Breath Analysis: Quantifying Oxidative Stress Status

MIRTHE Summer Workshop, August 7, 2014
Princeton University, Princeton
Terence H. Risby, PhD, FRSC
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 \textit{Exposome}
  - molecules or their metabolites derived from foods and beverages \textit{Metabolome}
  - molecules produced by anabolic or catabolic reactions in foreign organisms (viruses, bacteria, fungi) throughout the body (gut, mouth, lungs, etc.) \textit{Microbiome}
  - molecules produced by anabolic or catabolic reactions that occur in tissues or cells throughout the body \textit{Human Metabolome}
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 they tend to hyperventilate.

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

- Breath can be analyzed on-line without regard to molecular stability.
Cellular Reduction of Oxygen (electron transport chain)

1 electron – reduction of $O_2$ to $O_2^{-}$ by ubisemiquinone, flavin semiquinones of NADH-dehydrogenase, xanthine oxidase, NADH-oxidase

2 electrons -- reduction of $O_2$ to $H_2O_2$ by peroxisomal flavin oxidases: urate oxidase, acyl-CoA oxidase, etc.

4 electrons -- reduction of $O_2$ to $H_2O$ by mitochondrial enzyme: cytochrome oxidase
Oxidative Stress Status
(Balance between oxidative stress caused by ROS and antioxidant defenses)

- A typical adult (75 kg) generates approximately 0.3 mole of ROS/day based upon the utilization of approximately 14.7 mole of O₂/day. ROS are also generated in response to infections and for cell signaling (intracellular half lives for: \( O_2^- = 10^{-4} \) S; \( H_2O_2 < 1 \) S; \( HO^- = 10^{-9} \) S).

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

- Oxidative stress status can be estimated by quantifying the damage caused by ROS to DNA, proteins, lipids or to the change in antioxidant status.
- Measurements performed using samples of tissues, blood, serum, plasma, urine or breath.
Breath and Oxidative Stress Status

- Breath is unique since it is by definition non-invasive and can be collected from any breathing human or animal.
- Samples of breath can be used to quantify stable products of damage by ROS to polyunsaturated fatty acids (lipid peroxidation): hydrocarbons (ethane, ethylene, pentane, branched chain hydrocarbons), aldehydes, or arachidonic acid metabolites.
What does increased lipid peroxidation mean?

Exposure to solvents, ionizing or non-ionizing radiation

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), cancer, etc.

Having an active infection - viral, fungal, or bacterial (host response to infection)

Being premature

Growing old resulting in decreased levels of endogenous antioxidants

Ischemia/reperfusion injury observed during various surgeries - also observed during sickle cell anemia crises.
Chronic oxidant injury in humans

- Smoking
- Changes in levels of endogenous levels antioxidant vitamins, proteins, enzymes etc.,
- Diet
- Exercise
- Disease or aging

Following studies are based upon measurement of breath ethane using gas chromatography/mass spectroscopy, or mid IR laser absorption spectroscopy.
Maternal cigarette smoking

Neonates of mothers that smoke

Breath ethane to monitor vitamin E therapy

Study design for oxidative stress and diet case-controlled study (n=123)

Baseline sampling

## Experimental diets

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>C</th>
<th>A (DASH)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>saturated</td>
<td>16</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>monounsaturated</td>
<td>13</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>polyunsaturated</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td>48</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><strong>Cholesterol</strong></td>
<td>300mg</td>
<td>300mg</td>
<td>300mg</td>
</tr>
<tr>
<td><strong>Fruit/Vegs</strong></td>
<td>100g/day</td>
<td>500g/day</td>
<td>500g/day</td>
</tr>
</tbody>
</table>
Change in breath ethane from baseline to end of study

-1.5 ppb
0.0 ppb
+1.5 ppb

Diet A
Diet B
Diet C
Diet and Aging

◆ 24 month old Fisher 344 female rats

◆ *ad libitum* rats  289.7±10.5 g
◆ dietarily restricted rats  168.4±1.9 g
Collecting gases from rodents
### Metabolism

