 6.6
Exogenous molecules in breath

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

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

• Samples of exhaled breath are contaminated with molecules derived from foods and beverages
  • There is no accepted method to correct for these molecules
## 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>
Analytical methods for analysis 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, mass spec, etc)
- 2 Dimensional gas chromatography with various detectors

Real time analysis of molecules in breath

- Chemiluminescence reactions
- Electrochemical sensors
- Absorption of infra-red radiation
- Solid state sensors (quartz crystal microbalance, surface acoustic wave)
- Thermoelectric sensors

Analysis of collected or real-time -- breath profiles (electronic nose)

- Nanomaterial sensor arrays (metal-oxide materials, conducting polymers, carbon nanotubes, organic dielectrics, organic conductor)
Do unique breath biomarkers for diseases exist?

- Unique biomarkers in breath *can only* originate from the ingestion, inhalation or dermal absorption of foreign substances
- Or unique biomarkers in breath *can only* originate from bacterial, viral, or fungal metabolism

- 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*
Oxidative Stress Status: balance between oxidative stress and antioxidant defenses

- Oxidative stress is caused by reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radical which are generated in all cells. A typical adult (75 kg) generates approximately 0.3 mole of ROS/day based upon the utilization of approximately 14.7 mole of O\textsubscript{2}/day.

- Antioxidant defenses: enzymes (superoxide dismutase, glutathione peroxidase, catalase), vitamins (vitamins E & C, beta-carotene, polyphenols (flavonoids, flavones), and proteins (albumin, ferritin, transferrin, metallothionein).

- Oxidative stress status can be quantified in breath indirectly by quantifying stable products of damage by ROS i.e., lipid peroxidation: hydrocarbons (ethane, ethylene, pentane, branched chain hydrocarbons), aldehydes, or arachidonic acid metabolites.
What does increased oxidative stress mean

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

Elevated oxidative stress status provides no definitive information it is similar to a 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. 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 diseases or activities
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 Duchenne muscle dystrophy
- Subjects who have been exercising
- Elderly study subjects
- Smokers

Elevated breath isoprene could be due to a number of diseases or activities
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 bacteria 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 number of diseases or conditions.
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 role in clinical diagnosis if it:
  - Provides novel information or diagnoses
  - Provides information quicker than traditional tests (real time)

- Diagnosis based upon breath analysis will use real time analysis.
Requirements for real-time breath analysis

- Real-time -- result available within 5-10 min
- Based upon sampling single breath

Requirements
- Must be able to be performed by people with limited training
- Instruments must be reliable
# 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</td>
<td></td>
</tr>
<tr>
<td>(disaccharide deficiency, GI transit time, bacterial over growth, intestinal statis)</td>
<td></td>
</tr>
<tr>
<td>heart transplant rejection</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 drugs or enzyme substrates (H pylori, liver function tests, renal function, etc.)</td>
<td>(C^{13}) 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>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</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,</td>
<td>ammonia</td>
</tr>
<tr>
<td>exercise physiology</td>
<td></td>
</tr>
<tr>
<td>diabetes, fasting/dieting, weight loss</td>
<td>acetone, (ethanol)</td>
</tr>
</tbody>
</table>
Conclusions

Clinical breath analysis: are we there yet?

• From a clinical standpoint: *progress has been made*
  – There is a demonstrated need for breath analysis particularly real-time portable devices (point-of-care testing, personalized medicine)

• From an instrument standpoint: *progress has been made*
  – Devices suitable for routine clinical use are at various stages of development
Acknowledgements

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  - Colleagues and Students at Johns Hopkins Medical Institutions

- Study Subjects
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