<table>
<thead>
<tr>
<th></th>
<th>Oxygen Consumption (mL/g hr)</th>
<th>Carbon Dioxide Production (mL/g hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ad lib</td>
<td>DR</td>
</tr>
<tr>
<td>Ad lib</td>
<td>0.98 ± 0.17</td>
<td>0.64 ± 0.16</td>
</tr>
<tr>
<td>DR</td>
<td>1.25 ± 0.13</td>
<td>0.92 ± 0.09</td>
</tr>
</tbody>
</table>
Oxidative Stress and Diet Restriction

24 month Fisher 344 female rats (n=22)

- **ad lib** 289.7 ± 10.5 g   DR 168.4 ± 1.9 g

- **ad lib** 2.31 ± 0.78 pmol/ 100 g min  p < 0.5
- **DR** 2.32 ± 0.65 pmol/ 100 g min

- **ad lib** 135 ± 26 pmol / ml CO₂  p < 0.0003
- **DR** 97 ± 16 pmol / ml CO₂

Acute oxidant injury

- Reperfusion injury during organ transplantation
- Reperfusion injury during cardiopulmonary bypass
- Reperfusion injury after cardiac catheterization
Ischemia-reperfusion injury

1. ATP → AMP → Adenosine → Inosine → Hypoxanthine
2. Xanthine dehydrogenase
3. Xanthine oxidase
4. Xanthine → Urate + O₂⁻ + H₂O₂
5. O₂
Ischemic damage and reperfusion injury
Human liver transplantation
Human liver transplantation (case #1)

Exhaled Ethane (nmol/min/body surface area)

Baseline | Anhepatic | Reperfusion

Time After Intubation (min)

Portal vein opened

Hepatic artery opened closure
Human liver at reperfusion
Summary of human liver transplantation \((n=15)\)
Human cardiopulmonary bypass (n=10)
Reperfusion injury in different tissues

- baseline
- myocardium
- extra myocardial tissue
- liver
- visceral tissue

Graph showing exhaled breath ethane normalized to baseline.
Blocking reperfusion injury in human kidney transplantation

Renal function (Cr)

<table>
<thead>
<tr>
<th>Group</th>
<th>Renal function (Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>14</td>
</tr>
<tr>
<td>CIT &lt; 25</td>
<td>11</td>
</tr>
<tr>
<td>CIT 25-28</td>
<td>22</td>
</tr>
<tr>
<td>CIT &gt; 28</td>
<td>3</td>
</tr>
</tbody>
</table>

acebo  SOD
Blocking reperfusion injury at reperfusion of an occluded coronary artery
Induction of antioxidant defenses by ionizing radiation

![Bar graph showing exhaled ethane (pmol/L) on different treatment days.](image-url)
Breath ethane monitor (ICOS)

Breath sampling rate 50 ml/s
Cell volume 630 cm³
Cell pressure 130 Torr
Path length 1500 m
Wavelength 3.348 μm
Real-time breath measurements

Smoker, 30 minutes after smoking, 2 second avg.

Non-smoker with elevated signal, 2 second avg.

Breath of smoker as a function of time after smoking
Summary of advantages of breath test for Oxidative Stress Status

- Non invasive test
- Point of care test
- Results could available in “real time”
- Measures Breath Ethane in a single breath sample
- Each patient is his/her own control and the change in Breath Ethane can be used to evaluate interventions
Acknowledgements

- **COLLABORATORS**
  - Colleagues and Students at Johns Hopkins Medical Institutions

- **STUDY SUBJECTS**
  - Patients at Johns Hopkins Hospital
  - Healthy Controls

- **SUPPORT:**
  - National Institutes of Health
  - Air Force Office of Scientific Research
  - National Science Foundation (MIRTHE)
Effect of exercise on exhaled breath

- Controlled bicycle exercise
  - different work loads
- Ventilation monitored continuously
  - MV, O₂, CO₂, anaerobic threshold
- Cardiac function monitored continuously
  - CO, HR, stroke volumes, peak filling & emptying rates
Cardiac output and minute ventilation as a function of exercise.
Breath isoprene as a function of exercise

![Graph showing the relationship between isoprene pmol/ml and exercise (W/S).]
Breath ethane as a function of exercise
Breath acetone as a function of exercise